The 3rd Environment and Natural Resources International Conference

Mahidol University
Faculty of Environment and Resource Studies

22 – 23 November 2018, Chonburi, Thailand

Faculty of Environment and Resource Studies, Mahidol University

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ENRIC 2018

Global Development with Environmental Sustainability

Session 1: Environmental Pollution Monitoring and Control

Session 2: Social Environment Issue and Others

Session 3: Natural and Resource Management

Session 4: Climate Change Mitigation and Adaptation

Poster Presentation

HOME

Plenary Speech on

"Thailand's Development and Environmental Sustainability Reform"

Wijarn Simachaya, Ph.D.
Permanent Secretary of Ministry of Natural Resources and Environment, Thailand
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DR. Wijarn Simachaya is the Permanent Secretary of the Ministry of Natural Resources and Environment, Thailand. His main responsibilities are natural resources and environmental plans, strategy development, as well as international cooperation on natural resources and environment issues. He is also a representative of the ministry of various UN, Sub-regional, and ASEAN forums. He has worked as a leader of green growth and government reform strategy development for Thailand. Besides, He serves as a chairman of the long-term strategy (20-year plan) on natural resources and environmental management, including water resources management, sustainable forest management, together with pollution control management and environmental governance for the Ministry. He has been a supervisor of 16 departments and public organizations of the ministry, along with the 76 provincial offices for natural resources and environment throughout the country.

DR. Wijarn joined the Office of Environmental Policy and Planning Board in 1984 and Pollution Control Department in 1992. He used to serve as a director of the Environment Division of the Mekong River Commission Secretariat (International Organization) in Lao PDR during 1997-1998. He has served as several high ranking positions in the Ministry, including Inspector-General and Deputy Permanent Secretary of the Ministry of Natural Resources and Environment, Director-General of the Pollution Control Department for 2 terms and the Secretary-General of the Office of Natural Resources and Environmental Policy and Planning.

DR. Wijarn holds 2 Bachelor's degrees in Chemical Education and Laws from Chiang Mai University and Ramkhamhaeng University, respectively, a Master's in Environmental Science from Kasetsart University and a Graduate Diploma in Sanitary Engineering from Chulalongkorn University. His highest degree is a Doctorate in Philosophy (Ph.D.) in Environmental Engineering from University of Guelph, Canada in 1990.

Plenary Speech on

"EEC: A New Plan for the Present and the Future"

Mr. Pakarathon Tienchai
Governor of Chonburi Province, Thailand
E-mail: chonburi.pa@gmail.com

Date of Birth — 14 June 1962

Work Experience

- October 2016 – Present — Governor of Chonburi Province
- 2012 — Governor of Sa Kaeo Province
- 2010 — Vice Governor of Chonburi Province
- 2009 — Vice Governor of Yasothon Province

Education

- Master of Arts (M.A.), National Institute of Development Administration (NIDA)
- Bachelor of Political Science (B.Pol.Sc.), Thammasat University

Training

- The National Defence College Program (NCD)
- Executive Development Program, Ministry of Interior (MOI)
- District Chief Officer Development Program
- Department of Provincial Administration (DOPA)

Plenary Speech on

"Public Participation in Sustainable Environment: A Case Study of the Philippines"

Alex B. Brillantes, Jr., PhD
The National College of Public Administration
and Governance, University of Philippines
Diliman, Philippines
E-mail: abbrillantes@up.edu.ph

Alex B. Brillantes, Jr, is Professor at the National College of Public Administration and Governance at the University of the Philippines where he also served as its dean for two terms from 2002 to 2008. He obtained his PhD and MA in Political Science from the University of Hawaii as scholar of the East West Center, and MPA and AB Political Science from the University of the Philippines. He also took special courses at the Institute for Policy Studies and the Institute for Public-Private Partnership in Washington DC, and at the Kennedy School of Government at Harvard University. Dr. Brillantes is a member of the Pi Gamma Mu and Phi Kappa Pi International Honor Societies.

Brillantes is President of the Asian Association for Public Administration with its secretariat in Seoul, Korea. He is also President of the Philippine Society for Public Administration. He earlier served as Chairman of the Philippine Social Science Council, Board Member of the *Galing Pook* Foundation, the Local Government Development Foundation and Trustee of the Local Government Academy.

Brillantes has had close to ten years of actual government experience when he was seconded full time to work in the government as Commissioner of the Commission on Higher Education where he served as Chair of the Board of Regents of 21 State Universities and Colleges. Brillantes also was Executive Director of the Local Government Academy of the Department of Interior and Local Government.

Brillantes was visiting Professor in Kobe University, Meiji University and at the Graduate Research Institute for Policy Studies in Japan; Visiting Fellow at Queensland University of Technology in Brisbane, Australia; delivered lectures at the National Institute of Development Administration in Bangkok, Thailand, Tunghai University in Taiwan, Gadajah Maddah University in Jogjakarta and University of Indonesia in Jakarta, Indonesia. He taught a special course on Poverty Reduction: Gawad Kalinga at the Euromed School in Marseilles, France.

He served as international consultant on institutional development, decentralization and governance for several local and international agencies including the Asian Development Bank, the World Bank, the United Nations Development Program. the US Agency for International Development, the Canadian International Development Agency, the Australian Agency for International Development, the Japan International Cooperation Agency, and the European Union, among others.

Professor Brillantes has published articles on public administration and governance and institutions, decentralization and development administration in several local and international journals including the *Asian Survey*, *Administrative Science Quarterly*, *Kasarinlan, the Asian Review of Public Administration*, *International Public Management Review, and the Philippine Journal of Public Administration* where he served as Editor in Chief. He has written and co-edtied several books including *Dictatorship and Martial Law*; *The Philippine Presidency* (with Bienvenida Amarles-Ilago; and *Innovations and Excellence in Local Governance*. He was the lead editor of the book, *Reinventing A Local Government in the Philippines: The Makati Experience* and was one of the contributors to the book, *The Quest for a Federal Republic: The PDP Laban Model of Philippine Federalism.* 3.0, (2017. He was editor of the book *Local Governments in the Philippines: A Book of Readings* with Proserpina Domingo Tapales.

Dr. Brillantes was a recipient of the International Publications Award (IPA) of the University of the Philippines for several years from 2003 to 2018. Professor Brillantes was Centennial Professorial Chair Awardee for UP Diliman in celebration of the Centennial of the University. He was also awarded the Centennial Achievement Award by the University of the Philippines Baguio Alumni Association; the Tanglaw ng Bayan Award by the Polytechnic University of the Philippines; Outstanding Ilocano Educator by the University of Northern Philippines; and Distinguished Alumnus of the East West Center / East West Center Association (Hawaii).

Chair of the Conference

Associate Professor Sayam Aroonsrimorakot, Ph.D.

Faculty of Environment and Resource Studies, Mahidol University, Thailand

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20. Mr. Juan Carlos Calderon Lopez	Mahidol University, Thailand

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17. Theerawut Chiyanon
18. Wantanee Poklang
19. Yutthapol Pongpleesal

Tentative Program

Day 1: 22nd November, 2018

Time	Event
Room: Saen Suk 1-2	
08.00 - 09.00	Registration
09.00 - 09.30	**Opening Ceremony** Mahidol University's Audio Visual Presentation Faculty of Environment and Resource Studies's Audio Visual Presentation Welcome Speech by Mr. Pakarathon Tienchai, Chonburi Governor
	Conference Report by Associate Professor Dr. Kampanad Bhaktikul, Dean of the Faculty of Environment and Resource Studies, Mahidol University
	Welcome Speech and Conference Opening Remarks by Dr. Wijarn Simachaya, Permanent Secretary of Ministry of Natural Resources and Environment
	Presentation of Souvenir to the Honored Guest Group Photograph
09.30 - 10.30	**Keynote Speaker** **"Thailand's Development and Environmental Sustainability Reform" by** **Dr. Wijarn Simachaya, Permanent Secretary of Ministry of Natural Resources and Environment**
10.30 - 11.00	Break
11.00 - 12.00	**"EEC: A New Plan for the Present and the Future" by Mr. Pakarathon Tienchai, Chonburi Governor**

Time	Event	
12.00 - 13.00	Lunch (**Saensamran 1 – 3**, 2nd Floor)	
	Session 1: Environmental Pollution Monitoring and Control **Room: Saen Suk 1** **Chair Session: Professor Dr. Hideki Nakayama** **Co-chair Session: Dr. Harin Sachdev and** **Dr.Thomas Neal Stewart**	**Session 2: Social Environmental Issue & Others** **Room: Saen Suk 2** **Chair Session: Dr. Sukanya Sereenonchai** **Co-chair Session: Dr. Witchaya Rongsayamanont and** **Mr. Juan Carlos Calderón López**
13.00 – 13.15	No. 2018-01 The Application of Tannin Extract from Plants to Reduce the Concentration of Arsenic Sayam Aroonsrimorakot* and Niwooti Whangchai	No. 2018-23 Sampling and Isolation of *Ganoderma Lucidum* in Que Phong, Nghe An Province Le Minh Thanh* and Le Minh Xuan
13.15 – 13.30	No. 2018-08 Contamination by Airborne Fungi in Hair and Beauty Salons at Sri Khai Municipality, Warinchamrap District, Ubon Ratchathani Province Laksanee Boonkhao* and Patompong Chamnannetiwit	No. 2018-31 Using Animals such as Buffaloes in Thai Tourism Industry: An Analytical Review of Animal Ethics Sayam Aroonsrimorakot and Meena Laiphrakpam*
13.30 – 13.45	No. 2018-19 Kinetic Adsorption of Hazardous Methylene Blue from Aqueous Solution onto Iron-Impregnated Powder Activated Carbon	No. 2018-35 Sustainable Low-Carbon Community Development: A Study Based on a Royal Project for Highland Community Development in Thailand

Time	Event	
	Athit Phetrak*, Thanaporn Maswanna, Sirirat Sangkarak, Sumate Ampawong, Jutarat Rakprasit, Suda Ittisupornrat and Doungkamon Phihusut	Kampanad Bhaktikul*, Sayam Aroonsrimorakot and Meena Laiphrakpam
13.45 – 14.00	No. 2018-16 Characterization of Manganese Oxide-Biomineralization by the Psychrophilic Marine Bacterium, *Arthrobacter* sp. Strain NI-2 and Its Spontaneous Mutant Strain NI-2′ Hideki Nakayama*, Yusuke Shin, Toru Sumita, Kazuya Urata and Yasuyuki Ikegami	No. 2018-51 Factors Affecting the Efficiency of Applying the Green Office Principles in Organization Chonticha Korattana*, Sayam Aroonsrimorakot, Chalong Arunlertaree and Anong Hambananda
14.00 – 14.15	No. 2018-21 Influence of PM_{10} from the Outside Area Affecting on the Northern Part of Thailand Sirapong Sooktawee*, Aduldech Patpai, Suteera Boonyapitak, Rungrawee Kongsong, Nirun Piemyai and Usa Humphries	No. 2018-56 Food Safety and Consumption Quality Potentials of Cassava Lines Grown in Three Rain-Fed Plantation Areas in Thailand Petchludda Chaengsee, Pasajee Kongsil*, Nongnuch Siriwong, Sukanda Kerddee, Piya Kittipadakul, Rutai Ruangthamsing and Krittaya Petchpoung
14.15 – 14.30	No. 2018-32 Life Cycle Assessment of Two Limestone Quarries in Thailand with Environmental Footprint Technique	No. 2018-60 Manage the Environment for Security Development with the Legal Method at Vietnam

Time	Event	
	Thanapat Atikij* and Sayam Aroonsrimorakot	Nguyen Hoang Thuy*
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15.00 – 15.15	No. 2018-37 Removal of Methylene Blue Dye by Silver and Zirconium Doped TiO_2 Photocatalyst under Visible Light Pornrat Sanitnon, Siriluk Chiarakorn* and Chamorn Chawengkijwanich	No. 2018-61 Solutions for the Sustainable Development of Marine Resources in Vietnam in the Current Period Tran Huong Giang*
15.15 - 15.30	No. 2018-41 Heavy Metal Accumulation and Health Risk Assessment through Consumption of Vegetables around Loei River, Loei Province Netnapa Pongpetch* and Vanlop Thathong	No. 2018-62 Type of Story Related to “Human-Fairy Marriage” in Vetnam and Other Southeast Asian Islands Countries Mount with Environment Thu Minh Nguyen*

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15.30 – 15.45	No. 2018-59 Study on Production of Decomposing Cellulose Microbial Product from Microorganism Isolates from Nghi Yen Landfill Le Minh Thanh* and Nguyen Van Tiep	No. 2018-63 Female Blends and Religious Beliefs of Type of Story Related to "Human-Swan (Kinnari) Maiden Marriage" in Folk Stories Southeast Asia Thu Minh Nguyen*
15.45 – 16.00	No. 2018-67 Radiological Hazard Assessment and Excess Lifetime Cancer Risk Evaluation in Surface Soil Samples Collected from Mueang District in Rayong Province, Thailand Prasong Kessaratikoon*, Ruthairat Boonkrongcheep, Kittiya Eiadkaew and Sitthipong Polthum	No. 2018-68 Socioeconomic and Environment Challenges of Artisanal and Small-Scale Tin Mining Sectors in Bangka Barat Regency, Bangka Belitung, Indonesia Ranto Ranto*, Gusti Indah, Rendy Rendy and Sandy Pratama
16.00 - 16.15	No. 2018-77 Bacterial Growth Competition and Their Nitrate Reduction End-Products in the Steady State Chemostat of Nitrate Reducing Bacteria Isolated from Estuarine Sediments Iman Rusmana* and Dave Nedwell	No. 2018-83 Livable City - Liveable Life: Assessment of Happiness of Citizens of Danang, a City in the Central of Vietnam – Research for Social and Sustainable Development Nguyen Thi Hang Phuong*, Le My Dung, Luu Thi Thuy and Bui Van Van

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16.15 - 16.30	No. 2018-82 Environmental Pollution of Solid Waste Burial Sites in Nghe An Province, Vietnam Chau Dao Thi Minha* and Loi Nguyen Van	No. 2018-74 Comparative SWOT Analysis of the Key Stakeholders for Assessing Irrigation Governance in Cambodia Hironori Hamasaki* and Kong Sophaek
16.30 -16.45	No. 2018-84 A Simple Method for Synthesis of Triamine-SiO_2 Material toward Aqueous Nitrate Adsorption Phuoc Toan Phana, Trung Thanh Nguyen*, Nhat Huy Nguyen and Surapol Padungthon	
10.30 – 11.00 & 14.30 – 15.00	Posters Presentation and Demonstration Session	
	No. 2018-02 Kjeldahl Method and Sensory Analyses of a Thornback Ray (*Raja clavata*), Eel (*Anguilla* spp), and Tilapia (*Orechromis mossambicus*) Meat; As a Nutritious Product Alternative Juan Carlos Calderón López*, Harin Sachdev and Gabriela Alejandra Lemus Calderón	
	No. 2018-12 Security Food Alternative through Evaluation by Sensory and Bromatological Analyses of Protein from Corn H-59 (*Zea mays*) and Taro	

Time	Event
	Root (*Colocasia esculenta*), in the Elaboration of Tortilla as a Food Supplement Juan Carlos Calderón López*, Harin Sachdev and Fernando Antonio Moran Perz
	No. 2018-13 Bromatological and Sensory Analysis of a Corn (*Zea mays*) Flour Fortified with Moringa (*Moringa oleífera*), to Increase Its Nutritional Value Juan Carlos Calderón López*, Thamarat Phutthai, Yancy Carolina Moran Mendoza and Manuel Adolfo Calderón Turcios
	No. 2018-16 Characterization of Manganese Oxide-biomineralization by the Psychrophilic Marine Bacterium, *Arthrobacter* sp. Strain NI-2 and Its Spontaneous Mutant Strain NI-2′ Hideki Nakayama*, Yusuke Shin, Toru Sumita, Kazuya Urata and Yasuyuki Ikegami
	No. 2018-20 Optimization Studies Using Response Surface Methodology for Cr(VI) Adsorption on Graphite Oxide-Plaster Composite Doungkamon Phihusut*, Athit Phetrak, Monruedee Chantarat, Patchapun Rattanapun, Jakkrit Khamjerm and Sudthida Pliankarom Tanasupsin
	No. 2018-78 Isolation and Screening of Alkaliphile Bacteria for Biosurfactant Production Using Agricultural/Agro-Industrial Wastes as Substrate

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	Natcha Ruamyat, Ekawan Luepromchai and Nichakorn Khondee*
	No. 2018-79 Formulation of Lipopeptide-based Washing Agent for Oil-Based Drill Cutting Treatment Kanyarat Sikhao, Ekawan Luepromchai, Witchaya Rongsayamanont, Suwat Soonglerdsongpha and Nichakorn Khondee*
17.00 - 21.00	**Welcome Reception (Gala Dinner with Special Event: Loi Krathong Festival)** **Room: Saensamran 1 – 3**, 2^{nd} Floor
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18.30 – 18.40	Welcome & Short Intro by Associate Professor Dr. Sayam Aroonsrimorakot, Conference Chair
18.40 – 19.00	Opening Speech by Associate Professor Dr. Kampanad Bhaktikul (Dean of the Faculty of Environment and Resource Studies, Mahidol University)
19.00 – 20.30	Dinner & Thai Dancing
20.30 – 21.00	Special Event: Loi Krathong Festival

Day 2: 23rd November, 2018

Time	Event	
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08.00 - 09.00	Registration	
09.00 - 10.00	**Keynote Speaker** **"Public Participation in Sustainable Environment: A case Study of the Philippines" by Professor Alex Brillantes, The National College of Public Administration and Governance, University of Philippines Diliman, Philippines**	
10.00 - 10.30	Break	
	Session 1: Environmental Pollution Monitoring and Control (Cont.) **Room: Saen Suk 1** **Chair Session: Assoc. Prof. Dr. Sayam Aroonsrimorakot** **Co-chair Session: Dr.Thomas Neal Stewart and Mr. Juan Carlos Calderón López**	**Session 4: Climate Change Mitigation and Adaptation** **Room: Saen Suk 2** **Chair Session: Professor Mohammed Sharif** **Co-chair Session: Dr. Allan Sriratana Tabucanon and Dr. Chitsanupong Prathum**

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10.15 – 10.30	No. 2018-86 Efficiency of Activated Carbon and White Charcoal from Textile Dyeing Industry in Synthetic Wastewater Sayam Aroonsrimorakot*, Meena Laiphrakpam and Supapan Athirot	No. 2018-33 Change of Forest Area and Its Associated CO_2 Emissions at Provincial Level in Southern Part of Thailand Bussarin Thanutchangsang, Jantira Rattanarat and Thongchai Kanabkaew*
10.30 – 10.45	No. 2018-87 The Self-Heat Two-Stage Biomass Gasification Facility-Benefits, Questions and Prospects T. Fujino, M. Iijima, T. Shimogo and H. Sato	No. 2018-39 Vegetation Survey and Applied Remote Sensing Techniques for Monitoring Carbon Storage in Reclaimed Land of Reforestation at Banpu Lignite Mine, Northern Thailand Suppagarn Thiteja*, Soontorn Khamyong, Amarin Boontun, Panlop Huttagosol and Arisara Charoenpanyanet

Time	Event	
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	Session 3: Natural and Resources Management **Room : Saen Suk 1** **Chair Session: Assoc. Prof. Dr. Sayam Aroonsrimorakot** **Co-chair Session: Dr. Navaporn Karnjanasiranon and** **Dr. Piangjai Peerakiatkhajohn**	**Session 4: Climate Change Mitigation and Adaptation (Cont.)** **Room : Saen Suk 2** **Chair Session: Professor Mohammed Sharif** **Co-chair Session: Dr. Allan Sriratana Tabucanon and** **Dr. Chitsanupong Prathum**
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11.15 – 11.30	No. 2018-11 Using Contingent Valuation Method for Estimating the Willingness to Pay for Mangrove Forest: A Study in West	No. 2018-65 Alternative Energy in Household Using Used Vegetable Oil

Time	Event	
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11.45 – 12.00	No. 2018-50 Valuation of Irrigated Water Used in Crop Production: A Case of Rice and Sugarcane in Nakhon Pathom Province Irwin Vich Gonsalves, Ratchaphong Klinsrisuk* and Piyakarn Teartisup	No. 2018-69 People's Perception and Adaptive Behaviors for Mitigating Climate Change Sarawut Peakhunthod* and Monica Price
12.00 – 12.15		No. 2018-76 Statistical Downscaling for Regeneration of Historical Daily Rainfall Allan Sriratana Tabucanon*
12.00 - 13.00	Lunch (Sila Room)	

Time	Event	
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13.00 – 13.15	No. 2018-70 Monitoring Plant Species Diversity and Carbon Storage in a Recovery Dry Dipterocarp Forest on Volcanic Rock at Huai Hong Khrai Royal Development Study Center, Northern Thailand Soontorn Khamyong*, Teuanchay Phongkhamphanh, Panida Kachina, Niwat Anongrak and Kriangsak Sri-ngernyuang	No. 2018-14 Efficiency of Thai Morning Glory Plants (*Ipomoea aquatica*) in Domestic Wastewater Treatment and Reuse Potentials Manassanun Yammanas*, Akeanan Jongsrisupat, Phattarasin Choontanom, Warunsak Liamlaem and Chongrak Polprasert
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Contamination by Airborne Fungi in Hair and Beauty Salons at Sri Khai Municipality, Warinchamrap District, Ubon Ratchathani Province

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Abstract

This study aims to evaluate the airborne fungal contamination in 15 hair and beauty salons at Sri Khai Municipality, Warinchamrap district, Ubon Ratchathani Province. The settle plate technique was used to collect fungus samples and the physical checklist used to survey in the hair and beauty salons. Data were analyzed using descriptive statistics include frequency and percentage. The results showed that airborne fungus contamination in hair and beauty salons number 2, 4, 8, 13 and 14 got the high amount of total fungal count. While compared with the standards in these services showed very poor atmosphere. Almost of physical factors as temperature and humidity were not met the standards. Almost services had the damage windows (73.33%), the bin had a non-sealed lid and were not made of sturdy durable material (86.77%). Therefore the hair and beauty salon owners should be improve their workplace particularly de-moisture in the air by installing a vacuum cleaner, reduce dirt and dust, increase the brightness in the services and provide a garbage bin for each type of waste and remove the garbage to disposal in every day.

Keywords: Airborne fungal/ Contamination/ Hair and beauty salons/ Ubon Ratchathani province

1. Introduction

Fungus is eukaryotic organisms which single or multi-cell as hypha, *Mycelium*. The fungus will be form a single yeast cell such as *Histoplasma capsulatum*, *Penicillium marneffei* that can cause histoplasmosis and penicilliosis marneffei respectively [1]. Airborne particles are ubiquitous in atmosphere one of which is fungi. Gustarowska and Jakubowska (2002) indicated that about 30% of health problems relevant to indoor air quality as the result of a human organism reaction to mold [2].

The hair and beauty salon is a place that could be accumulated fungus in the equipment within the activities as haircuts, hair coloring, hair shiny, hair spa, permanent hair straightening, curling, steam treatment, makeup, manicure and pedicure. That may be accumulated microorganisms that is harmful to health which is the cause symptoms of the respiratory system, allergic reactions to biological substances or symptoms associated with the gastrointestinal tract [3]. It also found that fungal skin disease is a risk to contact fungus in the beauty salons that contaminated in the towels. The moisture content of the fabric could make a mold or mold spores growing infection in this towels, comb and brush found fungus 27. 34 [4]. Hair and beauty salons that located in Srikhai municipality, Warinchamrap district, Ubon Ratchathani Province have to service for local population and also students of Ubon Ratchathani University. For the study of health risk assessment on occupational health and safety in the workplace of beauty salons in Sri khai munucipality [5] found that there were insufficient ventilation in the facility, no plan for electrical appliances maintenance, the owners not separate the garbage before disposing and the workers do not wear personal protective equipment. These causes could be induced serious health consequences for both the users and the service owners and also causes fungal infections in the hair and beauty salons as well.

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Therefore, the researcher interested to study the total fungal count and the physical conditions in the hair and beauty salons at Sri Khai Municipality Warinchamrap District, Ubon Ratchathani Province. The result will be useful for the this municipality to find out the preventive measures against fungal diseases in this hair and beauty salons.

2. Methodology

This cross-sectional descriptive research aims to study the total fungal count that contamination in airborne and study the physical factors of hair and beauty salons at Sri Khai Municipality Warinchamrap District, Ubon Ratchathani Province during August 2017 to January 2018.

2.1 Goal and Scope of the Study

The population participated in this study were hair and beauty salons that located in Sri Khai municipality, Warinchamrap district totally 15 sites. Samples were collected in the service facilities include:

- Airborne fungus — 3 samples /site, total 45 samples
- Temperature — 1 point /site, total 15 points
- Humidity — 1 point /site, total 15 points
- Light Intensity — 1 point /site, total 15 points

Figure 1: Hair and beauty salon locations and the simulation in each shop for sample collection

2.2 Sampling strategy

1) For total fungal count:

- Total fungal count used to collect by using Sabouraud dextrose agar (SDA) within settle plate technique.

- Plates were exposed in the air for 15 minutes, placing at 1.5 m above the surface simulate as human breathing zone [6].

- Plates were incubated at 35-37 ° C for 48 hours.

- The total number of Colony forming unit (CFUs) was estimated and calculated to organisms per plate/hr and then compare with the index of microbial air contamination, IMA [7]. The researcher used to count all of fungus colonies except bacteria colonies which identified each colony by its characteristics.

2) The physical factors survey in the hair and beauty salons: Using checklist which was divided into 3 parts include:

Part 1: General data of hair and beauty salons

Part 2: Services / building conditions of hair and beauty salons

Part 3: Physical conditions in hair and beauty salons

2.3 Data analysis

Data were analyzed and presented by frequency and percentage. Quantitative data includes total fungal count, temperature level, humidity level and light intensity were analyzed and comparison with the standard. The airborne fungus was comparison within the index of microbial air contamination, IMA as follow in Table 1.

Table 1 The index of microbial air contamination, IMA

IMA value	CFU/plate/hr	Class
0 - 5	0 - 9	Very good
6 - 25	10 - 39	Good
26 - 50	40 - 84	Fair
51 - 5	85 - 124	Poor
≥ 76	≥ 125	Very poor

Source: Pasquarella et al. (2000) [7].

3. Results

3.1 General data of hair and beauty salons

The service duration of the hair and beauty salon were mostly from 1 to 5 years. The area size is 3 - 4 square meters; 53.3%. The number of barber chairs was less than or equal to 2 sets or 86.7% . there was 1 hair wash bed; 73.3%. The services had 1 set of air conditioner at 53.3% , fan 1 to 2 sets; 73. 3%, and also found that the windows in 11 services were damage; 73.3%, no garbage bin in 10 services; 66.7% and 73.3% did not have an air cleaner.

3.2 The physical environment in hair and beauty salons

According to the study of physical environment in hair and beauty salons, this study indicated that only four hair and beauty salons met the temperature standards which ranged in between 22.9-26.2°C, one service met the humidity standard at 52 % RH and ten services met the light intensity standards which ranged in between 221.0-317.0 Lux. (Table 2).

Table 2. Physical environment in hair and beauty salon (n=15)

Salon ID	Temperature (°C)		Humidity (%RH)		Light intensity (Lux)	
	Result	Standard	Result	Standard	Result	Standard
1	27.0	Not pass	73.0	Not pass	221.0	Pass
2	24.0	Pass	65.0	Not pass	196.0	Pass
3	28.3	Not pass	68.0	Not pass	273.0	Pass
4	26.2	Pass	70.0	Not pass	311.0	Pass
5	22.9	Pass	67.0	Not pass	277.0	Pass
6	26.0	Not pass	66.0	Not pass	317.0	Pass
7	26.5	Not pass	69.0	Not pass	244.0	Pass
8	24.7	Pass	75.0	Not pass	269.0	Pass
9	28.3	Not pass	65.0	Not pass	196.0	Pass
10	26.7	Not pass	69.0	Not pass	228.0	Pass
11	28.2	Not pass	65.0	Not pass	508.0	Pass
12	28.0	Not pass	64.0	Not pass	156.0	Not pass
13	27.6	Not pass	72.0	Not pass	298.0	Pass
14	27.3	Not pass	72.0	Not pass	138.0	Not pass
15	21.5	Not pass	52.0	Pass	178.0	Not pass

Remark: 1. Temperature standard range in between 22.5 to 25.0°C and humidity standard range in between 30 to 60 %RH [8].
2. Light intensity standard ≥200 Lux [9].

3.3 Airborne fungus contamination in the hair and beauty salons

Within the study of airborne fungus contamination in hair and beauty salons found that the service number 2, 4, 8, 13 and 14 got the high amount of total fungal count. While compared with the standards in these services showed very poor atmosphere. (Table 3).

Table 3. Airborne fungus contamination in the hair and beauty salons. (N=15)

Salon ID	Total fungal count (cfu/plate/hr)					STD
	Front of the room	Center of the room	Behind the room	Mean	SD	
1	52	56	84	64.00	17.44	Fair
2	184	136	108	142.67	38.44	Very poor
3	60	84	92	78.67	16.65	Fair
4	332	44	84	153.33	156.02	Very poor
5	68	52	12	44.00	28.84	Fair
6	136	48	56	80.00	48.66	Fair
7	284	28	32	114.67	146.66	Poor
8	168	128	100	132.00	34.18	Very poor
9	88	64	56	69.33	16.65	Fair
10	100	64	68	77.33	19.73	Fair
11	20	220	12	84.00	117.85	Fair
12	52	56	76	61.33	12.86	Fair
13	124	296	16	145.33	141.21	Very poor
14	604	124	92	273.33	286.81	Very poor
15	44	56	84	61.33	20.53	Fair

Remark: Standard reference with the index of microbial air contamination, IMA; Pasquarella et al. (2000)

3.4 The physical condition in hair and beauty salons

According to the physical condition survey in hair and beauty salons, this study found that all services building had the good condition, no damage condition. The hair wash bed and chairs were clean in a good condition with 93.33%. While found that the bins using in the services were not suitable, the bin hadn't a non-sealed lid and didn't made of durable material 86.77%. (Table 4).

Table 4. Hair and beauty salon physical conditions (N=15)

Item	Physical conditions	Results Number (Percentage)	
		Yes	No
Building condition			
1	The floor is not damaged, no stains, no residue or waste.	14(93.33)	1(6.77)
2	The walls have no signs of cracks that cause moss or mold.	12(80.00)	3(20.00)
3	The ceiling has no signs of cracks that cause moss or mold.	12(80.00)	3(20.00)
4	The door has a working condition. No part of the damage or removed from the original assembly. No stains and no dust.	13(86.77)	2(13.33)
5	The window has a working condition. No part of the damage or removed from the original assembly. No stains and no dust.	4(26.77)	11(73.33)
Services condition			
6	Barber chair is in a good condition, not damaged, clean and no stains.	14(93.33)	1(6.77)
7	Bed for hair washing in a good condition, clean, not damaged and no stains.	14(93.33)	1(6.77)
8	Clear space is used. It is divided into areas for styling, hair styling, storage, material / fabric / chemicals, seats waiting for service and other areas needed.	11(73.33)	4(26.77)
Bins			
9	Separation of each type of waste clearly.	2(13.33)	13(86.77)
10	The bins have a non-sealed lid and are made of sturdy durable materials.	2(13.33)	13(86.77)
The ventilation			
11	Ventilation devices such as air conditioners or fan blowers are in good working condition. No stains and no dust.	14(93.33)	1(6.77)
12	Good ventilation not hot and soggy	11(73.33)	4(26.77)

4. Discussion and Conclusions

All of 15 services got the high amount of total fungal count which asmosphere of each service show in the level of fair and very poor. This amount of fungus may affect the health of those who working in the building. As Karvala et al. (2010) [10] found that the moisture from the building in the workplace which was fungus can be grown can induce nasal inflammation. The study also found that the services did not have ventilators for venting outward 73.30% . The windows were not working properly or some parts were removed from the original assembly and there was dirt and dust on these assemblies. The owners were no separate each type of waste before disposal and there were not enough bins for each waste types at 86.77% and for the physical condition survey found that only four services and one service met the standard of temperature and humidity respectively. These conditions could be supported to fungus forming. As Moore- landecker,1990 [11] indicated that a few fungus can grow at relative humidity less than 65% RH while in these hair and beauty salons showed the humidity ranged in between 64-73%RH. Which these can cause fungal infection in the services. This study consistent with the findings of Kitja Jitpirom and et al. (2013) [12]. They evaluated the fungal contamination in Spa in Bangkok, they found that the amount of fungus in the air was came from the carpet, wall, room, curtains, air conditioners or areas where moisture and consistent with the study of Narutt Sahanawin (2012) [13]. Who study the microbial risk assessment in the classroom of Srinakharinwirot University. He found that the high humidity in the room would increase the chance of mold.

According to the contamination of fungus in each service facility, the service number 14 and number 4 got the high total fungal count at 273.33 cfu/plate/hr and 153.33 cfu/plate/hr respectively. As the study indicated that the physical environment: temperature, humidity, light intensity in these services were not met the standards, including the general environment of service facilities such as the door and windows were broken, there was dirt and dust accumulated in service area, the bin hadn't a non-sealed lid and did not made with durable materials. These may cause of moss or mold growth in two of that services.

However, although the provision of services in certain places is properly organized. It also found the high amount of fungus. This may be due to other causes or factors. As Katika Samaneein et al. (2016) [14] found the opening of doors and windows affected the amount of fungus suspended in the air. The room which has a door and window had the mean concentrations of fungus greater than those without doors and windows.

5. Recommendations from the study

According to the study, the results showed high amount of total fungal count which can induce fair and very poor atmosphere while compared with the index of microbial air contamination in hair and beauty salons at Sri Khai Municipality, Warinchamrap District, Ubon Ratchathani Province. The physical condition and physical environment in some hair and beauty salon showed not met the standard, then:

1) The service owners should be equipped a light bulb to enhance the brightness of the services. Because the high light intensity inhibits the growth of fungal growth [12].

2) The service owners should be installed and operated the vacuum cleaner that will be reduced the moisture in the air (the level is not suitable for the mold growth less than 60 %RH).

3) The environment conditions of services should be clean especially the windows, curtains, doors and doormats to prevent the formation of fungus.

4) Bins should be placed inside the facility to accommodate waste and the owners should be separated each type of waste before disposal for reduce an accumulation of waste in the services.

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Efficiency of Thai Morning Glory Plants (*Ipomoea aquatica*) in Domestic Wastewater Treatment and Reuse Potentials

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Abstract

This study investigated the efficiencies and growth of Thai morning glory plants (*Ipomoea aquatica*) in domestic wastewater treatment and reuse potentials as human foods or animal feeds. Three laboratory-scale reactors, each with a size of 20×40×30 cm^3 (width × length × depth) were fed with a polluted canal water and operated at the hydraulics retention time (HRT) of 5, 10 and 15 days. About 30 Thai morning glory were initially planted in each reactor and the wastewater treatment efficiencies were investigated with respect to the chemical oxygen demand (COD), total coliforms, Total Kjeldahl Nitrogen (TKN) and suspended solids (SS) removal. The growth and protein production of the Thai morning glory were measured after the operation period of 45 days. The experimental results showed the HRT of 10 days to be optimum in operation with the COD removal efficiency of about 33% and the growth and protein production of the Thai morning glory plants was 9.6 g/(m^2-day), corresponding to the protein production of 0.31 g/(m^2-day). The harvested Thai morning glory plants can be reused as animal feeds or raw materials for composting.

Keywords: Ipomoea aquatic/ Thai morning glory/ Wastewater treatment/ Reuse

1. Introduction

Due to increased population growth and urbanization, wastewater pollution of canals and rivers in Thailand is becoming more serious (Polprasert and Koottatep, 2017). Although some centralized wastewater treatment plants have been constructed, they are expensive in investment and operation and cannot treat all the produced wastewater. Alternative wastewater treatment systems which are low-cost and efficient in operation should be considered. This study investigated the technical feasibility of employing Thai morning glory plants (Ipomoea aquatica) to treat a polluted canal water and explore the reuse potentials of the harvested plants as animal feeds or raw materials for composting. In addition, the Thai morning glory (Ipomoea aquatic Var. aquatica) contains more medicinal properties than other morning glory plants (Honestdocs, 2018). The Thai morning glory plants are floating aquatic plants that can perform photosynthesis in which the produced oxygen can be utilized by bacteria in biodegradation of organic matter in wastewater. Because they can easily grow in canals and rivers, the use of these plants in wastewater treatment would save costs and energy, while the harvested plants can be reused for various purposes as mentioned above.

The World Health Organization (WHO) recommended that normally human body needs at least 50 grams of protein per day per person. Taking less than the minimum dose may result in slower growth, brain fatigue and low resistance to diseases.

The objectives of this study were to: (1) investigate the efficiencies of Thai morning glory plants in treating a domestic wastewater, with report to the remove of COD, TKN, SS and total coliform bacteria and (2) determine the growth and protein production of the Thai morning glory plants.

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2. Methodology

2.1. Laboratory-scale reactors

Three laboratory-scale reactors, each with a size of 20×40×30 cm^3 (width × length × depth), were constructed at the Faculty of Engineering, Thammasat University, Rangsit campus, Pathumthani province, Thailand (Figure 1). The 3 reactors were fed with a polluted canal water mixed with septic tank sludge (Figure 2) to attain COD concentration of about 80 mg/L and operated at the HRT of 5, 10 and 15 days. About 30 Thai morning glory each with were initially planted in each reactor (Figure 3) and the wastewater treatment efficiencies were analysed. The growth and protein production of the Thai morning glory plants after the operation period of 45 days were also measured. Experimental data obtained from each HRT were used to compare the effects of HRT on the COD removal efficiencies by the photosynthetic reactions of the Thai morning glory plants and the bacteria in the reactors responsible for organic matter degradation.

Figure 1. Laboratory-scale reactors

Figure 2. Polluted canal water and septic tank sludge

Figure 2. Polluted canal water and septic tank sludge (cont.)

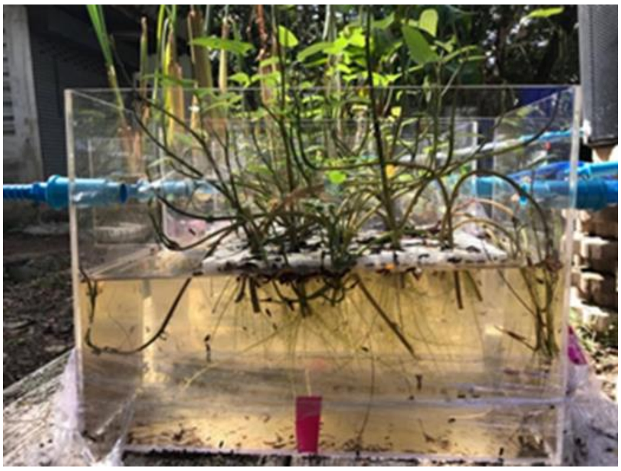

Figure 3. Reactor with a size of 20 × 40 × 30 cm^3 (width × length × depth) (30 plants per reactor)

2.2 Analytical methods

Influent and effluent samples of each reactor were analysed for COD, TKN, SS concentrations according to Standard Methods (APHA et al., 2005). Total coliforms and protein contents were analysed by using test kits (Petrifilm *E. coli*/Coliform count Plates, NPK/pH soil test kit, respectively).

3. Results and Discussion

Table 1 shows the treatment performance of the 3 reactors after steady state conditions in which the % removal efficiencies were found to increase with increasing HRT**.** Probably, due to the relatively short roots of the Thai morning glory plants and the low plant density employed in the experiments, the COD removal efficiencies were found to be 12, 33 and 37 at the HRT of 5, 10 and 15 days, respectively. Similar trends were also observed for total coliforms, TKN and SS removal efficiencies**.** The mechanisms of COD removal should be mainly through aerobic biodegradation by the biofilm bacteria attached on the roots and stems of the Thai morning glory plants which utilized oxygen produced by the plant photosynthesis. TKN removal in the reactors should be through the nitrification/denitrification and plant uptake processes. The HRT of 5 days appeared to be too short for the above processes, hence only 18% of TKN removal was achieved; while about 55 and 77% of TKN removal were observed at the HRTs of 10 and 15 days, respectively. Due to sedimentation and root filtration, about 90-96% of SS removal were achieved for all the HRT. The total coliforms removal should be due to sedimentation, natural die-offs and some antagonistic reactions occurring in the reactors**.** Assuming the BOD_5/COD ratio of domestic wastewater to be 0.6, the data shown in Table 1 suggested that operating the Thai morning glory plant reactor at the HRTs of 10 days or longer would produce treated effluent with characteristics meeting the discharge standards for buildings types c and d of Thailand (PCD, 1994). The growth and protein production of the Thai morning glory plants were found to be highest in the reactor operating at the HRT of 5 days (Table 2) because of the short HRT or high amount of nutrient inputs to the reactor when comparing with the reactors operating at longer HRTs of 10 and 15 days**.** However, with respect to the objectives of wastewater treatment and reuse, from the results shown in Tables 1 and 2, the HRT of 10 days was considered to be optimum which would result in COD removal of 33% and yield of the Thai morning glory plants of about 9.6 g/(m^2-day), corresponding to the protein production of 0.31 g/(m^2-day). The harvested Thai morning glory plants can be reused as animal feeds or raw materials for composting.

Table 1. Wastewater treatment efficiencies

Parameters	Influent(1)	Effluent(1)		
		HRT=5 days	HRT=10 days	HRT=15 days
COD (mg/L)	80	68(12)*	60(33)*	50(37)*
Total coliforms (MPN/100 mL)	600	300	200	100
TKN (mg/L)	22	18	10	5
SS (mg/L)	55	6	4	2

( )* = percent COD removal
(1) = Average of 3 replicates

Table 2. Growth and protein production of Thai morning glory plant

HRT, day	Weight of Thai morning glory plants, g/plant(2)				Protein Production(2), g/(m^2-day)
	Before treatment		After 45 days treatment		
	Wet	Dry	Wet	Dry	
5	3.00	0.37(0.005)*	16.00	1.50(0.045)*	0.33
10	3.00	0.37(0.005)*	12.00	1.30(0.042)*	0.31
15	3.00	0.37(0.005)*	10.00	1.10(0.039)*	0.28

( )* = percent COD removal
(2) = Average of 3 replicates

4. Kinetics and Application

Due to the width/length ratio of the reactors of 1/2, the flow hydraulics can be consumed to follow first-order reaction (Polprasert and Koottatep, 2017) according to Equation (1):

$$\frac{\quad}{0} = \frac{1}{1+} \qquad (1)$$

Where: C_0 = Influent concentration, mg/L
C_e = Effluent concentration, mg/L
k_T = first-order removal rate constant at temperature T °C, day^{-1}
T = liquid temperature, °C
k_{20} = first-order removal rate constant at 20°C, day^{-1}
$k_T = k_{20}(1.06)^{T-20}$
t = hydraulic retention time, days

From the data shown in Table 1, The k_{20} values of COD, total coliforms and TKN removal in the reactors were found to be 0.014, 0.018 and 0.030 day^{-1}, respectively. Due to the relatively short roots and low plant density as mentioned earlier, the k_{20} value of COD of the Thai morning glory plants was much less than those of the other aquatic plant systems such as water hyacinth or cattail plants. However, the k_{20} value of TKN was about twice of the COD value probably because of the high ability of the Thai morning glory plants to uptake the TKN, resulting in increased growth and protein production of about 3-5 times after the 45 days of operation. The k_{20} value of total coliforms was similar to other aquatic plant systems (Polprasert and Koottatep, 2017).

Based on the experiment results, The Thai morning glory plants could be applied for wastewater treatment as a polishing unit following secondary treatment to further reduce COD, total coliforms, TKN and SS concentrations to meet effluent standards for disposal or reuse. The protein production derived from the high growth of the Thai morning glory plants could be used as human food or animal feed. An example on designing a Thai morning glory plant reactor to polish effluent of a communal septic tank with a flow rate of 20 m^3/day and temperature of 30°C is shown below.

At the HRT of 10 days, the reactor volume is 200 m^3 or width × length × depth of 20 × 10 × 1 m^3. If the COD and TKN concentrations of the septic tank effluent were 70 and 20 mg/L, respectively, from the k_{30} values and Equation (1), the effluent COD and TKN concentrations of the Thai morning glory plant reactor would be 56 and 13 mg/L, respectively. From Table 2, the growth and protein production would be 1,920 and 62 g/day, respectively, or 115 and 3.7 kg/2 months if the harvesting period of the Thai morning glory plants is once in 2 months. Assuming the local price of Thai morning glory plants is 20 baht/kg, the revenue earned from this polishing unit would be 2,300 baht 2 months.

5. Conclusions

Based on the experimental results obtained from this study, the following conclusions are made.

1. The HRT of 10 days was found to be optimum for the reactor planted with the Thai morning glory in treating a polluted canal water mixed with septic tank sludge and using the harvested plants as human foods or animal feeds.

2. Under this optimum condition, about 33% COD removal was achieved and the growth and protein production of the Thai morning glory plants were found to be 9.6 and 0.31 g/(m^2–day), respectively.

3. The harvested Thai morning glory plants can be reused as animal feeds or raw materials for composting.

6. Acknowledgements

Research grants received from the Faculty of Engineering, Thammasat University, in the conduct of the experiments were gratefully appreciated.

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Characterization of Manganese Oxide-Biomineralization by the Psychrophilic Marine Bacterium, *Arthrobacter* sp. Strain NI-2 and Its Spontaneous Mutant Strain NI-2'

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Abstract

Metal pollution and metal shortage are a growing threat to the global environment and world high-tech industry. One of the promising strategies for removing and recycling metal-elements from the environments is by using Metal-Biotechnology based on metal-related biological activities, which include bioaccumulation, bioadsorption, and biomineralization. In this study, focusing on Manganese (Mn) pollution, we have isolated and analyzed the Mn(II)-oxidizing marine bacterium, *Arthrobacter* sp. NI-2 strain, from Imari Bay, Imari-shi, Saga, Japan. We have also isolated a spontaneous mutant, *Arthrobacter* sp. NI-2' strain with enhanced Mn(II)-oxidizing activity. Under the liquid culture condition at 30°C, *Arthrobacter* sp. NI-2' strain could efficiently remove more than 96% of Mn(II) from the liquid culture media containing 0.4 mM Mn(II). Although Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 strain is suppressed under the low temperature conditions, the increased Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2' strain was maintained when the growth temperature was shifted from 30°C to 10°C. Therefore, the *Arthrobacter* sp. NI-2' strain would be useful as a tool for Mn removal form low-temperature water, such as groundwater around the mining area.

Keywords: Metal-biotechnology/ Biomineralization/ Manganese(II)-oxidizing activity/ Biogenic manganese oxide/ *Arthrobacter* sp./ Low temperature

1. Introduction

Metal pollution and metal shortage are a growing threat to the global environment and world high-tech industry. As summarized in Figure 1, using metal-related biological activities (i.e. biosensor, bioaccumulation, bioadsorption, biomineralization, and chemisorption), Metal-Biotechnology would be one of the promising strategies for monitoring, removing and recycling metal-elements in polluted environments (Ike et al., 2011).

As Manganese (Mn) is one of the important elements for both the ecosystem and the industry, environmental Mn-pollution and industrial Mn-shortage are the matter of concern. In the environment, Mn(III, IV) oxides, found in various solid forms, play important roles in the global cycling of many major elements (C and S) and trace elements (Fe, Co, Pb, Cu, Cd, and Cr) as an oxidation catalyst and a metal scavenger (Namgung et al., 2018; Tebo et al., 2004). In water ecosystem, Mn(II) found as Mn^{2+} ions are further oxidized for the formation of Mn(III, IV) oxides mainly by the activity of microorganisms, because the rates of Mn(II) oxidation catalyzed by microorganisms are much faster than that of the abiotic Mn(II) oxidation (Nealson et al., 1988; Tebo et al., 2004). The biogenic Mn-oxides formation system, i.e. Mn-oxide biomineralization, could be a good tool for Mn removal from industrial wastewater (Barboza et al., 2016; Ike et al., 2011). Moreover, metal scavenger property of Mn oxides could be an additional tool for multiple heavy-metal removal for industrial wastewater (Figure 2, Chemisorption). Mn(III/IV) oxides are also recognized as powerful oxidants that are capable of oxidizing a wide range of compounds including organic contaminants (Remucal and Ginder-Vogel, 2014).

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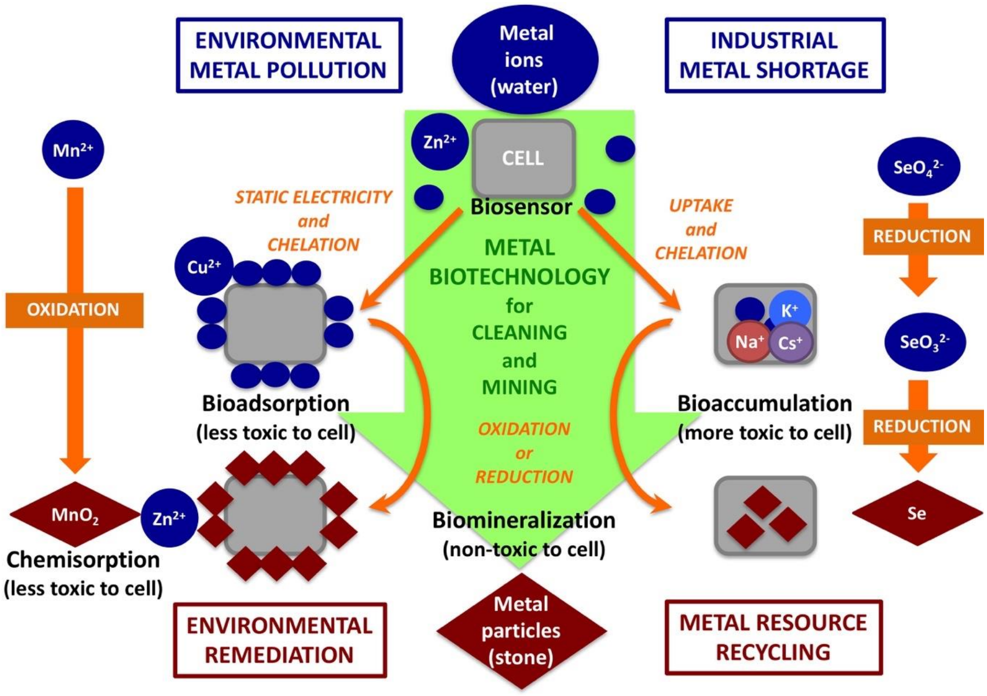

Figure 1. The overview of Metal Biotechnology including biosensor, bioaccumulation, bioadsorption, biomineralization, and chemisorption strategies.

As summarized in Figure 2, however, biogenic Mn(II)-oxidizing activities may be inhibited by environmental stresses such as osmotic stress caused by high salinity and thermal stress caused by dynamic changes of cold/hot weather. Therefore, in order to overcome the weakness of the biogenic Mn(II)-oxidation systems, we set out to isolate robust Mn(II)-oxidizing marine bacteria with ability to maintain the Mn(II)-oxidizing activities under high-salinity and/or low-temperature conditions. Previously, two Mn(II)-oxidizing marine bacteria strains NI-1 and NI-2 were isolated from Imari Bay, Imari-shi, Saga, Japan (Nakayama and Ikegami, 2009). As the NI-1 strain belonging to *Bacillus* sp., which exhibits Mn(II)-oxidizing activity even at 3% and 6% NaCl conditions, the *Bacillus* sp. NI-1 became good candidate for Mn-bioremediation of high-salinity wastewater such as contaminated seawater and concentrated seawater generated by desalination. Although the NI-2 strain does not possess Mn(II)-oxidizing activity under high-salinity conditions, we found that it thrives at low temperatures of 4 and 10°C as a psychrophilic marine bacterium. As cold stress may inhibit Mn oxide biomineralization system in cold wastewater such as drainage water of Mn mines in winter season or at cold climate regions, we further characterized NI-2 strain and its properties including Mn(II)-oxidizing activity under low-temperature conditions.

In this study, we have identified NI-2 strain as *Arthrobacter* sp. based on sequence analysis of the 16S rRNA gene in its genome. Moreover, we have successfully isolated the spontaneous mutant *Arthrobacter* sp. NI-2' strain, which exhibits enhanced ability of Mn oxide biomineralization at 10°C condition. We conclude that the *Arthrobacter* sp. NI-2' strain would be useful for bioremediation of metal-contaminated wastewater even at low temperature conditions.

2. Methodology

2.1. Isolation of Mn(II)-oxidizing bacteria

In order to isolate Mn(II)-oxidizing bacteria (MOB), seawater and seafloor sediment samples collected from Imari bay, Imari-shi, Saga, Japan (Nakayama and Ikegami, 2009) were diluted in 3% NaCl, and 100 μL of each dilution was spread onto solid LEPT media (Boogerd and de Vrind, 1987) containing 0.2 mM $MnCl_2$ at pH 7.5 and incubated for 14 days at 30°C in the dark.

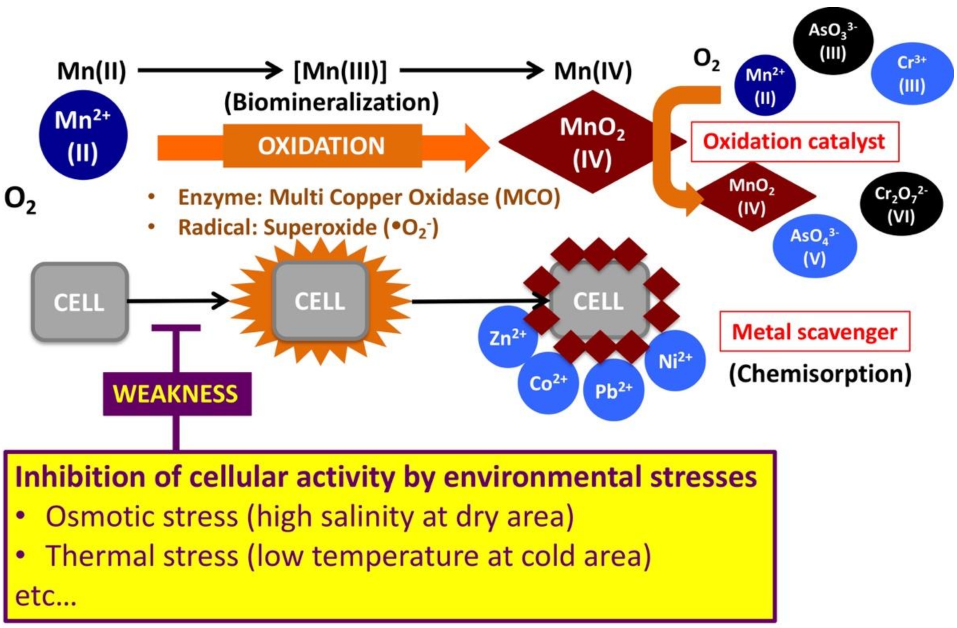

Figure 2. Mn oxide biomineralization and chemisorption as a tool for heavy-metal removal from polluted water. Oxidation of Mn(II) to Mn(III, IV) is mediated by microbial cell activities, which include enzyme reaction by multi copper oxidase (MCO) and/or radical reaction by superoxide (O_2^-).

MOB were identified by using leucoberbelin blue I (LBB), which reacts with Mn(III/IV) oxides and the LBB's color is changed to blue in colorimetric assays (Krumbein and Altmann, 1973). In brief, a drop or spray of the LBB reagent was applied directly to a brownish-black colony or to the whole surface of the growth medium, and the mixture was incubated for 5 min at room temperature in the dark prior to a visual inspection for color change. LBB-positive colonies were transferred and streaked for single colony isolation at least 3 times.

In this study, two isolates named NI-1 and NI-2 strains were used. The NI-1 strain, which was identified as *Bacillus* sp. (Nakayama & Ikegami, 2009) with Mn(II)-oxidizing activity is used as a positive control strain for Mn(II)-oxidizing activity. *Escherichia coli* DH5α, which possesses no Mn(II)-oxidizing activity is used as a negative control strain.

Spontaneous mutant of NI-2 strain with enhanced Mn(II)-oxidizing activity, named NI-2', was isolated by chance during routine subculture periods.

2.2 Identification of MBO isolates by 16S rRNA gene analysis

In order to identify MOB isolates, total genomic DNA was extracted and purified with ISOPLANT II kit (Nippon Gene Co., Ltd., Tokyo, Japan). The almost full length of 16S rRNA gene fragments (about 1.5 kbp) were PCR-amplified using the universal primer set, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAAGGAGGTGATCCAGCC-3'), in a Takara Thermal Cycler (Takara, Shiga, Japan). The PCR products were subcloned with the Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen™, Thermo Fisher Scientific, Waltham, MA) following the manufacturer's specifications. The sequence of the subcloned 16S rRNA gene fragment was determined using universal primer set, M13 Forward (-20) (5'-GTAAAACGACGGCCAG-3') and M13 Reverse (5'-CAGGAAACAGCTATGAC-3'), provided in the Kit. The sequences of the fragments were subjected to a homology search in the APORON database (Techno Suruga Laboratory, Shizuoka, Japan) and phylogenetic trees were constructed to ascertain the phylogenetic positions of the isolates.

2.3 Bioassay for Mn(II)-oxidizing activities under various conditions.

In order to evaluate the effect of NaCl stress on the cell growth and biogenic Mn-oxide formation, a series of solid LEPT media with 3% or 6% NaCl, and with or without 0.2 mM $MnCl_2$ were prepared. Then bacterial cells were streaked on the media and incubated for 14 days at 30°C in the dark as a bioassay for Mn(II)-oxidizing activities under NaCl-stress conditions. In addition, to evaluate the effect of cold stress on the cell growth and biogenic Mn-oxide formation, bacterial cells were streaked on the media and incubated for 14 days at 4°C, 10°C, or 30°C in the dark as a bioassay for Mn(II)-oxidizing activities under cold-stress conditions.

2.4 Quantification of Mn in liquid culture media by ICP-OES analysis.

In order to quantify Mn concentration in liquid culture media, bacterial cells were cultured in 6 mL of the half strength of liquid LEPT media (1/2 LEPT media) with or without 0.4 mM $MnCl_2$ for 14 days and 5 mL of liquid samples were collected from supernatant of the cultures after centrifugation at 3,000×g at room temperature for 10 min to pellet bacterial cells and Mn oxides. The liquid samples were transferred to 50 mL Polypropylene tubes with watch glasses as a lid (DigiTUBE, SCP Science, Quebec, Canada) and the tubes were settled in a digestion block (DigiPREP jr., SCP Science, Quebec, Canada). Digestion was done by adding 25 mL of Milli-Q water and 5 mL of 70% HNO_3 (Nacalai Tesque Inc., Kyoto, Japan) to each sample and heated the mixtures to 65°C, maintained for15 min, then heated up to 105°C and maintained for 120 min before letting the mixture cool down to room temperature. After cooling down, 0.5 mL of 30% H_2O_2 (Fujifilm Wako Pure Chemical, Ltd., Osaka, Japan) were added to the mixtures and the digestion block was again heated to 105°C and maintained at the temperature for 60 min. The digested solution samples were cooled down and filtered through 0.45 µm-pore-size Teflon® membrane filter (DigiFILTER, SCP Science, Quebec, Canada). After rinsing the filter with Milli-Q water, volume of each filtered samples were adjusted to 50 mL by adding Milli-Q water. Measurements were conducted on an ICP-OES (ICPS-7500, Shimadzu Corporation, Kyoto, Japan) following the instruction provided by the manufacturer.

3. Results and Discussion

3.1 Identification of Mn(II)-oxidizing marine bacterium NI-2 strain

As shown in Figure 3, two Mn(II)-oxidizing marine bacteria strains NI-1 and NI-2 were isolated from seawater and seafloor sediment samples collected from Imari Bay, Imari-shi, Saga, Japan (Nakayama & Ikegami, 2009). We found that when Mn(II) is present in the medium, both NI-1 and NI-2 strains produce visible brown-colored particles (Figure 3A and Figure 7). Production of biogenic Mn oxide was confirmed by blue color formation after LBB spray treatment (Figure 3B).

Based on 16S rRNA gene sequencing, the NI-1 strain was previously classified as *Bacillus* sp. (Nakayama & Ikegami, 2009), and the NI-2 strain is classified as *Arthrobacter* sp. in this study (Figure 4). The closest relative species of *Arthrobacter* sp. NI-2 strain is *Arthrobacter subterraneus* CH7^T, which was isolated as a new species from deep subsurface water of the South Coast of Korea (Chang et al., 2011). In the previous report, *A. subterraneus* CH7^T was found to grow under low temperature conditions similar to deep sea environment (Chang et al., 2011). As shown in Figure 5, we found that *Arthrobacter* sp. NI-2 strain grow well under cold-stress condition (4°C), while *Bacillus* sp. NI-1 strain cannot grow under this condition. The result indicates that *Arthrobacter* sp. NI-2 is a psychrophilic marine bacterium.

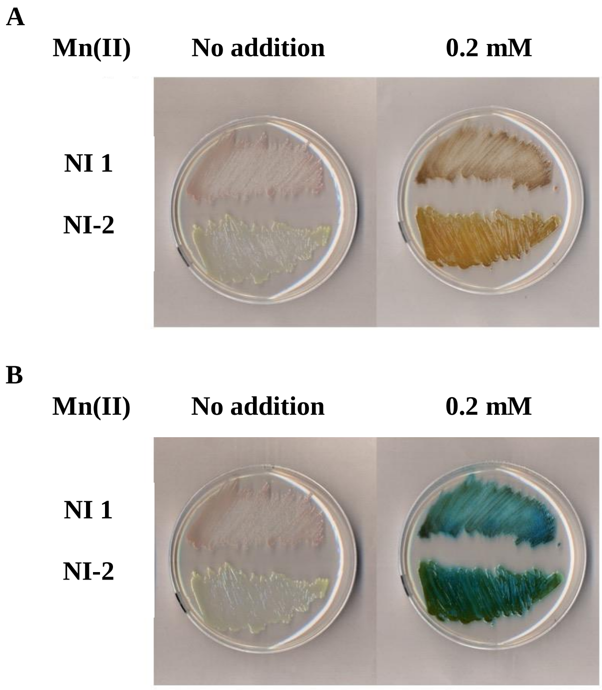

Figure 3: Mn(II)-oxidizing marine bacteria strains NI-1 and NI-2 isolated from Imari Bay, Imari-shi, Saga, Japan. **A**, Both NI-1 and NI-2 stains were cultured for 14 days at 30°C on LEPT media with or without $MnCl_2$ (pH 7.5). **B**, Same samples as A, 5 min after LBB spray treatment.

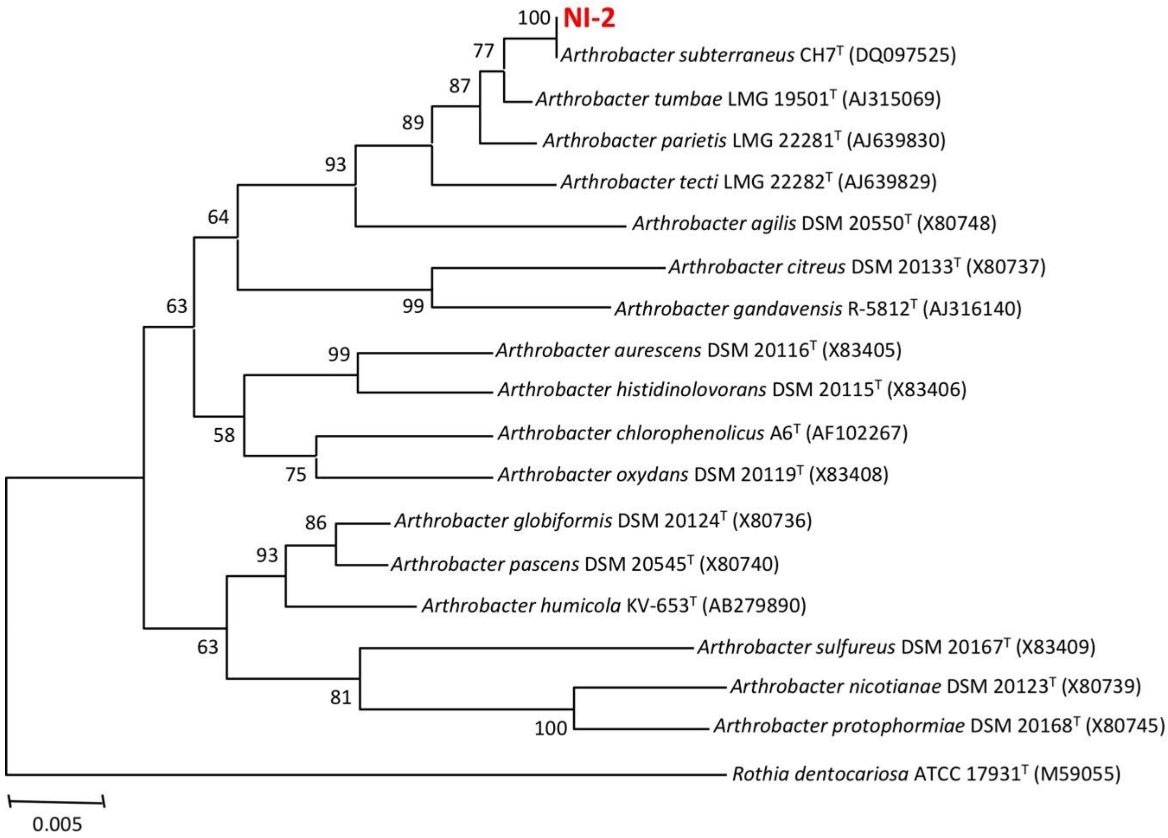

Figure 4. NI-2 strain is classified as *Arthrobacter* sp. in phylogenetic tree based on 16S rRNA gene sequencing by neighbor joining method.

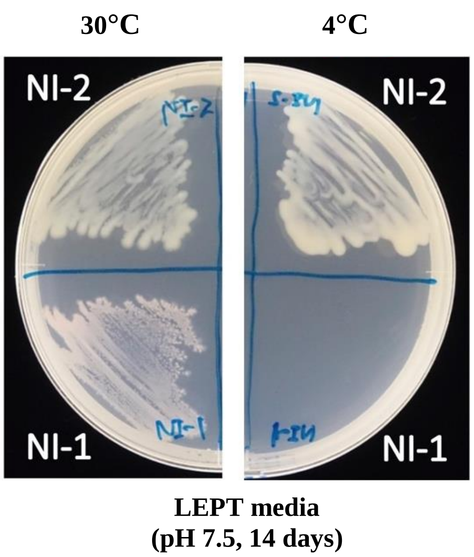

Figure 5. Growth of *Bacillus* sp. NI-1 and *Arthrobacter* sp. NI-2 under cold-stress condition.

3.2 Isolation of NI-2 derivative mutant with increased Mn(II)-oxidizing activity

Recently, *Arthrobacter* sp. strain QXT-31 was identified as a non-Mn(II)-oxidizing strain in monoculture condition, however, interaction (co-culture) with another non-Mn(II)-oxidizing *Sphingopyxis* sp. QXT-31 strain could induce cooperative Mn(II) oxidation by *Arthrobacter* sp. strain QXT-3 in an aquatic environment (Liang et al, 2016). Interestingly, during our screening and isolation process, we have also observed that *Arthrobacter* sp. NI-2 strain displayed improved Mn(II)-oxidizing activity when interacts with an unidentified non-Mn(II)-oxidizing bacteria (Figure 6). However, in contrast to *Arthrobacter* sp. QXT-31 strain, *Arthrobacter* sp. NI-2 strain showed Mn(II)-oxidizing activity even in monoculture at 30°C (Figure 7). This observation implicated that *Arthrobacter* sp. NI-2 strain is probably equipped with endogenous auto-activation system for Mn(II)-oxidizing activity.

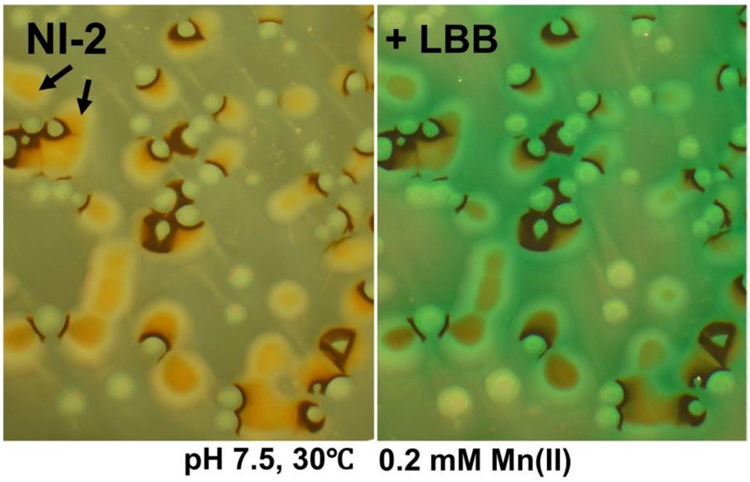

Figure 6. Enhanced Mn(II)-oxidizing activity by interaction with non-Mn(II)-oxidizing bacteria in *Arthrobacter* sp. NI-2 strain.

In this study, *Bacillus* sp. NI-1 and *E. coli* DH5α strains were used as a positive control strain and a negative control strain, respectively, for bioassay of Mn(II)-oxidizing activity. During the routine sub-culturing of NI-2 strain, we have identified a spontaneous mutant colony, which shown a faster and more intense accumulation of Mn oxide (brown-colored particles). As shown in Figure 7, this spontaneous mutant, which we named *Arthrobacter* sp. NI-2' possesses enhanced Mn(II)-oxidizing activity on the Mn(II)-containing LEPT medium at 30°C.

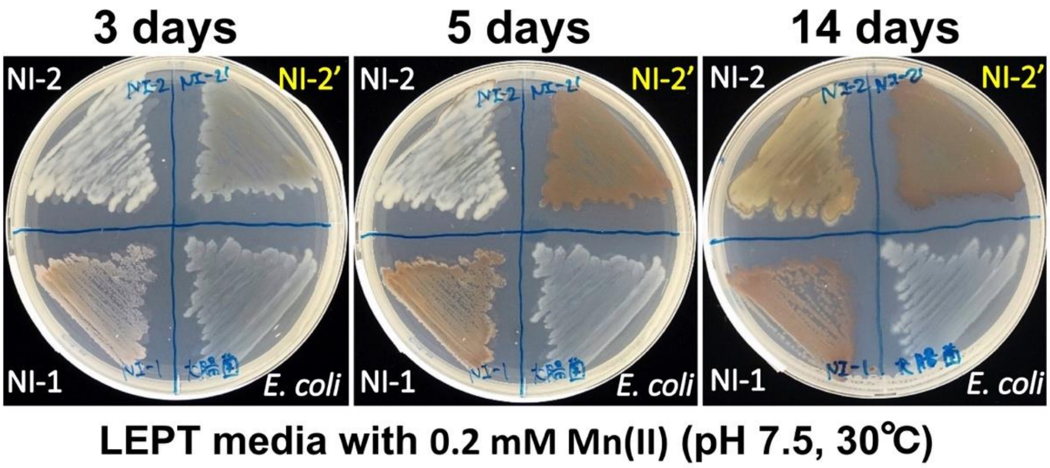

Figure 7. Mn(II)-oxidizing activity in *Arthrobacter* sp. NI-2 and its derivative mutant, *Arthrobacter* sp. NI-2'. *Bacillus* sp. NI-1 and *E. coli* DH5α were used as a positive and a negative control strains for Mn(II)-oxidizing activity, respectively.

3.3 Mn(II) removal by Mn-oxide biomineralization in liquid culture system

In order to evaluate ability of Mn(II) removal by Mn-oxide biomineralization in liquid culture system, *Bacillus* sp. NI-1, *E. coli* DH5α, and *Arthrobacter* sp. NI-2' were subjected to bioassay of Mn-oxide biomineralization. Mn(II)-oxidizing activity seems to be mediated by multi copper oxidase (MCO) on the surface of spore of *Bacillus* sp. NI-1 and is found to be induced during spore formation triggered by starvation in other Mn(II)-oxidizing *Bacillus* sp. strains such as SG-1 strain (de Vrind et al.,1986; Francis & Tebo, 2002). Therefore, our bioassay was performed in half strength of liquid LEPT (1/2 LEPT) culture media, in which nutrients were reduced to accelerate the entering to the starvation phase. As shown in Figure 8A, both *Bacillus* sp. NI-1 and *Arthrobacter* sp. NI-2' were able to form brown-colored particles of Mn oxide efficiently in the culture medium containing 0.4 mM $MnCl_2$ (approx. 20 ppm Mn; doubled the concentration of Mn allowed in drainage water by Uniform Effluent Standard in Japan). Moreover, more than 96% of Mn(II) could be removed from liquid culture media through Mn-oxide biomineralization by *Arthrobacter* sp. NI-2' and *Bacillus* sp. NI-1 (shown in Figure 8B). While only 20% could be remove through biosorption or bioaccumulation by *E. coli* DH5α, which possessed no Mn(II)-oxidizing activity. The results suggest that both *Arthrobacter* sp. NI-2' and *Bacillus* sp. NI-1 strains would be useful for Mn-bioremediation of wastewater at 30°C. As *Arthrobacter* sp. NI-2' was expected to grow under cold-stress conditions similar to the original *Arthrobacter* sp. NI-2 strain, we further investigated Mn(II)-oxidizing activity of each strain under cold-stress conditions.

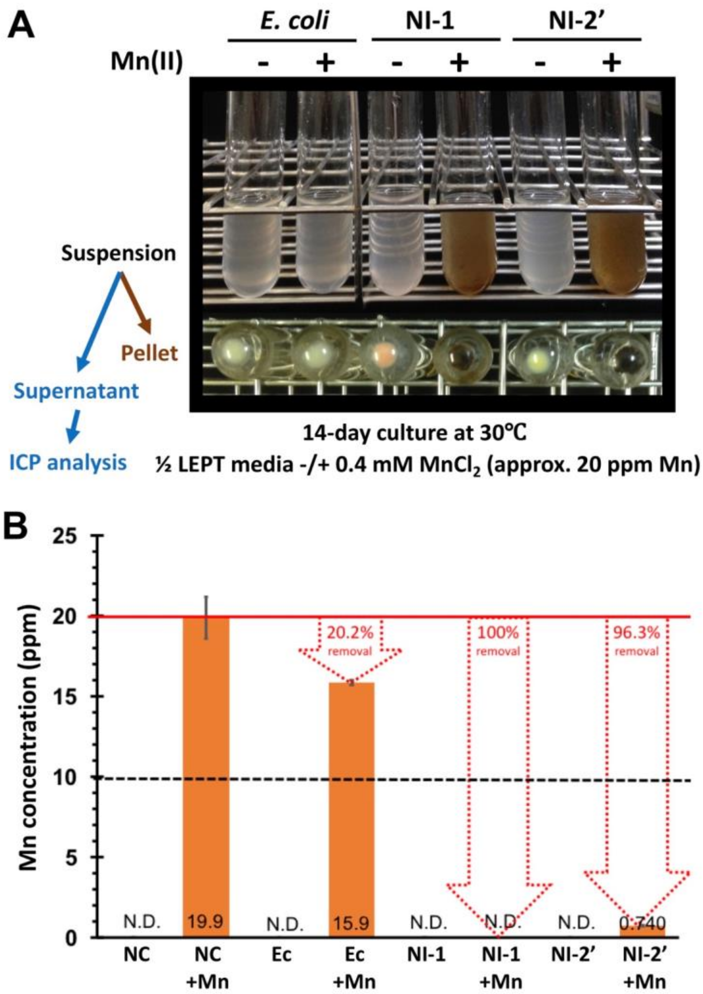

Figure 8. Bioassay of Mn-oxide biomineralization in liquid culture media using *E. coli* DH5α (Ec), *Bacillus* sp. NI-1 (NI-1), and *Arthrobacter* sp. NI-2' (NI-2'). **A**, cell-suspension cultures are separated into pellets and supernatants by centrifugation and each supernatant was subjected to ICP analysis. **B**, ICP-OES analysis of supernatant samples of suspension cell-cultures. N.D. indicates not detectable levels by ICP-OES because of low Mn concentration. NC indicates sample with no bacterial cells (no inoculation) as a control.

3.4 Mn(II)-oxidizing activity under NaCl- or cold-stress conditions

Due to the fact that *Arthrobacter* sp. NI-2 and NI-2' strains can grow well under NaCl-stress condition similar to *Bacillus* sp. NI-1, we tested Mn(II)-oxidizing activities of both *Arthrobacter* sp. NI-2 and NI-2' strains in medium containing 3% (sea water level) and 6% NaCl. Interestingly, Mn(II)-oxidizing activities of both strains were completely suppressed under these conditions (Figure 9). The results indicate that sea-water level of NaCl strongly affects Mn(II)-oxidizing activity of both

Arthrobacter sp. NI-2 and NI-2' strains but not that of *Bacillus* sp. NI-1. Thus, *Bacillus* sp. NI-1 would be a useful strain for Mn removal in high-salinity wastewater including seawater and concentrated seawater as byproduct of desalination (Nakayama And Ikegami, 2009). Therefore, we decided to further investigate the ability of Mn-oxide biomineralization in *Arthrobacter* sp. NI-2' under low-temperature condition without NaCl stress.

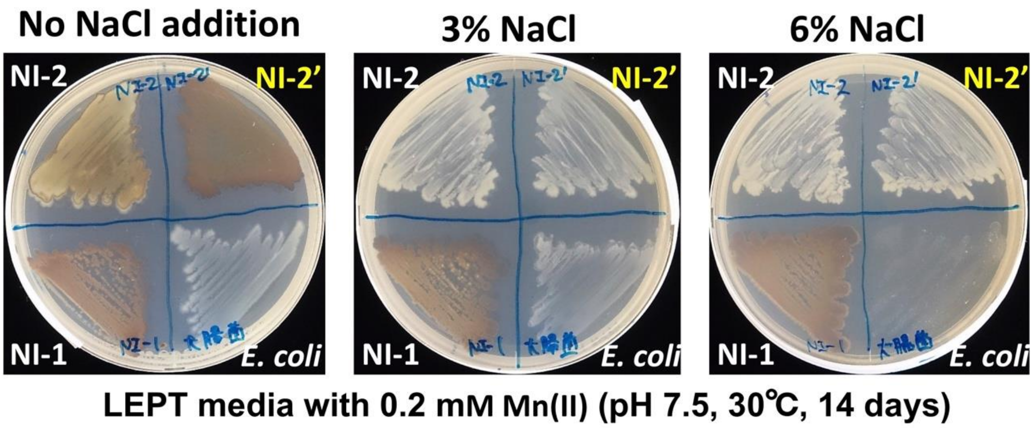

Figure 9. Effect of NaCl stress on cell growth and Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 and its derivative mutant, *Arthrobacter* sp. NI-2'. *Bacillus* sp. NI-1 and *E. coli* DH5α were used as a positive and a negative control strains of Mn(II)-oxidizing activity, respectively. The control plate (No NaCl addition) is exactly the same as the 14-days plate shown in Figure 7.

In order to evaluate Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 and NI-2' under cold-stress conditions, these 2 strains together with *E. coli* DH5α and *Bacillus* sp. NI-1, were subjected to bioassay of Mn-oxide biomineralization on the Mn(II)-containing LEPT medium incubated at 30°C, 10°C, or 4°C. As shown in Figure 10, the growth of *E. coli* DH5α and *Bacillus* sp. NI-1 strains were completely inhibited at 10°C and 4°C because of their sensitivity to cold stress, while both *Arthrobacter* sp. NI-2 and NI-2' strains thrived under these cold-stress conditions. Remarkably, *Arthrobacter* sp. NI-2' strain showed strong Mn(II)-oxidizing activity at 10°C condition while *Arthrobacter* sp. NI-2 strain did not. These results suggest that Mn(II)-oxidizing activity may connected to thermal-responsive signal transduction in *Arthrobacter* sp. NI-2 and NI-2'. Moreover, the *Arthrobacter* sp. NI-2’ strain could be a superior strain for Mn-bioremediation of cold wastewater above 10°C.

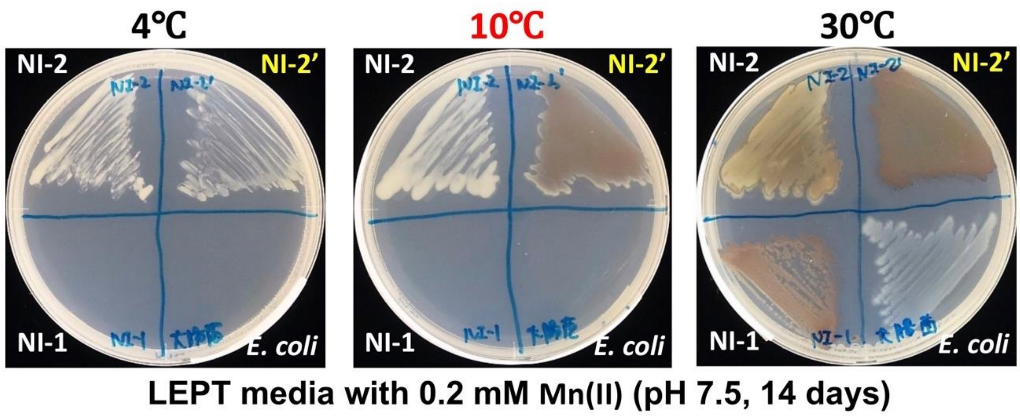

Figure 10: Effect of cold stress on cell growth and Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 and its derivative mutant, *Arthrobacter* sp. NI-2'. *Bacillus* sp. NI-1 and *E. coli* DH5α were used as a positive and a negative control strains of Mn(II)-oxidizing activity, respectively. The control plate (30°C) is exactly the same as the 14-days plate shown in Figure 7.

4. Conclusions

In the study, we identified and characterized Mn(II)-oxidizing marine bacteria, *Arthrobacter* sp. NI-2, and its derivative mutant *Arthrobacter* sp. NI-2' with an enhanced Mn(II)-oxidizing activity. In contrast to the previously identified *Bacillus* sp. NI-1, both NI-2 and NI-2' strains did not show Mn(II)-oxidizing activity under NaCl-stress conditions. However, under cold-stress condition (10°C), which inhibited the growth of *Bacillus* sp. NI-1 strain, the *Arthrobacter* sp. NI-2' strain can grow and actively oxidize Mn(II). Therefore, we conclude that, while *Bacillus* sp. NI-1 is a good strain for Mn biomineralization under high salinity environments, the *Arthrobacter* sp. NI-2' strain can be used for Mn bioremediation in low temperature environments, such as drainage water of Mn mines in winter or at cold climate regions.

To clarify molecular mechanisms underlying the activation of Mn(II)-oxidizing activity in *Arthrobacter* sp. NI-2' strain, we have currently established a transposon mutagenesis system for *Arthrobacter* sp. NI-2' strain with a modified method based on the methods previously described (Gartemann and Eichenlaub, 2001; Zhang et al., 2011). The transposon-insertional mutants with suppressed Mn(II)-oxidizing activity obtained from the population will facilitate the identification of key genes in the mechanisms.

Chemisorption and oxidation catalyzing properties of Mn oxide (Figure 2) make Mn biomineralization applicable for removal of multiple heavy metals and a wide range of compounds including organic contaminants from the environments (Remucal & Ginder-Vogel, 2014). The finding of the key genes and factors in the mechanisms underlying the activation of Mn(II)-oxidizing activity will contribute to the development of efficient bioremediation technology for decontamination of wastewater even at low temperature conditions.

5. Acknowledgements

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Kinetic Adsorption of Hazardous Methylene Blue from Aqueous Solution onto Iron-Impregnated Powder Activated Carbon

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Abstract

In this study, iron-impregnated powder activated carbon (Fe-PAC) prepared by using chemical co-precipitation techniques was used as an adsorbent for methylene blue (MB) removal in batch experiment. The analysis of transmission electron microscopy, scanning electron microscopy with energy dispersive spectroscopy showed that iron oxide particle was substantially distributed into the surface of adsorbent, suggesting that Fe-PAC was successfully synthesized. The results found that fast and efficient adsorption of MB by Fe-PAC was achieved, with relative short contact time of 10 min and MB adsorption capacity of 51 mg/g. The kinetic adsorption of MB on Fe-APC adsorbent was well described by pseudo-second-order model. Meanwhile, the analysis of intraparticle diffusion model suggests that intraparticle diffusion is not the only rate-limiting step of MB molecules adsorption by Fe-PAC adsorbent. The elevated temperature conditions also improved the removal efficiency of MB. Thermodynamic parameters exhibited the MB adsorption process onto Fe-PAC was endothermic and spontaneous. The findings of the present work indicate that Fe-PAC can be potentially effective adsorbent for MB removal in wastewater due to its fast and efficient MB adsorption, and separation in wastewater treatment system.

Keywords: Adsorption/ Dye removal/ Iron-impregnated powder activated carbon/ Methylene blue

1. Introduction

Nowadays, there are raising concerns of environmental problems and public health caused by hazardous pollutant of dyes due to their toxic, mutagenic, allergenic and carcinogenic properties. Many industries, such as textile, printing, and plastic, usually employed dyes and pigments in their production processes. Consequently, colored wastewaters have been potentially found in treatment processes because of unfixed these dyes on the products and washing processes. Dyeing industries discharge dyes between 30,000 and 150,000 tons per year into receiving waters (Anjaneya et al., 2013). Thus, the colored wastewater released into natural water have been gained more attention not only aesthetically displeasing but also detrimental effects to natural water quality.

In the past, there were various technologies proposed in eliminating dyes from wastewater, namely, adsorption (Li et al., 2018; Wong et al., 2016; Wu et al., 2014), membrane filtration (Rashidi et al., 2015), ion exchange (Karcher et al., 2002) and biological treatment (Anjaneya et al., 2013). Among these, adsorption by activated carbon, such as powder activated carbon (PAC), is one of the most effective techniques for water decontamination due to operational simplicity, no intermediate compound and high removal efficiency. However, the separation of this adsorbent is one of challenging of its application due to low setting rates in treatment unit and clogging of membrane filtration (Faulconer et al., 2012; Han et al., 2015; Kim et al., 2013). Therefore, development of novel adsorbent with high adsorption capacity, self-settling for decoloration has been receiving more attention for pollutant removals.

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Recently, the use of iron oxide particles has gained much research attention in the field of pollutant removal due to their high efficiency of pollutant removals and simple separation by introducing a magnetic field (Kitkaew et al., 2018). Hence, combination between PAC and iron oxide particles as iron-impregnated powder activated carbon (Fe-PAC) could be an ideal adsorbent for pollutant removal from water due to self-aggregation and high removal efficiency. In this study, we thus synthesized the Fe-PAC as adsorbent in laboratory scale by using simple chemical co-precipitation technique and further used to remove methylene blue (MB) as model dye compound in aqueous solution. Batch adsorption of MB onto Fe-PAC was carried out as function of adsorption time and equilibrium temperature condition. MB removal mechanism by Fe-PAC and its adsorption capacity were examined in this work. The results from this study can provide useful information in removing MB from wastewater of dye industries.

2. Methodology

2.1 Preparation of Fe-PAC adsorbent

The PAC (received from Sigma-Aldrich, Singapore) was washed with Milli-Q water to remove impurities as received-PAC. The Fe-PAC adsorbent was prepared by chemical co-precipitation method, adapted from our previous method of Kitkaew et al., (2018). Briefly, 18.3 g of $FeSO_4 \cdot 7H_2O$, 33.3 g of $FeCl_3 \cdot 6H_2O$, 25 g of PAC and 1,000 mL of Milli-Q water were combined in a 2,000-mL beaker and mixed by magnetic stirrer. The mixed solution was heated to 65±2°C and then cooled down to below 40°C before adding of 5 N NaOH to increase the pH solution to 10–11. The resulting suspension was stirred constantly for 1 h and then rested overnight to precipitate. Subsequently, the supernatant was discarded and the adsorbent suspension was rinsed thrice with 1,000 mL of Milli-Q water, twice with ethanol. The adsorbent suspension was transferred into 50-mL conical centrifuge tubes and centrifuged at 3,000 rpm for 5 min. The pellet was harvested, dried at 70–75°C in an oven for 48 h and then manually ground and sieved to obtain a 20–50 mesh fraction which was kept in an air-tight container until further use. The surface morphology and elemental compositions of adsorbent were characterized using transmission electron microscopy (TEM) (Hitachi-HT7700) and scanning electron microscopy (SEM) coupled with energy dispersive spectrometer (SEM-EDS) (Hitachi-SU3500, Japan).

2.2 MB adsorption experiments

Analytical grade of MB (chemical formula of $C_{16}H1_8N_3ClS.2H_2O$) was obtained from Ajax Finechem. The stock solution of MB solutions of 1,000 mg/L was made by dissolving an exact amount of MB in Milli-Q water. Meanwhile, the experimental MB concentrations were thereafter diluted to the required concentration using Milli-Q water.

Time-dependent adsorption experiments were conducted in bottle-point-technique. The pre-weighted amount of 100-mg Mag-PAC was added into a 100-mL flask containing MB solution of 50 mL to obtained Fe-PAC dose of 2.0 g/L, with initial MB concentration of 100 mg/L and initial pH of 8.13. Consequently, the mixed solution was agitated at 150 rpm in horizontal shaking incubator at 25°C for 120 min.

Separately from time-dependent adsorption experiment, adsorption of MB on Fe-PAC was performed at various equilibrium temperature conditions (15-65°C) to investigate the effect of temperature on MB adsorption. This experimental condition was carried out at adsorbent dose of 2 g/L, initial pH of 6.1, initial MB concentration of 200 mg/L, agitation speed of 150 rpm and adsorption time of 20 h. The use of MB concentration of 100–200 mg/L and Fe-PAC dose of 2 g/L in our study are within the ranges of dyes adsorption in the previous studies (Phihusut and Chantharat, 2017; Novais et al., 2018). At specific adsorption times, each bottle was opened and an aliquot sample was filtered through a pre-rinsed 0.45 µm membrane nylon filter. The MB concentration in aqueous solution before and after adsorption by Fe-PAC was determined by UV–Visible spectrophotometer (U-2700, Shimadzu) at 668 nm. Each adsorption experiment was carried out in duplicate samples under identical conditions. The average value was reported with standard deviation. The removal rates of MB (%) and its adsorption capacity at *t* time; q_t (mg/g) were examined following the equations of 1, and 2, respectively.

$$\text{MB removal}(\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (1)$$

$$dsor\ tio\ \ ca\ ac\ ty\ at\ tim\ (\ \ ) = \frac{_0 -}{} \times \quad (2)$$

Where C_o and C_e are the initial and equilibrium MB concentration in the solution (mg/L), respectively, V is the solution volume (L) and m is the dosage of Fe-PAC adsorbent (g).

In addition, the results of MB adsorption by Fe-PAC was fitted with pseudo-first-order, pseudo-second-order and intraparticle diffusion models, which are expressed in equations of 3 (Lagergren, 1898), 4 (Ho et al., 2000) and 5 (Weber and Morris, 1963), respectively.

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

$$q_t = k_{diff} \cdot t^{0.5} + C \quad (5)$$

Where q_e and q_t are the adsorption amounts of MB (mg/g) at equilibrium and time t (min), respectively, and k_1 (1/min), k_2 (g/mg.min) and k_{diff} (mg/g.$t^{0.5}$) are the rate constants of the pseudo-first-order, pseudo-second-order adsorption, and intraparticle diffusion respectively.C is a constant (mg/g).

The effect of temperature (288, 298, 308, 318, 328 and 338 K) on thermodynamic parameters for MB adsorption onto Fe-PAC was also investigated in this work. The calculation of Gibbs free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) were determined from the following equations;

$$K_c = \frac{(C_0 - C_t)}{C} \times \frac{V}{W} \quad (6)$$

$$\Delta G^o = -RT \ln K_c \quad (7)$$

$$\ln \quad = \frac{\Delta}{} - \frac{\Delta}{RT} \quad (8)$$

Where K_C is equilibrium constant of MB adsorption onto Fe-PAC. R is the universal gas constant (8.314 J/ mol. K), while T is the absolute temperature (K) of adsorption system.

3. Results and discussion

3.1 Characterization of Fe-PAC adsorbent

The structural analysis of Fe-PAC was studied at high magnification using TEM as shown in Figure 1. The images of Fe-PAC adsorbent, synthesized by chemical co-precipitation method, was clearly seen that iron oxide particles are embedded into the activated carbon powder pores. This was corresponded to the morphological observation using SEM (Figure 2) that iron oxide particles was well dispersed covering PAC matrix. However, the result was consistent with the EDS results as seen in Table 1, showing elemental composition of the adsorbent included 36.2% of carbon, followed by 33.3% of oxygen, 29.4% iron, and 1.1 % sodium. This phenomenon can be hypostasized that reduction of specific of surface area of adsorbent after iron impregnation may be found owing to the pore blockage of iron oxide particles onto PAC pores (Han et al., 2015; Mohan et al., 2011; Shang et al., 2016). In addition, this magnetizing process can also lead to decrease pH value at point of zero charge (pH_{pzc}) of Fe-PAC. Normally, pH_{pzc} of PAC adsorbent was higher than 8.7 (Han et al., 2015; Punyapalakul and Takizawa, 2006; Suriyanon et al., 2015), whereas pH_{pzc} of iron oxide adsorbent was ranged from 5.0 to 7.4 (Lohwacharin et al., 2014; Rajput et al., 2016). Thus, it can be implied that pH_{pzc} of Fe-PAC could be reduced as compared with PAC adsorbent. This is because surface of PAC was oxidized by adding iron precursors during process of impregnation (Borah et al., 2009).

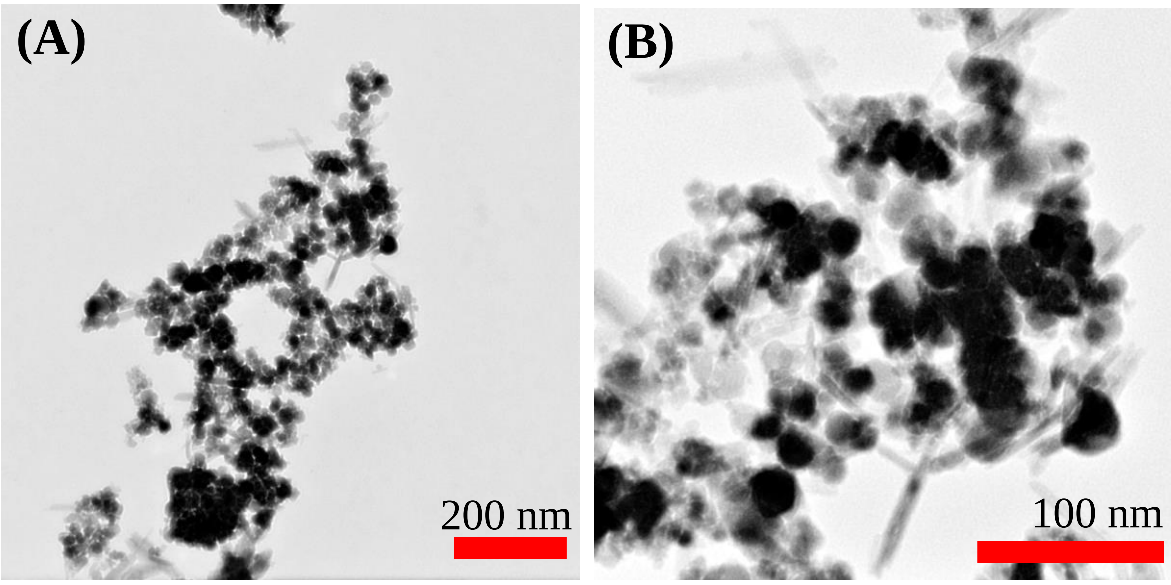

Figure 1. TEM images of Fe-PAC at (A) ×20,000 and (B) × 70,000 magnification

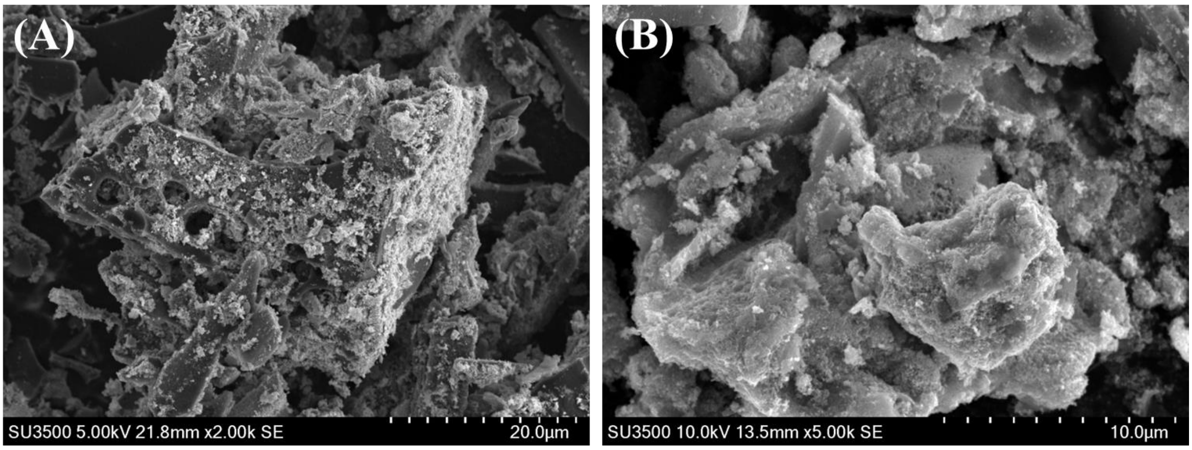

Figure 2. SEM images of Fe-PAC at (A) ×2,000 and (B) ×5,000 magnification.

Table 1. Elemental composition of the Fe-PAC adsorbent.

Element	Line	Weight (%)	Atomic (%)
Carbon	K Serie	36.2	53.2
Oxygen	K Serie	33.3	36.7
Iron	L Serie	29.4	9.3
Sodium	K Serie	1.1	0.8

3.2 Effect of adsorption time

Equilibrium adsorption time is an important factor in designing wastewater treatment unit. The plot of MB removal and its adsorption capacity as function of adsorption time is shown in Figure 3. It is noteworthy that removal rate of MB and its adsorption capacity have been affected by adsorbent dose. In this study, the adsorbent dose of 2 g/L was selected to remove MB in aqueous solution. This applied dosage was similar to adsorption dose used in other studies (Naeem et al., 2017; Novais et al., 2018). It can be observed that MB removal and its adsorption capacity was improved with elevated adsorption time. A fast uptake of the adsorbed amount of MB had been found at 89% removal rate within a few minutes of adsorption time. This is because the adsorption sites are more available during initial state of adsorption (Fu et al., 2015).

Subsequently, the MB removal rate was further increased to 99% at 10 min and then reached a plateau, indicating reducing of accessible vacant adsorption sites. The trend of MB adsorption capacity was similar to removal rate of MB, being reached equilibrium adsorption state at 10 min of contact time, with MB adsorption capacity of 51 mg/g. This suggests that equilibrium achieved with Fe-PAC for MB adsorption is of 10 min, which was substantially faster than those reported in literatures (Naeem et al., 2017; Phihusut and Chantharat, 2017; Wong et al., 2016). This obtained results of rapid adsorption time under these experimental conditions indicate that Fe-PAC have high removal efficiency with MB molecules in aqueous solution.

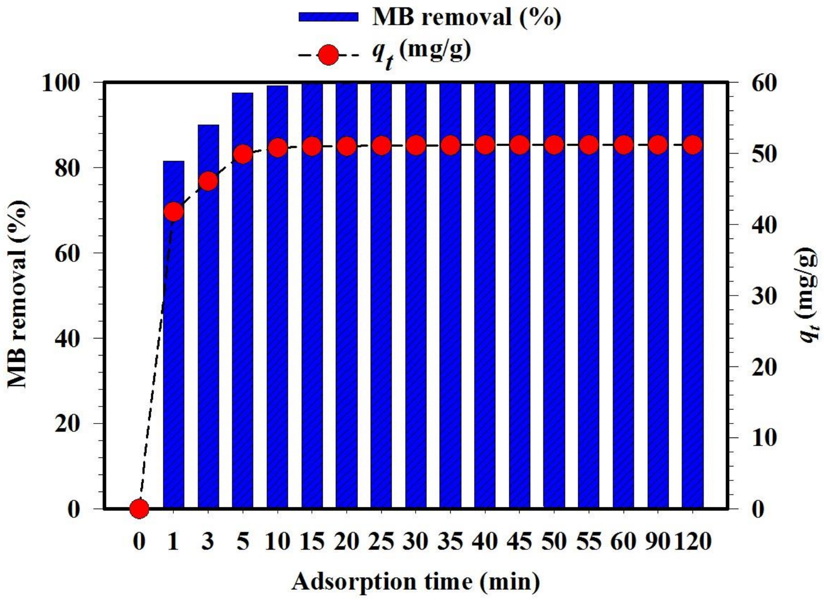

Figure 3. Influence of adsorption time on the removal efficiency of MB by Fe-PAC.

3.3 Kinetic adsorption of MB

To further investigate kinetic rate constant of MB adsorption onto Fe-PAC and its removal mechanism, the adsorption data were applied to kinetic adsorption models; namely, pseudo-first order model, pseudo-second order model and intraparticle diffusion mode. The results of kinetic parameters of MB adsorption onto Fe-PAC adsorbent are presented in Table 2. It can be observed from Figs.4A-4B that the correlation coefficient (R^2) of pseudo-second order model (R^2 closed to 1.000) has higher than that of pseudo-first order model (R^2 = 0.908). Additionally, the theoretical value of adsorption capacity for pseudo-second order model (q_e= 51.282 mg/g) was similar to that of the experimental equilibrium adsorption capacity (q_e= 51.183 mg/g) (Table 2). It indicates that the adsorption of MB onto Fe-PAC was fitted well with pseudo-second-order-model, confirming that chemisorption, involving electrostatic interaction between MB molecules and Fe-PAC, is rate-limiting step (Abuzerr et al., 2018; Phihusut and Chantharat, 2017).

In this study, the intraparticle diffusion model was also employed to further describe the contribution of sorption mechanism of MB molecules onto Fe-PAC and results were summarized in Figure 4(C) and Table 2. As found in Figure 4(C), the plot between q_t and adsorption time ($t^{\wedge 0.5}$) displayed two straight lines, which did not pass through the origin, suggesting that intraparticle diffusion was not the only rate-controlling step. Multiple steps of diffusion mechanisms are included in MB adsorption onto Fe-PAC. It is consistent with findings by others (Li et al., 2018; Pathania et al., 2017). Considering the Figure 4(C), it found that the slope of the first straight line (k_{diff-1} = 6.555 mg/g.min$^{0.5}$) was considerably higher than that of the slope of the second straight line (k_{diff-1} = 0.041 mg/g.min$^{0.5}$). This suggests that sorption rate of external mass transfer was relatively faster than the rate of intraparticle diffusion.

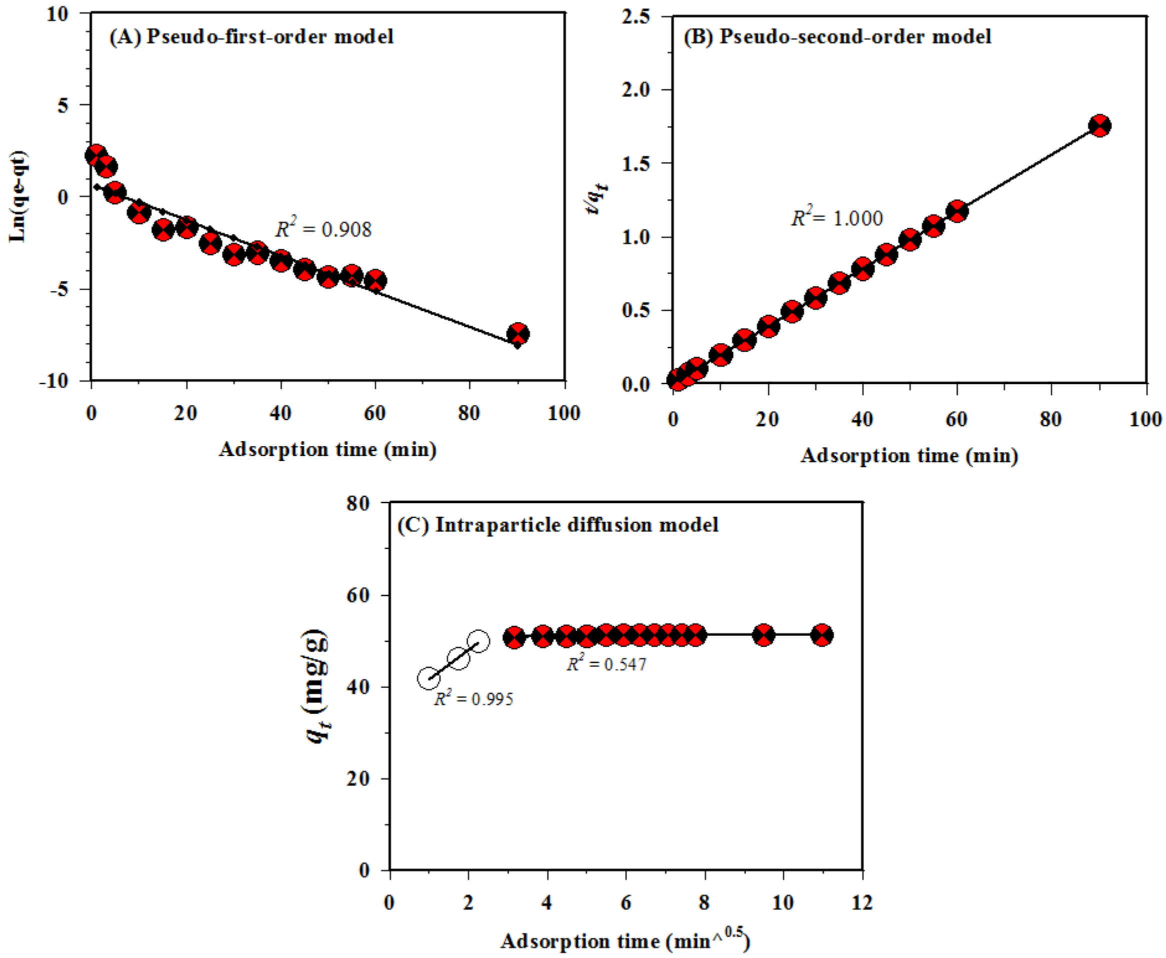

Figure 4. Kinetic adsorption models of MB by Fe-PAC (A) pseudo-first-order-model, (B) pseudo-second-order-model, and (C) intraparticle diffusion model.

Table 2. The parameters of kinetic adsorption of MB on Fe-PAC.

Kinetic adsorption models	Parameters	Values
Experimental results	q_{exp} (mg/g)	51.183
Pseudo-first-order model	q_{cal} (mg/g)	1.938
	k_1 (L/min)	0.097
	R^2	0.908
Pseudo-second-order model	q_{cal} (mg/g)	51.282
	k_2 (g/mg.min)	0.127
	R^2	1.000
Intraparticle diffusion model	$K_{diff\text{-}1}$ (mg/g.min$^{\wedge 0.5}$)	6.555
	R^2	0.995
	$K_{diff\text{-}2}$ (g/mg.min$^{\wedge 0.5}$)	0.041
	R^2	0.547

3.4 Effect of temperature on MB adsorption

Figure 5 illustrates MB removal and its adsorption capacity onto Fe-PAC at various temperature conditions. The trend of MB adsorption was improved with increase equilibrium temperature conditions. The average MB adsorption capacity was 101±3 mg/g under these experimental conditions. Meanwhile, the removal efficiency of MB was 99.6% at 15°C and slightly improved to 99.9% in the range of equilibrium temperature condition between 25°C and 65°C. This can be explained by reason that elevated equilibrium temperature conditions may result in swelling effect of Fe-PAC porosity, promoting a quick movement of MB molecules into internal pores of adsorbent (Fu et al., 2015; Wu et al., 2014).

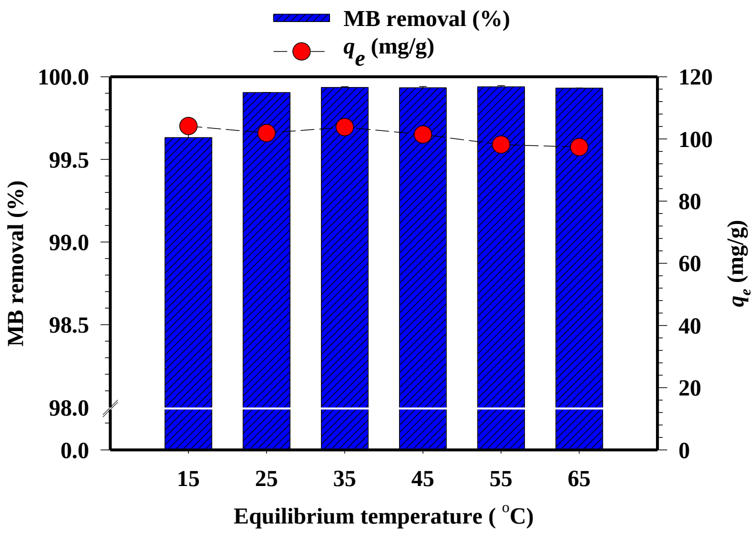

Figure 5. Influence of equilibrium temperature condition on the adsorbed and removal efficiency of MB by Fe-PAC (Fe-PAC dosage of 2 g/L, initial MB concentration of 100 mg/L at initial pH of 6.16 and adsorption time of 20 h).

3.5 Adsorption thermodynamics

To further investigate the effect of temperature on the MB adsorption of onto Fe-PAC, the thermodynamic analysis was carried out and its parameters were presented in Table 3. The results found that there were negative values of ΔG^0 with these tested conditions, indicating that adsorption process of MB on Fe-PAC was spontaneous and thermodynamically feasible in nature. With the increase equilibrium temperature conditions, more negative values of ΔG^0 was further increased from 288 K to 338 K, suggesting that more efficient adsorption of MB at higher temperature (Lalhmunsiama et al., 2016). This was consistent with the positive values of enthalpy change, (ΔH^0) which was found to be 23.28 kJ/mol, indicating adsorption process was endothermic in nature and higher temperature conditions also improve MB adsorption onto Fe-PAC. Meanwhile, the calculated of ΔS^0 value was positive, confirming that high affinity of Fe-PAC for MB molecules and increasing randomness at the solid-liquid interface during the sorption process (Nekouei et al., 2015).

Table 3. Thermodynamic parameters of MB adsorption onto Fe-PAC

Temperature (K)	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (kJ/mol)
288	-11.75	23.28	0.13
298	-15.51		
308	-17.03		
318	-17.51		
328	-18.29		
338	-18.50		

4. Conclusions

In the present work, the iron oxide particle was successfully impregnated onto PAC. The prepared adsorbent as Fe-PAC was used for MB adsorption in aqueous solution. Based on experimental results obtained from this study, MB molecules could be rapidly and efficiently removed by Fe-PAC adsorbent in the short contact time of 10 min with MB adsorption capacity of 51 mg/g. The adsorption process of MB with FA-PAC was well fitted by pseudo-second-order-model, suggesting that chemisorption between MB molecules and adsorbent was estimated to be rate-limiting step. Thermodynamic parameters indicated that MB adsorption onto Fe-PAC was feasible, endothermic and spontaneous in nature. Therefore, application of Fe-PAC adsorbent for MB adsorption was attractive and yield benefits for treating dye-containing wastewater because of favorable performance of Fe-PAC with MB removal and its easy separation from aqueous solution. Furthermore, the role of iron oxide particles on PAC should be investigated in the future study to differentiate MB removal rate and its adsorption capacity.

5. Acknowledgements

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Influence of PM_{10} from the Outside Area Affecting on the Northern Part of Thailand

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Abstract

The upper northern part of Thailand (UNT) has been experiencing haze for almost a decade. The concentration of particulate matter less than 10 microns (PM_{10}) often exceeds the air quality standard of Thailand during the haze episode. The PM_{10} level over the UNT region would be affected by the emission sources locating inside and outside the region via atmospheric processes. To determine the influence of PM_{10} from the outside UNT area sources, simulations of PM_{10} levels during the haze episode by using the Weather Research and Forecasting model coupled with Chemistry (WRF-Chem) were applied. The first simulation used the PM_{10} emission covering the UNT area and neighborhood region, whereas the second simulation used only the emission from UNT area. The difference between both simulated results reveals the influence of PM_{10} from the outside area. Result shows that the PM_{10} level over the UNT region is not only affected by the local emission sources. The outside UNT emission is important. Its contributing percentage to the PM_{10} level over UNT area was presented in this study. Policy makers would consider on discussing this issue among the steering committee on transboundary haze pollution of ASEAN to find the collaborative approach in reducing overall regional PM_{10} emissions.

Keywords: PM_{10}/ Haze/ Northern Thailand/ WRF-Chem

1. Introduction

In the upper northern part of Thailand (UNT), haze has emerged as major air quality problem. The major source of haze events is biomass burning that emits pollutants such as carbon dioxide (CO_2) and particulates less than 10 microns (PM_{10}) impacting climate change and air quality (van der Werf et al., 2009; Lee et al., 2018). Thailand has implemented the National Haze Action Plans in 1997 for haze management (Tiyapairat, 2012). The government aims to reduce the severity of problem by controlling the emission sources. They has been launched the policy, which not allow people to perform open burning in a period of 60 days during haze episode. For regional scale, the ASEAN have launched the Agreement on Transboundary Haze Pollution (AATHP) in June 2002, which reveals that the problem is too large for one agency to handle alone and requires more understanding of technical inputs to determine the appropriated action plans (Tiyapairat, 2012), which could be used approaches in the same way to solve climate change issue (Ali et al., 2017, Ali et al., 2018).

High PM_{10} concentration enhances severe public health risks, and causes respiratory illnesses. Its deposition on vegetation reduces growth, yield, flowering, and reproduction (Wiwanitkit, 2008; Prajapati, 2012). Severe haze episodes occur most frequently from March to early April. The magnitude during the period is quite often greater than the national ambient air quality standard of the PM_{10}. (Sooktawee et al., 2015). Two factors affect the concentration of PM_{10} in ambient air. The first is emission of the pollutant. One of the important sources related to haze severity is open burning. There is a study showing the good relationship of increasing in PM_{10} level to open burning activities represented by the numbers of fire hotspots, significantly (Sirimongkonlertkun, 2014).

Another factor is meteorological condition that imply to the pollutant transportation.

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In the UNT region, high PM_{10} concentrations were often coincident with present of prevailing westerly and southwesterly winds more than the easterly wind (Sooktawee et al., 2016). The pollutant emission from the open burning activity of neighboring countries possibly results in the increasing in PM_{10} levels at the border area of Thailand (Sirimongkonlertkul et al., 2013). These mean that the change of PM_{10} level in the UNT region possible influenced by the emission sources in the country and neighboring countries.

Many of their studies provide the valuable information such as emission information and trajectory of air mass (Wiwanitkit, 2008; Prajapati, 2012; Sirimongkonlertkun, 2014; Sooktawee et al., 2015; Sooktawee et al., 2016). It is more completed than that to know how much the PM_{10} level in the UNT region is affected from the sources locating inside and outside the area, and the modeling work is required. The Weather Research Forecasting model coupled with Chemistry (WRF-Chem) is a Weather Research Forecasting model (WRF) model coupled with a numerical module of chemical interactions in the atmosphere (Grell et al., 2005; Grell et al., 2011). The model can use to simulate and forecast the PM_{10} level during hazy period (Wang et al., 2014; Nuryanto, 2015). Therefore, our purpose is to present the information for the policy makers to pay attention on controlling the emission in the UNT region and strengthen to reduce the impact from the long-range transportation in the appropriated decision.

2. Data and methodology

2.1 Data

The NCEP FNL (Final) Operational Global Analysis data (National Centers for Environmental Prediction, 2000), the real-time Global Sea Surface Temperature (RTG-SST) (Gemmill et al., 2007), EDGAR-HTAP V2 (Janssens-Maenhout et al., 2012), and The Fire INventory from NCAR (FINN) datasets (Wiedinmyer et al., 2011) are used to drive the WRF-Chem model. The initial and lateral boundary conditions were six hourly data of the FNL data with 0.5 x 0.5 degree horizontal resolution. The HTAP is anthropogenic emission data of various sectors such as transportation and industrial sectors. The open burning emission data estimated from fire hotspots is the Fire Inventory from NCAR (FINN) emissions dataset. FINN data set. The GOCART data is also used as boundary condition.

For the PM_{10} observed data, we used data presented at the ari4thai website. They were published by the Pollution Control Department of Thailand (PCD). Their information of air quality monitoring stations and locations shows in Table 1 and Figure 1, respectively. The numeric station identities have been assigned by the PCD, whereas station identity M is the mobile air quality monitoring unit of the Environmental Research and Training Center (ERTC).

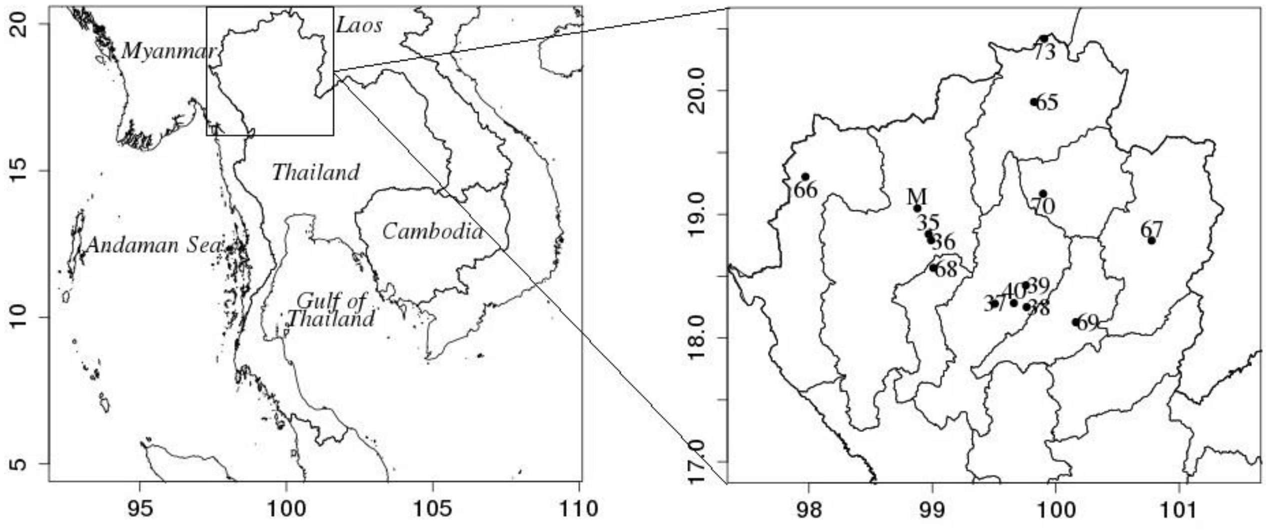


Figure 1. Locations of air quality monitoring stations over the UNT region.

Table 1. Monitoring stations information.

Province	Station ID	Location (Lat/Lon)
Chiang Mai	M	19.049607/98.879071
	35	18.840711/98.969700
	36	18.790906/98.990064
Lampang	37	18.278279/99.506440
	38	18.250781/99.763821
	39	18.426807/99.757436
	40	18.282584/99.659806
Chiang Rai	65	19.909251/99.823347
	73	20.427279/99.905942
Mae Hong Son	66	19.304583/97.971686
Nan	67	18.788963/100.776359
Lamphun	68	18.567387/99.008008
Phrae	69	18.128431/100.162417
Phayao	70	19.166673/99.896887

2.2 Study domain and simulation description

The simulation period is on 1-31 March 2016. Two domains of study were designed for the WRF-Chem modeling. The mother domain (D1) has 100×100 grid cells with 36×36 km^2 resolution covering Thailand and neighboring countries. It aims to capture the emission sources and air-mass circulation affecting to the atmospheric processes over UNT region. The nested domain (D2) has 52×70 grid cells with 12×12 km^2 resolution, and was designed to focus on the UNT region (Figure 2). Both domains has 31 vertical levels from the first level (approximated height is 4 m above ground) to the top level at 5,000 Pa. The eta_levels using to classify the vertical levels are 1.000, 0.999, 0.997, 0.995, 0.992, 0.987, 0.980, 0.970, 0.950, 0.910, 0.860, 0.800, 0.750, 0.700, 0.650, 0.600, 0.550, 0.500, 0.450, 0.400, 0.350, 0.300, 0.250, 0.200, 0.150, 0.100, 0.075, 0.050, 0.025, 0.010, 0.000. The numerical calculation was done with time step 30 second.

Among a large number of physics parameterization schemes and chemistry options (Grell et al., 2005; Grell et al., 2011; Skamarock et al., 2008; Wang et al., 2014), the combination of selected physics parameterization schemes used in this study shown in Table 2, and the most of them were used for WRF modeling over Thailand (Kaewmesri et al., 2017[a,b]). Whereas, Grell-Freitas scheme was selected for cumulus scheme because it is an improved GD scheme. The original GD scheme reveals better performance of rainfall spatial distribution over the northern Thailand (Pimonsree et al., 2016). The Chemistry schemes configuration also show in Table 3.

Meteorological, terrestrial data, and other data mentioned before were used for two simulation cases. But, the emission data sets were prepared for each case. The first emission data set is prepared by using the FINN emission for the entire domains representing the regional emission, whereas the second is prepared for the UNT region.

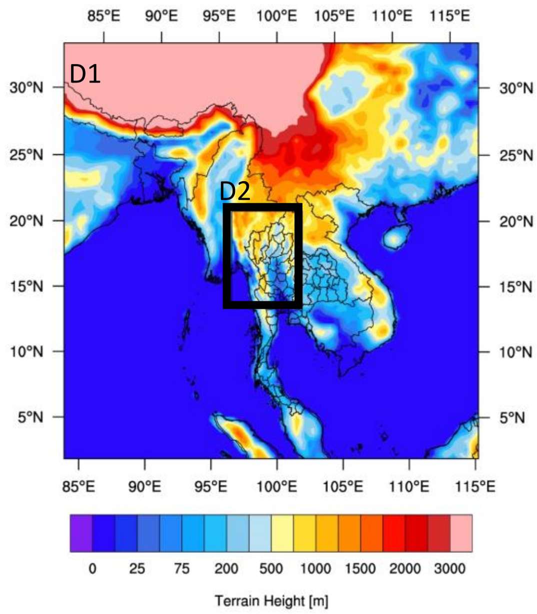


Figure 2. Map of the first domain (Domain 1; D1) and the nesting domain (Domain 2; D2) for simulation. Shading presents terrain elevation.

Table 2. Selected physics parameterization schemes for model configuration.

Physics Parameterization	Selected Scheme	Physics Parameterization	Selected Scheme
Microphysics	Kessler scheme	Cumulus Parameterization	Grell-Freitas scheme
Longwave Radiation	RRTM	Surface Layer	Monin-Obukhov
Shortwave Radiation	Dudhia scheme	Land Surface	Noah Land Surface Model
Planetary Boundary Layer	Yonsei University scheme		

Table 3. Selected chemistry options for model configuration.

Chemistry Option	Selected Option
Chemical Mechanism	MOZART Chemistry and GOCART aerosols using KPP library
Biogenic Emission	Guenther biogenic emission
Anthropogenic Emission	MOZART emission
Biomass Burning Emission	Biomass burning emission and plume rise calculation for MOCART (MOZART and GOCART)

2.3 Statistical measures

Mathematical models such as an atmospheric model and an air quality model are the powerful tools to predict or present the spatial situation of environment. However, the value derived from the model is the result based on the available theories or equations. It may not be possible to represent the natural phenomena completely, and cannot be validated or verify model performance to natural processes. To see the performance of model, the evaluation by using statistical indices has been used for various objectives such as performance in simulating an average or maximum values (Chang and Hanna, 2004). The statistical indices used to reveal modeling performance of this study shown in Table 4.

To see how the simulation can be accepted whether or not, we used criteria suggested by Chang and Hanna (2004). The statistical index |FB| should not be greater than 0.3, the NMSE value is less than 1.5, and the FAC2 is in the range of 0.5-2.0.

Table 4. Statistical indices used to evaluate the simulation performance.

Index	Equation	Reference
Mean Bias Error	$MBE = N^{-1}\sum_{i=1}^{N}(P_i - O_i)$	Willmott et al. (1982); Warner et al. (2004); Zhang et al. (2014)
Mean Absolute Error Or Gross Error	$MAE = N^{-1}\sum_{i=1}^{N}\lvert P_i - O_i\rvert$	Willmott et al. (1982); Zhang et al. (2014)
Mean Normalized Bias	$MNB = \frac{1}{N}\sum_{i=1}^{N}\frac{P_i - O_i}{O_i}$	Zhang et al. (2014)
Normalized Gross Error	$NGE = \frac{1}{N}\sum_{i=1}^{N}\frac{\lvert P_i - O_i\rvert}{O_i}$	Zhang et al. (2014)
Fractional Bias	$FB = \frac{(\overline{O} - \overline{P})}{0.5(\overline{O} + \overline{P})}$	EPA (1992); Warner et al. (2004); Chang and Hanna (2004)

Table 4. Statistical indices used to evaluate the simulation performance. (cont.)

Index	Equation	Reference
Normalized Mean Square Error	$NMSE = \frac{\overline{(O-P)^2}}{\bar{O}\times\bar{P}}$	Warner et al. (2004); Chang and Hanna (2004)
Correlation Coefficient	$R = \frac{\overline{(O-\bar{O})(P-\bar{P})}}{\sigma_P\sigma_O}$	Warner et al. (2004); Chang and Hanna (2004)
Factor of Two	$0.5 \le \frac{P}{O} \le 2.0$	Warner et al. (2004); Chang and Hanna (2004)
Root Mean Square Error	$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(P_i-O_i)^2}$	Warner et al. (2004); Zhang et al. (2014)

2.4 Influence of Emission Source

This part presents methodology to find the influence of PM_{10} from the outside UNT region to the concentration level over the UNT area. The concept presents as equations as follows:

$$PM_{10(totalemiss)} = PM_{10(emiss\_outsideUNT)} + PM_{10(emiss\_insideUNT)}$$

$$\frac{PM_{10(emiss\_outsideUNT)}}{PM_{10(totalemiss)}}\times 100 = \frac{PM_{10(totalemiss)} - PM_{10(emiss\_insideUNT)}}{PM_{10(totalemiss)}}\times 100$$

$$Inf\_PM_{10(outsideUNT)} = \frac{PM_{10(totalemiss)} - PM_{10(emiss\_insideUNT)}}{PM_{10(totalemiss)}}\times 100$$

As equations above, we simulated the PM_{10} concentration using emissions over the entire study domain (UNT + neighboring area), and then we simulated the PM_{10} concentration by using only the emission in the UNT region. Therefore the difference between two simulations will show the percentage of PM_{10} level over the UNT region influenced by the emission located outside the region.

3. Results and discussion

3.1 PM_{10} Simulation using emissions over the entire domain

To compare the simulation result with the observed data, we simulate PM_{10} concentration during March 2016 by using open-burning emission and anthropogenic emission as the emission input for the WRF-Chem model. The total amount of emissions throughout March, 2016 show in Figures 3(a) and 3(b). The simulation area of the D1 domain covering Thailand and its neighbors, and the D2 domain focuses to present the UNT region.

Figures 3(c) and 3(d) show results of simulation that are the accumulated PM_{10} concentrations throughout March 2016 for domain1 and doman2, respectively. They show that high accumulated concentrations of PM_{10} (red color) present over neighboring areas, the west side of Mae Hong Son province, over northern areas of Chiang Mai and Chiang Rai provinces, and some areas over Nan and Phrae provinces with lower concentrations over the surrounding areas. It shows that the pollution presenting in the atmosphere can be diffused and transported through meteorological factors such as wind.

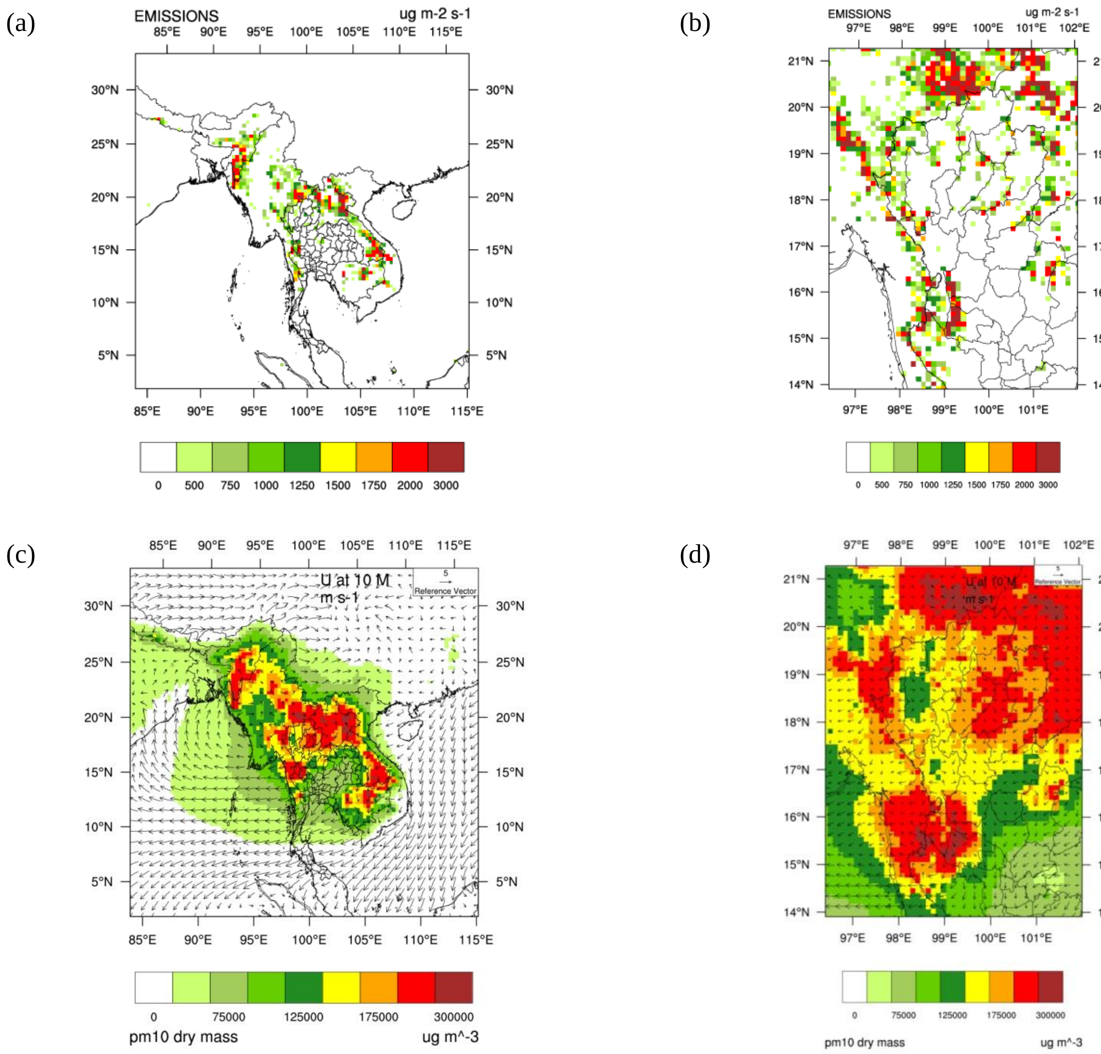


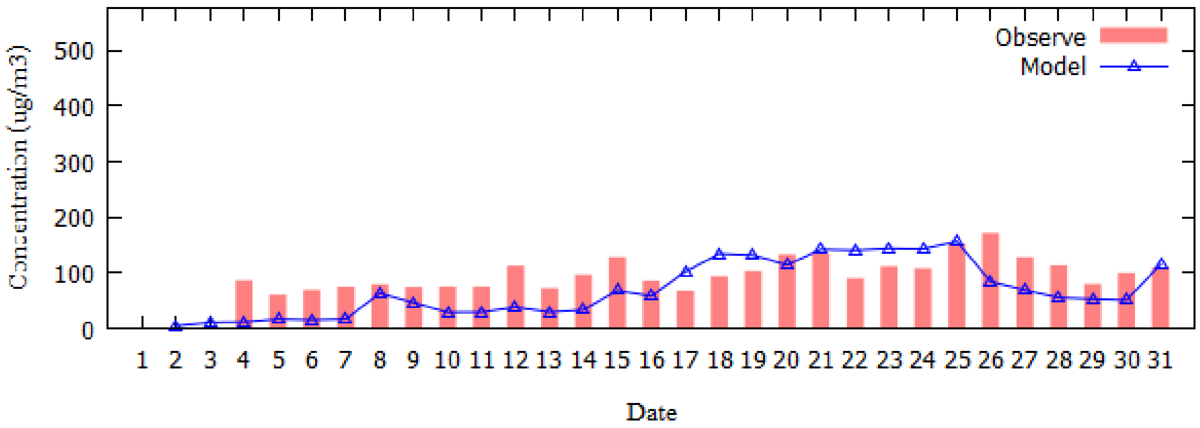
Figure 3. Spatial Patterns of accumulated PM_{10} emissions on March 2016 of (a) domain1 and (b) domain2, whereas (c) and (d) are the accumulated PM_{10} concentrations during March 2016 for domain 1 and domain 2, respectively.

PM_{10} levels are high over the area having high emission from open-burning (Figure 3) and PM_{10} concentrations are spatially distributed across the border of Mae Hong Son, Chiang Rai, Nan, and Tak Provinces. The distribution of PM_{10} resulting from wind blowing, such as wind divergence at 97°E, 20.5°N that push the lower air mass into Mae Hong Son Province. Northerly wind blows down to the northern part of Chiang Rai, and pass through the area of Nan and neighboring areas. It is possible that the influence of the outside UNT emission source will affect the concentration of PM_{10} in the UNT area.

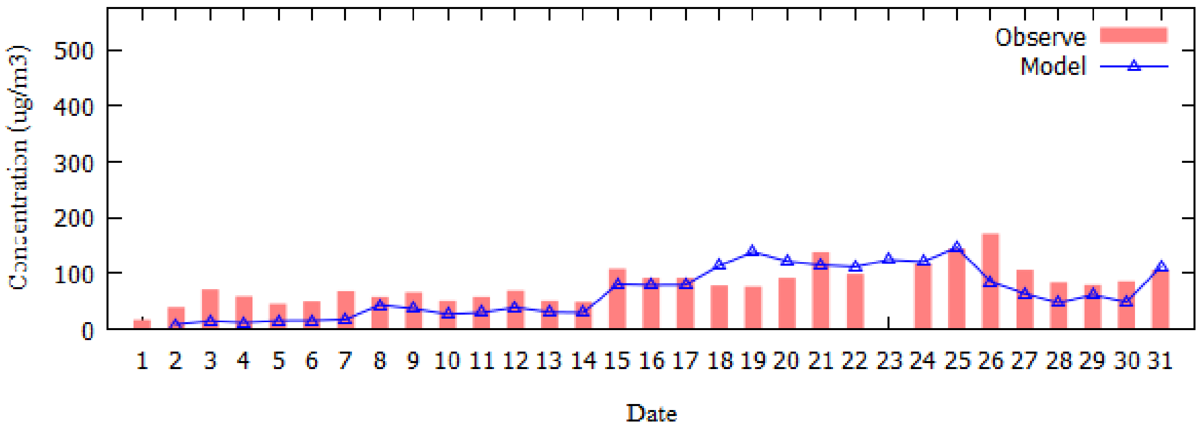
The predicted PM_{10} concentrations by the WRF-Chem model were extracted at each locations of air quality monitoring stations to compare with its observed data (Figure 4). Most of result illustrate that the WRF-Chem can provide simulation results, which tend to be in the same direction as the observed concentrations in the ambient air. The PM_{10} concentrations from monitoring stations began to rise during 19-26 March 2016, while the predicted PM_{10} concentrations increased during 15-26 March 2016. Prior to March 15, the model yielded a lower predicted value than the observed value. This implies to the fact that the emission input for the model such as anthropogenic sources are less than the actual air pollution activities. After the mid of May 2016, concentrations of PM_{10} at many monitoring station increase due to the increasing of open burning emission.

Moreover, the variations of 24-hr average concentrations over Chiang Rai province are consistent between the values given by prediction and the measurement (Figure 4(e)). However, there is a forest fire on March 21, 2016 in Myanmar, which located near suburb of Amphoe Mae Sai, Chiang Rai province, Thailand. The open-burning emission input on that day perhaps over estimation due to that fire, and resulting in the simulation yields very high concentration.

(a) Sta.M Chiang Mai



(b) Sta.35 Chiang Mai



(c) Sta.36 Chiang Mai

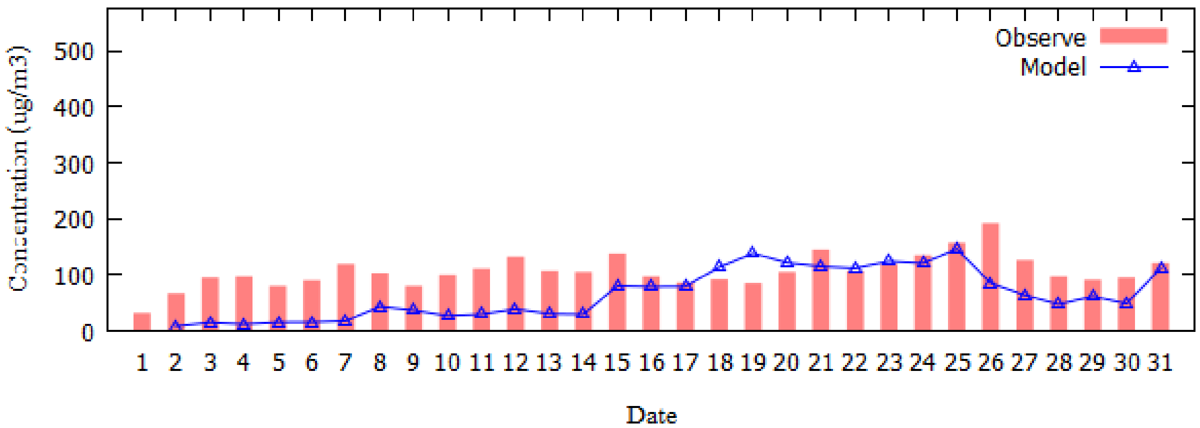
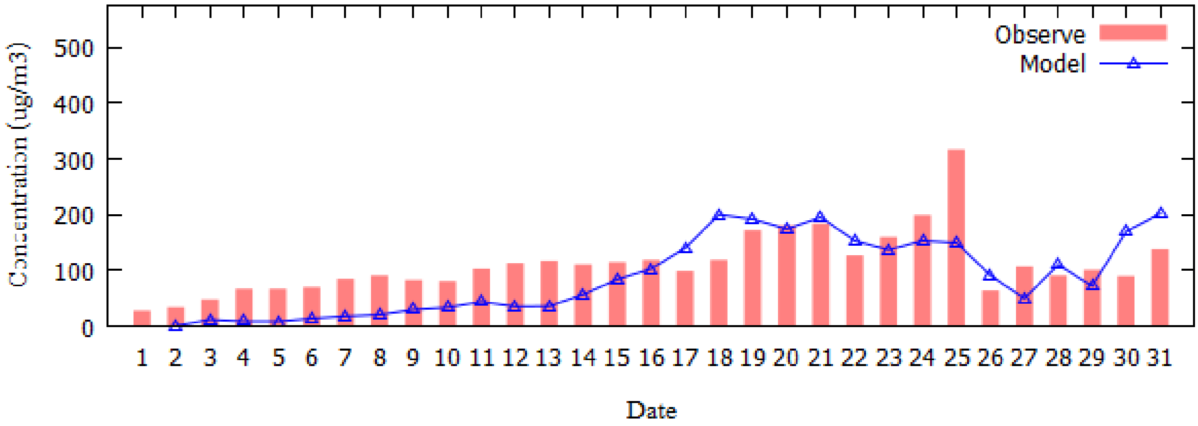
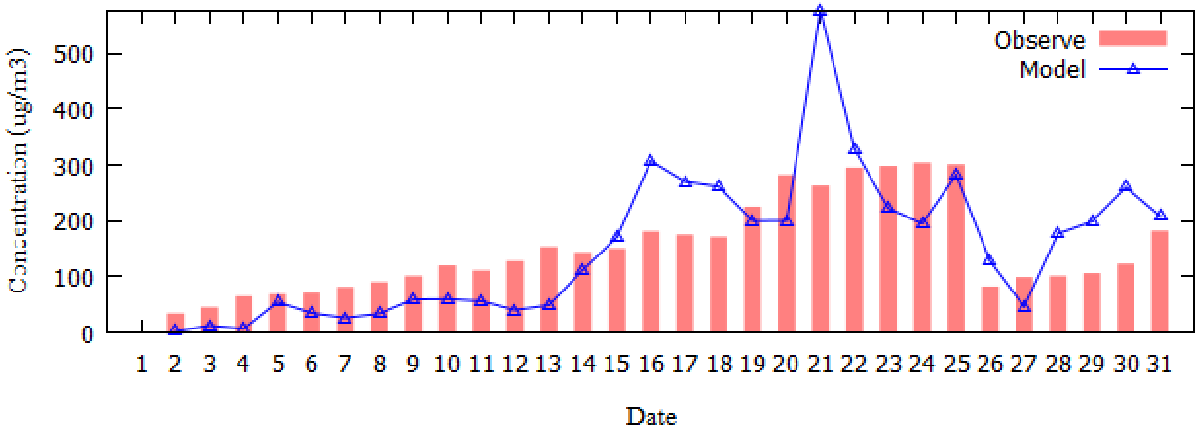


Figure 4. Time series of PM_{10} concentrations ($\mu g/m^3$) derived from measurement and the WRF-Chem modeling.

(d) Sta.65 Chiang Rai



(e) Sta.73 Chiang Rai



(f) Sta.38 Lampang

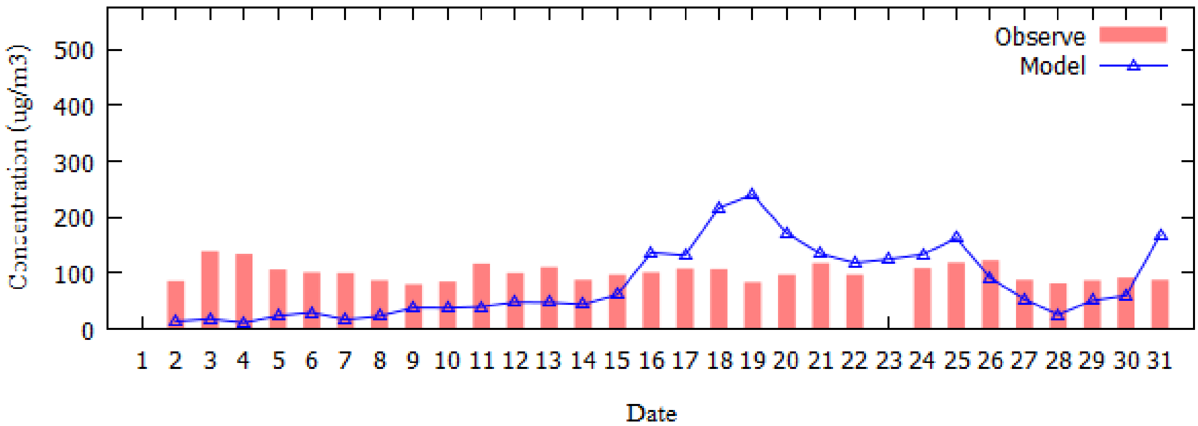


Figure 4. Time series of PM_{10} concentrations ($\mu g/m^3$) derived from measurement and the WRF-Chem modeling (cont.).

Whereas, the differences between the measurement results and the simulation at the air quality monitoring stations, which located in Lampang province at the Tambon Ban Sop Pad Health Promotion Hospital (as shown in Figure 4(f)), the office of Thai Meteorology Department, Lampang, , Mae Mo District, and Ban Tha Si Sub-district (not shown here). The observed values were not much varying throughout the month, but the simulation shown lower concentrations, comparing to the predicted values in the early of March. The reason may be the topography of Lampang province that resemble to a reservoir. It is a possible cause to the air pollutants are accumulated in the area.

We also consider the statistical measures. The measures obtained from calculation using equations with the predicted values and the observed values. The mean value obtained from all air quality monitoring stations was 112.17 μg/m^3 and the value obtained from the WRF-Chem model equals to 82.17 μg/m^3. For bias value, it is -30 μg/m^3 or about -26%. This means that the simulation model is likely to be lower than the detection value of 30 μg/m^3 or about 26%, on average.

To see how this simulation can be accepted wether or not, we used criteria suggested by Chang and Hanna (2004). The statistical index |FB| should not be greater than 0.3, the NMSE value is less than 1.5, and the FAC2 is in the range of 0.5-2.0. The results of this study show that |FB|=0.3, NMSE=0.46, and FAC2=0.733 (Table 5), which satisfy the criteria.

Table 5. Statistical indices

Station	MEAN (Obs)	MEAN (Model)	BIAS	NMSE	CORR	FAC2	FB	RMSE	MAE	MNE	n
All Stations	112.17	82.17	30.00	0.46	0.533	0.733	0.30	65.42	54.85	-0.29	415
M	99.58	74.68	24.90	0.29	0.539	0.750	0.28	46.79	41.85	-0.27	28
35.	82.83	63.50	19.33	0.23	0.728	0.767	0.26	34.83	29.75	-0.28	29
36.	109.13	65.52	43.62	0.49	0.423	0.600	0.49	58.94	50.37	-0.41	30
37.	111.90	81.81	30.09	0.50	0.077	0.731	0.31	67.81	57.43	-0.26	30
38.	101.07	80.94	20.12	0.58	0.000	0.801	0.22	69.17	60.50	-0.19	29
39.	122.13	80.26	41.87	0.52	0.342	0.657	0.41	71.38	61.82	-0.34	30
40.	111.53	79.45	32.08	0.56	0.054	0.712	0.33	70.48	59.90	-0.27	30
65.	115.10	89.87	25.23	0.33	0.646	0.781	0.24	58.51	49.87	-0.27	30
66.	141.69	71.45	70.24	0.63	0.768	0.504	0.65	79.77	70.29	-0.55	29
67.	105.03	80.64	24.39	0.42	0.633	0.768	0.26	59.98	50.27	-0.29	30
68.	78.00	65.57	12.43	0.42	0.219	0.841	0.17	46.17	41.70	-0.14	30
69.	120.13	83.81	36.32	0.61	0.040	0.698	0.35	78.46	69.01	-0.28	30
70.	119.40	78.75	40.65	0.41	0.658	0.660	0.41	61.87	54.56	-0.39	30
73.	151.63	152.56	-0.93	0.34	0.719	1.006	-0.01	89.15	69.53	-0.09	30

3.2 PM_{10} simulation without emissions outside the upper northern part of Thailand

After we know the PM_{10} distribution over the region caused by all emissions in the entire the domain, it is interesting that how is the PM_{10} level caused by only emission within the UNT area. Therefore, we remove emission outside the UNT region from the emission input of previous simulation. The second simulation was performed in the same configuration as previous experiment by using the emission input having only emissions coming from the UNT area. The new emission input shows in Figure 5(a), which differs from the previous emission input (Figure 3(b)). The result of simulation using only the emission of UNT shows in Figure 5(b). The accumulated PM_{10} concentration during March, 2016, the level of PM_{10} accumulation is decreased, comparing to Figure 3(d). This implies that the PM_{10} concentration on the March 2016 during the haze episode in the UNT region was affected by outside UNT sources. Therefore, comparison of the result given by two simulations will reveal the influence of outside UNT emission sources on the PM_{10} level in the UNT region.

3.3 Influence of PM_{10} outside the upper northern part of Thailand

To find the influence of emission source outside the UNT region, we used the principle that PM_{10} concentration given by the simulation using all emission sources would equal to summation of concentrations given by the simulation using emission sources in the UNT region and the simulation using the outside UNT emission sources. Therefore, the influence of outside UNT emission sources on the concentration over the UNT is the difference between both results of (Figure 3(d) and Figure 5(b)). The difference shows in Figure 6 that presents the percentage of difference between both results over the UNT region having the range of 40-50%.

(a) (b)

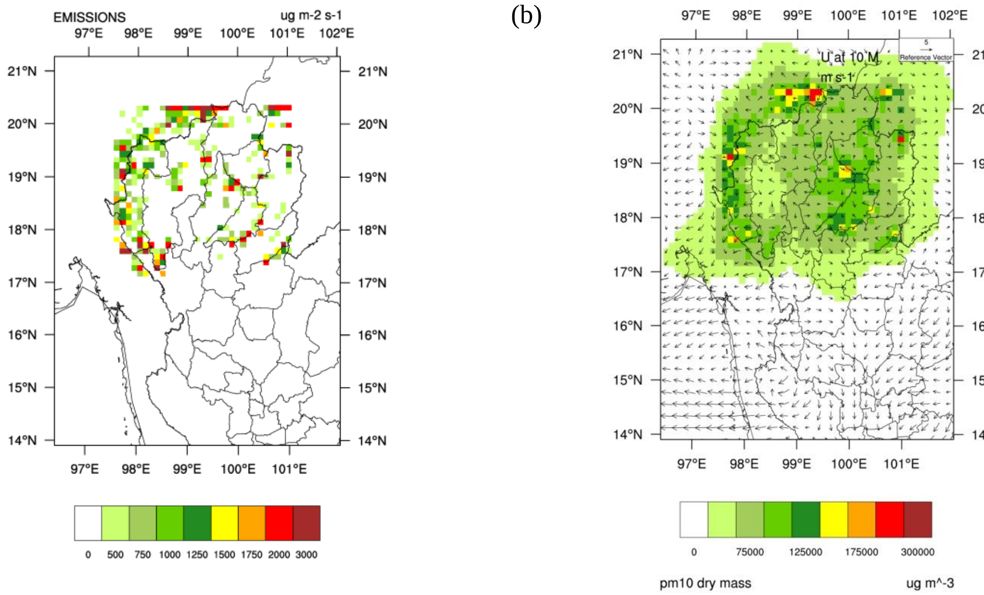


Figure 5. Spatial patterns of (a) accumulated PM_{10} emissions inside the UNT region and (b) accumulated PM_{10} concentrations during March 2016 for domain 2 given by WRF-Chem simulation.

This result implies that the PM_{10} concentration during March 2016 in the UNT region was affected by the outside emission sources around 40%-50% through physical processes and chemical processes in the atmosphere. It is said that transboundary processes play an important role on enhancing the PM_{10} levels over the UNT region. Without the cooperation with neighborhoods, methodology to mitigate the haze problem in the UNT region by local management cannot completely solve the problem.

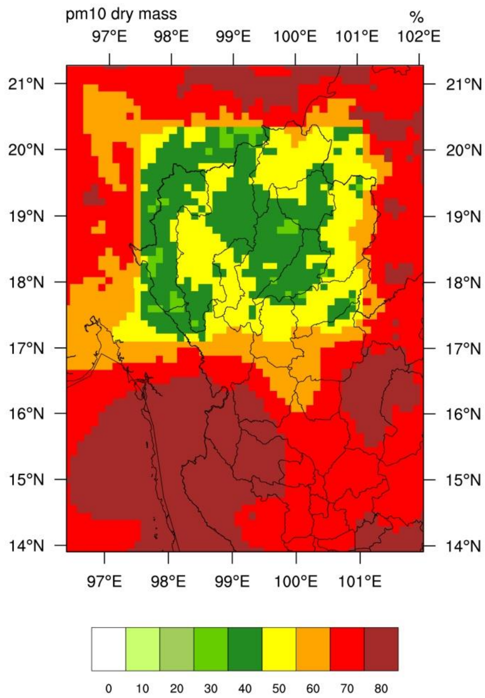


Figure 6. The percentage of difference in PM_{10} concentrations between accumulated concentrations given by simulations using with and without the outside UNT PM_{10} emission throughout March, 2016.

4. Conclusions

This study using the weather model coupled with chemistry module (WRF-Chem) for study on the haze problem in the UNT region. To demonstrate the model performance on predicting concentrations of PM_{10} during the haze period, we selected the period of March 2016 for simulating PM_{10} concentrations. Major input data of the model consist of the open burning emission data from the FINN data set, anthropogenic emission from the EDGAR-HTAP version2 data, meteorological data from the FNL data set and sea surface temperature data from RTG-SST.

The hourly simulation results were calculated to the 24 hours average PM_{10} concentration in order to comply with the national air quality standard of Thailand. Comparison result between observed data and predicted data shows that the simulation performance are likely to be lower than the observed values, with a bias of -30 $\mu g/m^3$ or about 26%. For statistical measures, |FB|=0.3. NMSE=0.46 and FAC2=0.733 are meet the acceptable criteria suggested by Chang and Hanna (2004). However, Thailand would develop the national emission inventory database such as anthropogenic emissions to represent current activities of their resident.

In order to know how much the high concentrations of PM_{10} during haze period (March 2016) affected by outside or inside UNT emission sources, the comparison of two simulations was performed. The first is the emission data was prepared for the entire simulation domain covering the UNT region and neighboring countries, the second emission data was prepared for only the UNT region. Both results given by the simulations were compared. The difference between both results implies to the influence of the outside UNT emission sources. The different values shows that PM_{10} concentrations over the UNT region influenced by the outside emission sources of 40%-50% through the physical and chemical processes. It is said that transboundary processes play an important role on enhancing the PM_{10} levels over the UNT region.

This study demonstrates that the high PM_{10} level over the UNT region is the broad-area pollution problem that does not affected by only the local sources. Without the cooperation with neighborhoods, methodology to mitigate the haze problem in the UNT region by local management cannot completely solve the problem. Therefore, cooperation of the Asian Community is an important mechanism to find the policies and conventions to solve this problem.

5. Acknowledgements

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Enhanced Photocatalytic Performance of Al Doped Zinc Oxide for Dye Degradation

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Abstract

This research aims to develop a visible-light responsive zinc oxide (ZnO) photocatalyst for Methyl orange (MO) dye degradation under photocatalytic system. Aluminium doped zinc oxide (Al/ZnO) photocatalysts were synthesized via sol-gel method with different Al contents and calcined at 400°C. The photocatalytic activity of the Al/ZnO nanoparticle photocatalysts was evaluated by the decolorization of Methyl orange at an initial concentration of 50 ppm. The results indicated that degradation efficiency changed according to the Al contents. Among Al/ZnO photocatalysts, the 5% Al/ZnO photocatalyst exhibited the best photocatalytic performance of 85% MO degradation under visible light illumination after 40 min. The Al/ZnO photocatalyst provided higher photocatalytic activity than that of pure ZnO photocatalyst, approximately 15 times. Notably, Al doped in ZnO played an outstanding role in extending the light absorption spectrum of Al/ZnO toward the visible region as well as inhibiting electron-holes recombination, which improving the photocatalytic activity. The Al/ZnO is a good candidate for further apply in wastewater treatment process. Thus, this study developed Al/ZnO nanoparticles which exhibited efficient photocatalyst which prepared from simple, economical and environment-friendly approach.

Keywords: Photocatalysis/ Dye degradation/ Zinc oxide nanoparticles/ Aluminium/ Sol-gel method/ Methyl orange

1. Introduction

Methyl orange dye has been widely used in the textile, paper, plastic and leather industry. The wastewater generated from these industries may contains some extent of MO, which can lead to very serious environmental problems and health hazards from mutagenic and carcinogenic agents [1-3]. Therefore, many researches have been focusing on eliminating MO dyes from the wastewater via various treatment methods such as physical and chemical methods in order to satisfy the environmental regulations [3-6]. However, some treatment methods are typically provide costly and incomplete elimination or only transfer the contaminants. Thus, it is necessary to develop an efficient way to completely eliminate the pollutants contained in the wastewater that are inexpensive and environment-friendly process. Photocatalytic process is being considered as an alternative way to mineralize toxic organic pollutants in wastewater due to its simplicity, low-cost and complete degradation of contaminants without producing secondary pollutant [7-9]. ZnO photocatalyst is a low cost material that efficient for azo dye degradation. [10-14] Nevertheless, ZnO could be activated under UV light irradiation due to its large band gap of 3.39 eV [13-16].

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Therefore, it is a challenge issue in order to extending the photocatalytic activity of ZnO photocatalyst to the visible region from the solar spectrum for improving light utilization of the photocatalyst. Recently, various research works have been demonstrated that ZnO powders nanoparticles doped with metal and non-metal elements such as Ag, Mn, C, N, and Fe show good photocatalytic activities under visible light [16-22]. However, the modification of ZnO with different Al contents which exhibits high efficiency and rapids degradation for azo dyes has not been reported. While ZnO photocatalysts have been synthesized through various methods such as hydrothermal synthesis [20, 23], precipitation [24, 25], and sol-gel method [26, 27]. Among these methods, the sol-gel method reveals wide application due to excellent crystalline structure of particles with simple, low-cost and mild conditions [27, 28]. In this study, Al doped ZnO with different doping amount were developed and their activity were investigated by MO degradation in photocatalytic system.

2. Methodology

2.1. Preparation ZnO and Al/ZnO photocatalysts

Pure and aluminum doped ZnO were synthesized by the conventional sol-gel method. In typical, zinc acetate ($Zn(CH_3COO)_2.2H_2O$) 0.15 M was dissolved in ethanol and stirred for 30 min to obtain solution (solution A). Aluminum nitrate ($Al(NO_3)_3$) as a source of aluminium, 0.15 M was dissolved in Milli-Q water and stirred for 30 min (solution B). Then, solution B was added to the solution A, drop-wise with different amount (1, 3 and 5%) and continuously stirred for 90 min. The pH of the mixture solution was adjusted to pH 10 by 1.5 M sodium hydroxide (NaOH). Then, a white colloid sol was obtained and aged to form a gel and stirred for 60 min. Finally, ZnO particles was obtained by thermal treatment at calcination temperature of 400°C for 2 h with ramping rate 2°C/min. The as-prepared photocatalysts was characterized by X-ray diffraction (XRD) Bruker D8 using the Cu Kα1 wavelength, scanning electron microscopy (SEM) and UV-Vis spectrophotometer.

2.2. Photocatalytic measurement

The photocatalytic efficiency of the nanoparticles was evaluated by monitoring the decolourization of synthetic MO solution (Sigma-Aldrich, 85% purity) with a concentration of 50 mg/L under light irradiation using a UV-Vis spectrophotometer. In a typical photocatalysis, 30 mg of photocatalyst was added to 30 mL of aqueous MO dye under continuous stirring. The solution was kept in the dark for 5 min to reach adsorption-desorption equilibrium of the dye on the photocatalyst followed UV and Fluorescence irradiations. The photocatalyst was exposed to different illumination sources: ultraviolet light (UV) lamp of 10 W and Florescence lamp of 10 W. The distance between the reactor and the lamp was 10 cm. The dye sample was sampling every 5 min and immediately centrifuged at 8,200 rpm for 10 min to remove photocatalyst particles. Finally, the absorbance of the dye in the supernatant liquid was observed by a UV-Vis spectrophotometer with the maximum absorption wavelength of the dye 460 nm. The absorption spectra of the MO solution was measured every 5 min for up to 30 min. The degradation percentage of the dye was obtained from the standard curve of the absorbance. The degradation percentage can be calculated as follows:

$$\text{Degradation (\%)} = [(C_0 - C)/(C_0)] \times 100$$

Where: C_0 and C are the initial concentration (mg/L), respectively

3. Results

3.1 Characterization

Morphology of the prepared ZnO and Al/ZnO photocatalysts were investigated by SEM analysis as shown in Figure 1. Figure 1 (a) presented ZnO nanoparticles with highly uniform spherical particles with the grain average sizes of 20-50 nm. It was observed that the aluminum concentration in the precursor solution have a great influence on the morphological characteristics of the photocatalysts. Effect of Al amount on morphology of ZnO nanoparticles is shown in Figure 1 (b-d). By adding of 1% and 3% Al, hexagonal and spherical structure with smaller particle size of 20-50 nm nanoparticles was revealed in Figure 1 (b)-(c). By increasing the Al content up to 5%, not only hexagonal and spherical structure were revealed but also a rod-like structure was achieved as shown in Figure 1 (d).

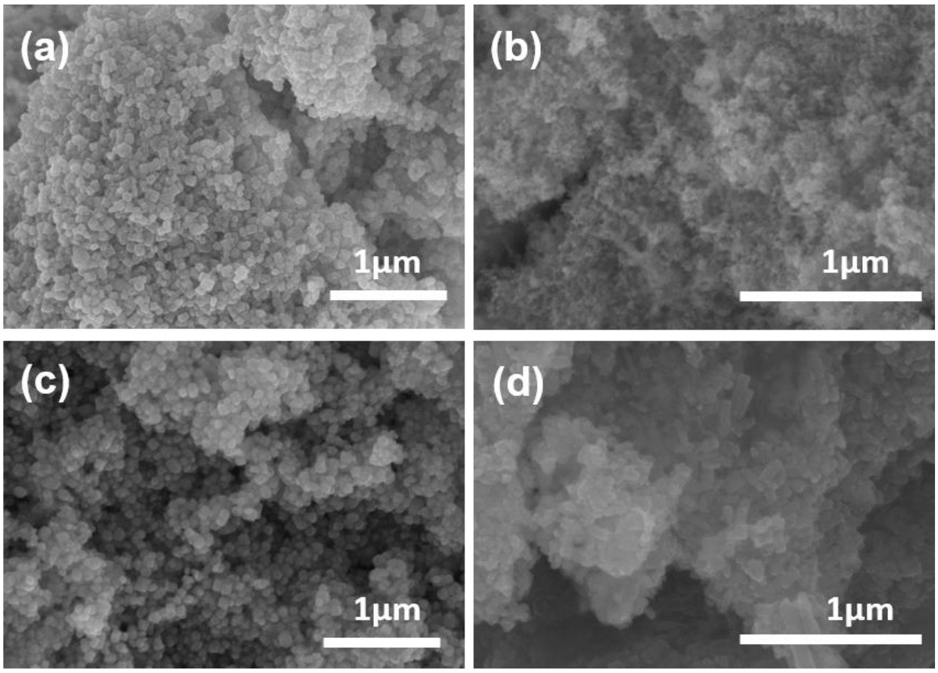

Figure 1. SEM images of (a) ZnO, (b) 1% Al/ZnO (c) 3% Al/ZnO (d) 5% Al/ZnO photocatalysts.

The UV-Vis diffused spectra of the pure ZnO and Al/ZnO photocatalysts with different Al contents are displayed in Figure 2. It was clear that doping Al on ZnO thin film caused plasmonic effect, all the samples doping Al reveal a red shift of a spectral band upon increasing content of Al. The shift of the wavelength absorption edge to visible light (400-700 nm) was observed on the Al/ ZnO nanoparticles, which expected to be beneficial for photocatalytic activity. This effect has an advantage in lower band gap energy of ZnO, in other words, lower energy required for electron excitation from valence band to conduction band. A similar trend was also observed in Mahdavi and Sam studies [15, 29]. Both studies concluded that the absorption edge shift possibly linked to the interaction of Al and ZnO, resulting in a higher photocatalytic degradation efficiency.

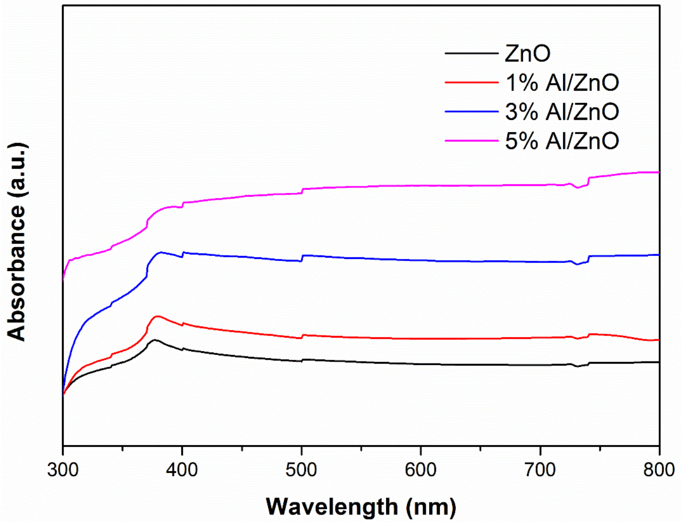

Figure 2. Absorption spectra of (a) ZnO, (b) 1% Al/ZnO, (c) 3% Al/ZnO, (d) 5% Al/ZnO photocatalysts analyzed by UV-Vis spectrophotometer.

3.2 Photocatalytic activity: Decolorization of MO dye solution

The Photocatalytic activity was determined by decolorization of 50 mg/L MO solution under UV and visible irradiations, as shown in Figure 3 (a) and (b), it was found that MO concentration decreasing as a function of irradiation time. Figure 3 (c) showed the changing in the color of the MO solution with the Al/ZnO photocatalyst upon the light irradiation. It was obviously seen that the color of the MO dye solution gradually faded as the irradiation time was increased. All Al/ZnO photocatalysts provided better degradation performance than the ZnO photocatalyst. The efficiency of photodecolorization decreased gradually and was constant within 40 min.

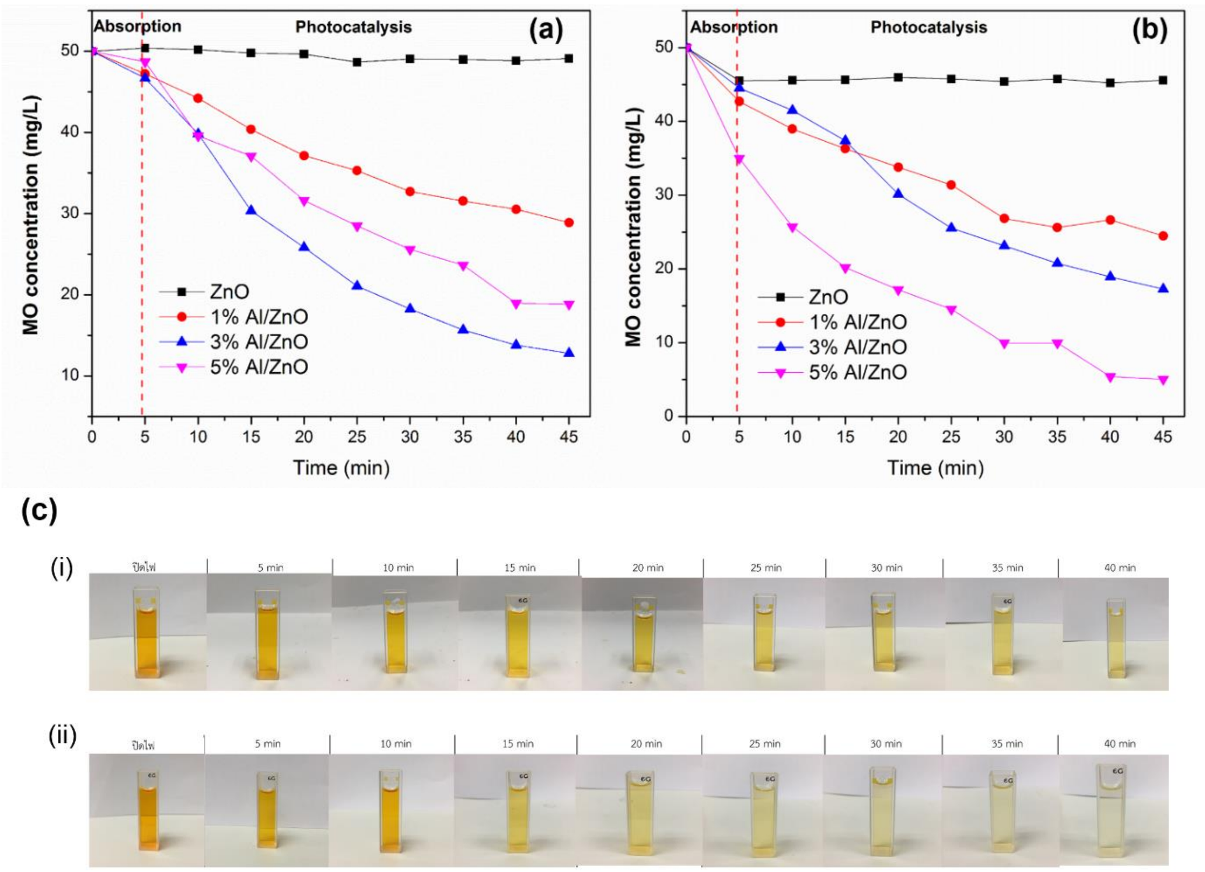

Figure 3. Photodecolorizations of MO dye solution by ZnO and Al/ZnO photocatalysts under (a) UV and (b) fluorescence irradiations, respectively and (c) Images of MO dye solution decolorization under photocatalytic measurement after for 40 min using: (i) 3% Al/ZnO photocatalyst under UV irradiation (ii) 5% Al/ZnO photocatalyst under visible irradiation.

4. Discussion

Figure 4 (a) showed that 3% Al/ZnO photocatalyst presented the highest decolorization efficiency of 70% after 40 min under UV irradiation. On the contrary, the maximum degradation efficiency was 85%, under 5% Al/ZnO photocatalyst after 40 min under visible irradiation (Figure 4 (b)). The photodecolorization rate under UV and fluorescence irradiation were fitted well with the first order reaction model as shown in Figure 4 (c)-(d), respectively. Additionally, the calculated reaction rate constants (k of ZnO and Al/ZnO photocatalysts with different amount of Al (1, 3, and 5%) were presented in Table 1. Notably, it could be indicated the optimal constituent, too large amount of Al nanoparticle was acted as recombination center for photogenerated electron-hole pairs, resulting in the inhibition of photocatalytic activity of ZnO [10, 30].

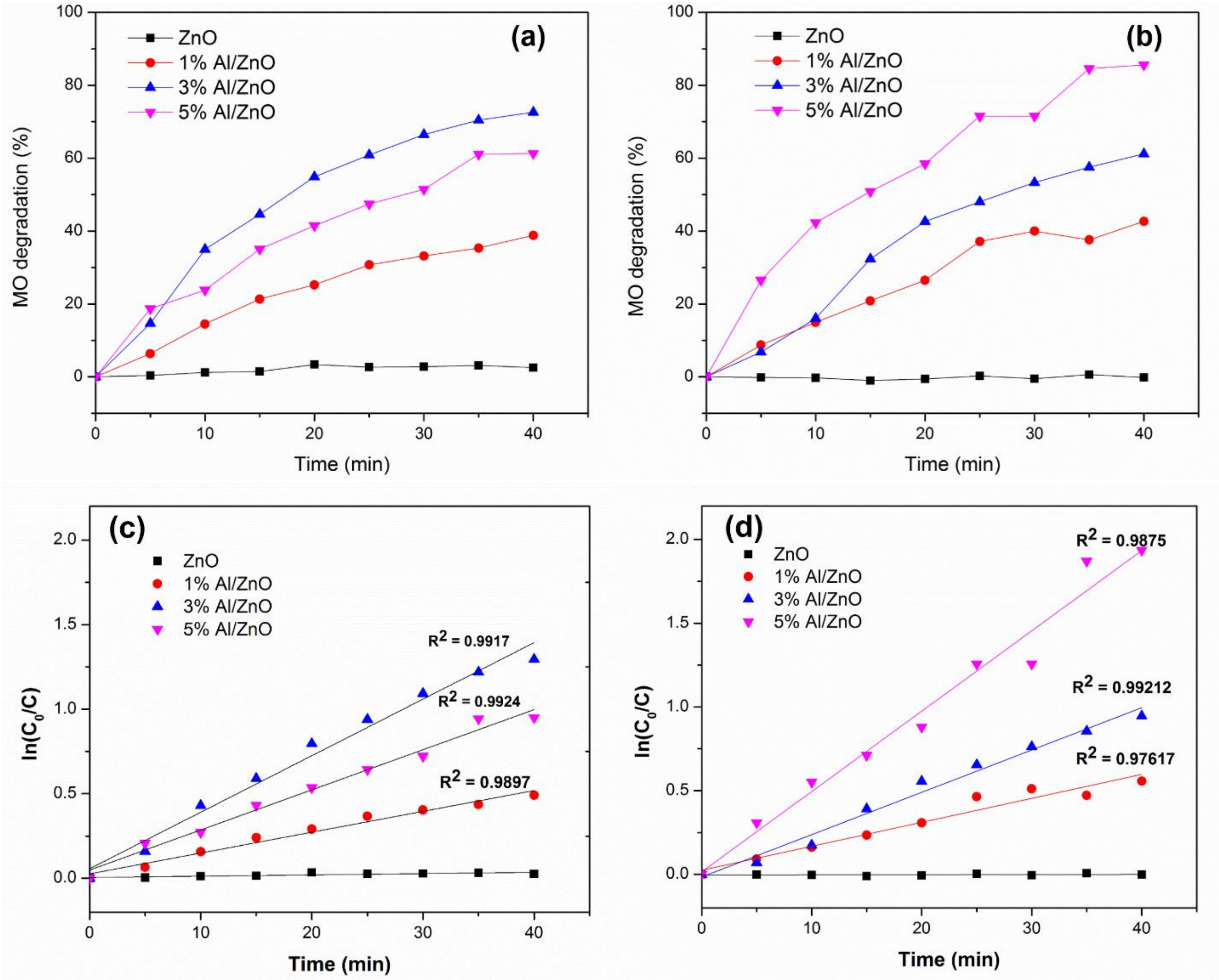

Figure 4. Photodegradations (a, b) and First order reaction rates (c, d) of MO dye by ZnO and Al/ZnO photocatalysts under UV and fluorescence irradiations, respectively.

Table 1. Degradation rate constants of methyl orange in the presence of photocatalysts

Photocatalyst	Kinetic (min^{-1})	
	UV	Visible light
ZnO	0.00072	0.00034
1% Al/ZnO	0.01245	0.01449
3% Al/ZnO	0.03402	0.02563
5% Al/ZnO	0.02404	0.04964

A mechanism for photocatalytic degradation of MO dye on the ZnO and Al/ZnO photocatalysts under light irradiation was shown in Figure 5. Typically, the photon energy is equal or higher than the band gap of ZnO (3.37 eV), the electrons in the valence band transfer to the conduction band under UV irradiation [31, 32]. The photo-generated holes could either directly oxidize the adsorbed MO dye or react with hydroxyl radicals (·OH). The photoelectrons reduce oxygen (O_2) adsorbed on the photocatalyst surface into superoxide radical ($\cdot O_2^-$). Finally, the MO dye molecules are degraded into simpler organic molecules by the continuous generated reactive oxidation species and further converted into CO_2 and H_2O. Hence, the enhanced photocatalytic degradation of the Al/ZnO photocatalysts should be attributed to the effective of charge-transfer by Al elements under UV and visible light irradiations. The relevant reactions were shown as follows [31, 32]:

$$ZnO + h\nu \rightarrow h^+_{(VB)} + e^-_{(CB)}$$
$$h^+ + OH^- \rightarrow \cdot OH$$
$$e^- + O_2 \rightarrow O_2^-$$
$$OH^-/O_2^- + \text{MO dye} \rightarrow CO_2 + H_2O + \text{inorganic molecules}$$

Furthermore, through the Al elements coupling, the quantity of free electrons is significantly increased with the surface plasmon resonance [30, 33, 34]. Under visible light irradiation, the main degradation pathway was the formation of radicals by electron migration to decompose the dye molecules [10, 30]. Therefore, it could be stated that the roles of Al are not only decreasing band gap energy but also promoting the charge separation efficiency.

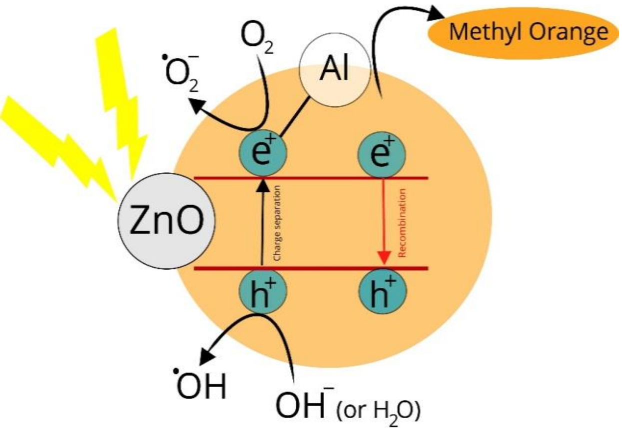

Figure 5. Mechanism of the photocatalysis of Al doped ZnO photocatalysts under light irradiation.

5. Conclusions

In this present work, the visible-light responsive Al/ZnO photocatalyst could be achieved via a facile sol-gel process. The Al/ ZnO showed good degradation efficiency under UV and visible light illuminations. The Al played an outstanding role in extending the light absorption spectrum toward the visible region which were beneficial for photocatalytic activity. Notably, the 5% Al doped ZnO not only increased the photocatalytic activity three times over the bare ZnO under UV and visible light illuminations, but also showed good absorption in the dark condition. The photocatalytic activity enhancement of the Al/ ZnO photocatalyst may leaded to the development of a simple operation, low cost, high efficiency, and environmentally friendly catalyst for wastewater treatment applications under sunlight irradiation.

6. Acknowledgements

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Removal of Methylene Blue Dye by Silver and Zirconium Doped TiO_2 Photocatalyst under Visible Light

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Abstract

This study aimed to remove methylene blue dye by silver (Ag) and zirconium (Zr) doped TiO_2 photocatalysts under visible light irradiation. The concentration of Ag doping was fixed at 5%mol for Ag doped TiO_2 (AT) while Zr doping fixed at 10%mol for Zr doped TiO_2 (ZT). Undoped TiO_2, 5%mol Ag doped TiO_2 and 10%mol Zr doped TiO_2 were prepared by sol-gel method and calcined at 700°C for 5 h. The characterizations of all nanocomposite powders were analyzed by X-ray diffraction (XRD), UV-vis spectrophotometer and Field emission scanning electron micrographs (FE-SEM). The photocatalytic decolorization of methylene blue (MB) dye under visible light irradiation and its kinetic were evaluated. The results indicated that TiO_2 and Ag doped TiO_2 had rutile phase while Zr doped TiO_2 had anatase phase. The results from UV-Vis spectrophotometer exhibited the significant improvement of wavelength absorption in visible light region. The Ag doped TiO_2 calcined showed the highest photocatalytic decolorization of MB (75.31%). This was due to the plasmon resonance effect of silver dopant.

Keywords: Silver/ Zirconium/ Titanium dioxide/ sol-gel/ methylene blue

1. Introduction

Wastewater from textile industry causes adverse effect on environment. It contains colored commercial dyes chemicals, pigments and various chemicals that are harmful to environment. The physico-chemical treatments of dye removal from textile are such as adsorption, activated carbon adsorption, ion-exchange, ultra-filtration, flocculation, reverse osmosis and membrane process. However, these convention treatments have some drawbacks because contaminants cannot be destroyed. They can only transfer from one phase to accumulate in another (Khan et al., 2016; Mondal et al., 2017). For this reason, photocatalysis is recommended to eliminate organic pollutants in water (Hu et al., 2004). There are many studies on the degradation of dye using semiconductor photocatalyst. Mukhlish and co-worker (2013) studied the photocatalytic degradation of methylene blue (MB) and congo red (CR) dyes by using TiO_2 as a photocatalyst. TiO_2 is one of the photocatalysts that widely used for the degradation of organic and inorganic pollutants due to its low cost, non toxic, high efficiency and excellent chemical stability (Kapusuz et al., 2013; Padmanaban et al., 2017). The limitation of TiO_2 is its large band-gap energy (3.2 eV for anatase and brookite and 3.0 eV for rutile) that cause the photocatalytic activity of TiO_2 occurs only under UV irradiation (Kumar et al., 2011). The way to increase the photocatalytic degradation efficiency of TiO_2 under visible light is doping TiO_2 with different metals such as V, Cr, Fe, Co, Cu, Mo, Ni, Ag and Zr (Gnanasekaran et al., 2016).

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These metals are expected to act by the following mechanisms. It may (i) acting as electron traps that enhance the electron–hole separation (ii), extending the light absorption into the visible range by plasmon resonances excited by visible light and (iii) modifying the surface properties of photocatalysts (Harikishore et al,. 2014). Silver (Ag) is one of promising dopants for preparing visible light responsive photocatalyst. The benefits of silver are enhancing the electron-hole separation by acting as electron traps, decreasing energy band gap and extending the wavelength absorption toward visible region. It was reported that plasmon resonance of silver nanoparticles could strongly absorb visible light (Neelavannan et al., 2010; Peerakiatkhajorn et al., 2012; Chen et al., 2011; Ubonchonlakate et al., 2012). Zirconium (Zr) is another dopant that can improve the photocatalytic reactivity of TiO_2 under visible light. Zr can incorporate in TiO_2 lattice leading to anatase phase stabilizing, increased surface area and retarding the electron hole pairs recombination (Kim et al., 2008; Oluwabi et al., 2018). In this study, silver and zirconium were selected as dopants for TiO_2. The doped TiO_2 nanocomposite powders were prepared by sol-gel method with the calcination temperature at 700°C. The photocatalytic decolorization of methylene blue dye under visible light irradiation was investigated in batch reactor and the kinetic reaction was investigated.

2. Methodology

2.1 Chemicals

Titanium(IV) butoxide, silver nitrate, zirconium acetylacetonate, acetylacetone, nitric acid, ethanol, methylene blue and de-ionized water and were purchased from Merck and Fluka.

2.2 Preparation of TiO_2, Ag doped TiO_2 and Zr doped TiO_2 nanocomposite powder

The preparation method was modified from the study of Tongon et al. (2014). Titanium butoxide, ethanol, acetylacetonate, de-ionized water and nitric acid were mixed in the molar ratio as 1: 18: 0.5: 2: 0.2, respectively. After that silver nitrate or zirconium acetylacetonate was added in the titanium solution then stirred for 1 h at room temperature. The amount of Ag was fixed at 5 %mol while Zr was fixed at 10 %mol. Then the mixture was stirred further for 1 h. The gel was dried at 80ºC and calcined at 700ºC for 5 h. The undoped TiO_2, Ag doped TiO_2 (AT) and Zr doped TiO_2 (ZT) nonanocomposite powder were obtained.

2.3 Nanocomposite powder characterizations

The crystallinity of the undoped and doped TiO_2 was examined by X-ray diffraction (XRD) measurements in the range of 20-80º (2θ) with CuKα 40kV and 40mA. The optical absorbance spectra were examined on UV-3100, Shimadzu instrument in the region of 200-800 nm. The field emission scanning electron microscopy (FE-SEM) images and energy dispersive X-ray spectrometer (EDS) were carried out by JSM-7001F instrument.

2.4 Photocatalytic degradation of MB dye

The photocatalytic reactivity of TiO_2, Ag doped TiO_2 and Zr doped TiO_2 nanocomposite powder was determined by degradation of MB dye under visible light. The 50 mg nanocomposite powder was added in the MB solution (10^{-5} M) and stirred under dark condition until the dye adsorption was saturated. After that a 15W fluorescent lamp was turned on. The photocatalytic degradation of MB dye was determined by UV-vis spectrophotometer (Genesis10 UV).

3. Results

3.1 Crystallinity

The crystalline phases of TiO_2, Ag doped TiO_2 and Zr doped TiO_2 were identified by X-ray diffraction (XRD) spectroscopy (shown in Figure 1). The result indicated that TiO_2 and Ag doped TiO_2 had strong peaks of rutile phase TiO_2 according to JCPDS: 21-1276 pattern. While Zr doped TiO_2 had strong peaks of anatase phase TiO_2 according to JCPDS: 21-1272 pattern. For Ag doped TiO_2, the characteristics of Ag could be observed at 38.1°, 44.0° and 64.5° which correspond to Ag metal. This result was in agreement with Chao et al. 2003. It is interestingly that rutile was found in both TiO_2 and Ag doped TiO_2 while anatase was found only in Zr doped TiO_2 nanocomposite powder. This was due to the effect of Zr ion on the retardation of rutile formation. The atom radius of Zr^{4+} was 0.79 Å, smaller than that of Ag^{+} (1.22Å) (Subash et al., 2013). The presence of Zr^{4+} could substitute Ti^{4+} (0.61 Å) in the crystal which disturbed the phase formation from anatase to rutile. While silver compound aggregated around rutile crystal rather than substituted in rutile crystal.

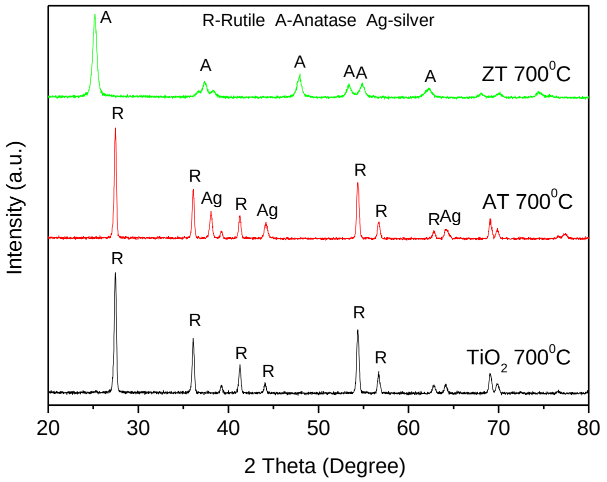

Figure 1. XRD patterns of undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2 calcined at 700°C

3.2 Wavelength absorption

Figure 2 presents the wavelength absorptions of the undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2. The results indicated that the wavelength absorption intensity of both Ag and Zr doped TiO_2 in UV and visible region was significantly enhanced when compared with the undoped TiO_2. Ag doped TiO_2 exhibited a predominant red-shift toward the visible region due to a reduction of the band gap energy caused by the surface plasmon resonance of silver (Suwanchawalit et al., 2012; Gupta et al., 2013; Tongon et al., 2014). Additionally, Ag nanoparticles on the surface of TiO_2 can enhance the photogenerated charge separation of TiO2. As the Fermi level of Ag is lower than TiO_2, the electrons transfer from TiO_2 to Ag nanoparticles leading to the retardation of electron-hole pairs recombination under visible light absorption (Kumar et al., 2011; Pham and Lee, 2014). On the contrary, Zr doped TiO_2 showed blue shift due to the lattice deformation effect (Mattsson et al., 2013). Some Zr ions might be segregated from the lattice of TiO_2. Very small microcrystals brought about the shift of wavelength absorption to UV region (Gao et al., 2010; Song et al., 2011).

The band gap energy can calculated from the cut-off wavelength from the following Equation.

$$= h \ /$$

where h is the plank's constant (6.626×10^{-34} Js), is a speed of light (3.0×10^{8} m/s) and is the wavelength of the light used. The result exhibited that the band gap energy of TiO_2, Ag doped TiO_2 and Zr doped TiO_2 were 2.95, 2.9 and 3.0 eV, respectively.

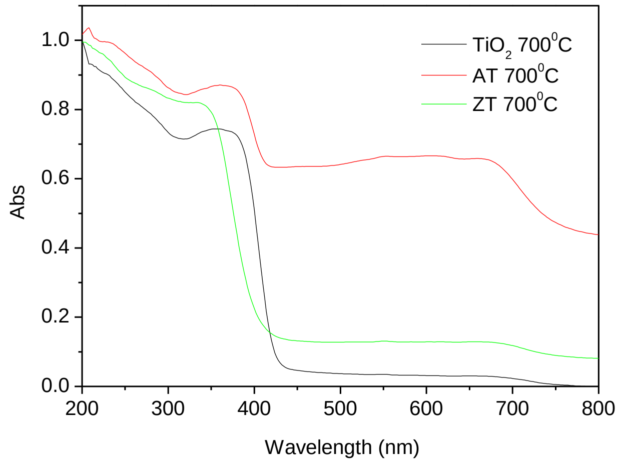

Figure 2. UV-vis spectra of undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2 calcined at 700°C

3.3 Photocatalytic activity

The photocatalytic decolorization of MB dye by undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2 calcined at 700°C under visible light was shown in Figure 3. It was found that the photocatalytic decolorization efficiency of nanocomposite powder increased as a function of irradiation time. The Ag doped TiO_2 performed the best photocatalytic decolorization efficiency (75.31%), followed by Zr doped TiO_2 (55.75%) and TiO_2 (27.99%) within 20 h. This is because silver could reduce the band gap energy by surface plasmon resonance (Mogal et al., 2014; Kumar et al., 2011). Whereas zirconium acts as an electron trap and prevent electron-hole recombination (Singh et al., 2017). The photocatalytic treatment of MB dye by doped TiO_2 photocatalyst under visible light irradiation could be explained in Equation (1) to (8) (Kumar et al., 2011; Zuo et al., 2014). MB dye is photosensitized and electrons are injected into the conduction band of doped TiO_2. The electrons react with oxygen to form oxidizing species (superoxide, hydroperoxyl, and hydroxyl radicals) resulting in photooxidation reactions. The MB dye is eliminated by reacting with the oxidizing agents.

$MB + h\upsilon \rightarrow MB^*$ (1)

$MB^* + \text{Doped } TiO_2 \rightarrow MB^{+\bullet} + \text{Doped } TiO_2\ (e)$ (2)

$\text{Doped } TiO_2\ (e) + O_2 \rightarrow O_2^{\bullet-}$ (3)

$H^+ + O_2^{\bullet-} \leftrightarrow HO_2^{\bullet}$ (4)

$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow O_2 + H_2O_2$ (5)

$O_2^{\bullet-} + e^- + 2H^+ \rightarrow H_2O_2$ (6)

$H_2O_2 + e^- \rightarrow OH^{\bullet} + OH^-$ (7)

$MB^{+\bullet} + O_2\ (O_2^{\bullet-} \text{or } OH^{\bullet}) \rightarrow \text{product}$ (8)

The kinetic rates were analyzed with the pseudo-first order model as the following equation.

$$\ln\left(\frac{Co}{\ }\right) = kt$$

where, Co is the initial concentration at t=0, is the concentration of MB dye and is rate constant (Harikishore et al. 2014).

The pseudo-first order plots were shown in Figure 4. The rate constants of TiO_2, Ag doped TiO_2 and Zr doped TiO_2 were 0.0172, 0.0842 and 0.0414 h^{-1}, respectively.

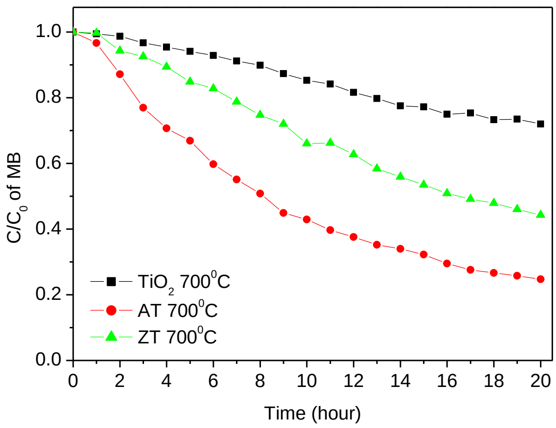

Figure 3. photocatalytic decolorization of MB dye by undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2 calcined at 700°C under visible light

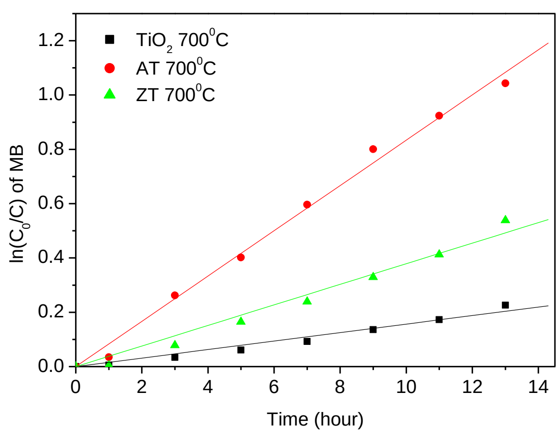

Figure 4. kinetics of MB decolorization by undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2 calcined at 700°C under visible light

3.4 Morphology

The field emission electron micrograph (FE-SEM) images and the energy dispersive X-ray (EDX) spectra were shown in Figure 5. The images indicated that the particle sizes of TiO_2 and Ag doped TiO_2 were large agglomerated particles (larger than 100 nm) when compared with the Zr doped TiO_2. It was obviously seen that the clusters of silver nanoparticles were agglomerated on the surface of Ag doped TiO_2 as displayed as the bright elongate shape particles. This result is in agreement with Guillen-Santiago et al. (2010). However, the morphology of Zr doped TiO_2 looked different. The surface was smooth, homogenous and the agglomeration of fine particles could be observed. This is due to the replacement of Zr^{4+} ion in TiO_2 crystalline structure (Wang et al. 2013).

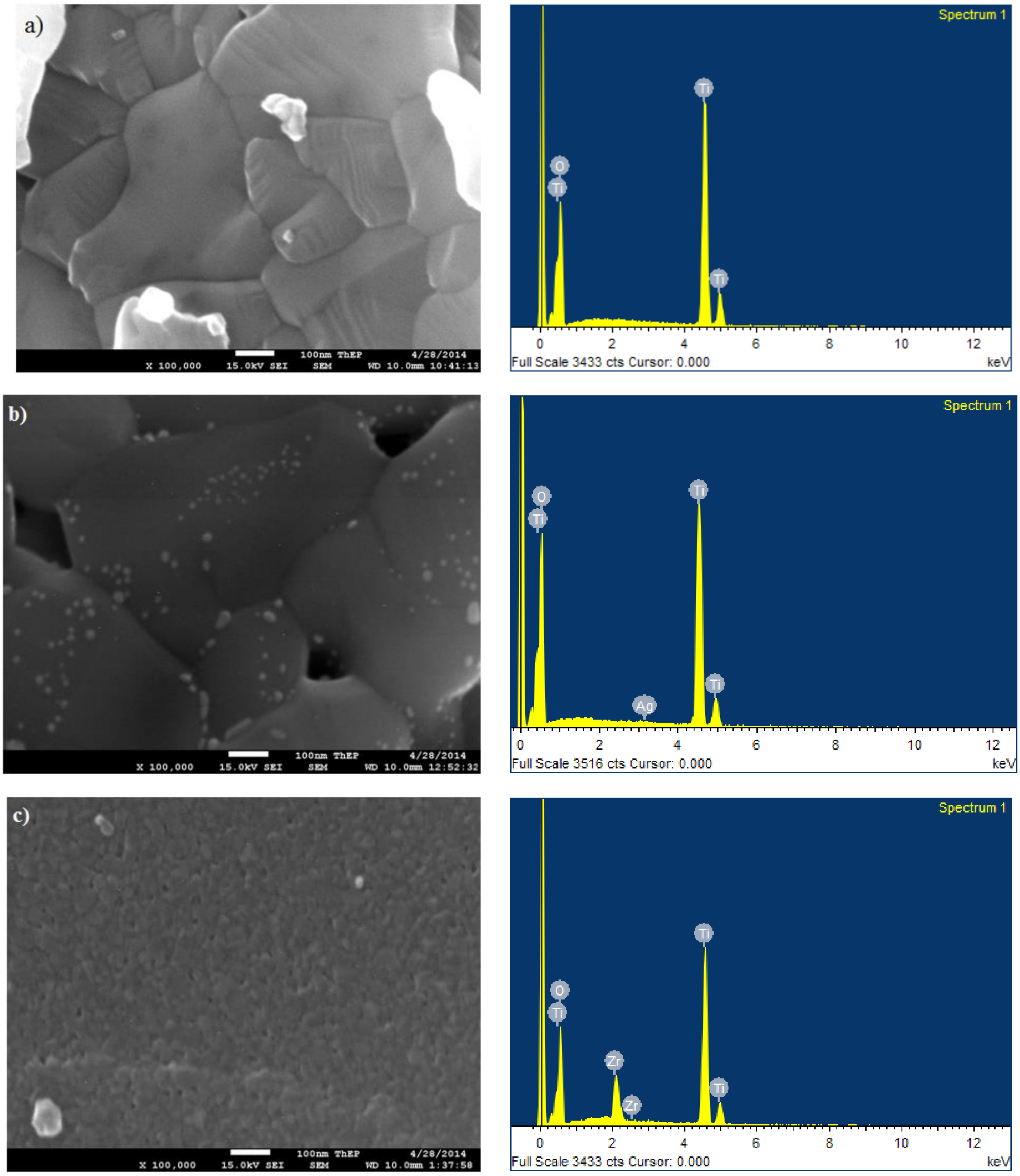

Figure 5. FE-SEM images of a) TiO_2 b) Ag doped TiO_2 and c) Zr doped TiO_2 with EDS spectra

4. Conclusion

The undoped TiO_2, Ag doped TiO_2 and Zr doped TiO_2 nanocomposite powder were successfully synthesized by sol-gel method and calcined at 700°C. Metal doping had a significant influence on physical and chemical of TiO_2 properties. Ag doping could not change TiO_2 rutile phase while Zr doping could retard the phase transformation from anatase to rutile. Ag dopant caused red-shift effect due to its surface plasmon resonanc while Zr dopant caused blue-shift effect due to the lattice deformation. Among all samples, the photocatalytic reactivity of Ag doped TiO_2 provided the highest photocatalytic efficiency of MB dye removal at 75.31% under visible light irradiation.

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Heavy Metal Accumulation and Health Risk Assessment through Consumption of Vegetables around Loei River, Loei Province

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Abstract

This research studied about accumulation of heavy metals (As, Zn, Pb, Cd, Cr and Cu) in surface water, sediment and vegetables (Papaya, Lvy Gourd and Lead tree) around Loei River, Mueang Loei and Chiang Khan Districts, Loei Province. Moreover, health risk assessment was done by using Health Risk Index (HRI). The results showed that the maximum of heavy metal accumulations were found in sediment followed by vegetables and surface water, respectively. In addition, there were found in the order of Zn>Cu>As>Cr>Pb>Cd in vegetables. While, health risk assessment showed that there were HRI<1 in Zn, Pb, Cd, Cr and Cu. However, HRI>1 was found of As in Papaya and Lvy Gourd as health risk. Therefore, sources for As should be studied to prevent heavy metal contamination on human health.

Keywords: Heavy metal/ Health risk assessment/ Vegetable/ Loei River

1. Introduction

The accumulation of pollutants, especially heavy metals, has come from industrial development and agriculture. Heavy metals distribution occurs from surface runoff and waste discharge into the environment (Uluturhan and Kucuksezgin, 2007). Heavy metals could affect human health and animal life because heavy metals cannot be degraded and they can accumulate through food chain or bioaccumulation (Yang et al., 2013). Heavy metals enter our bodies through contaminated food, drinking water and inhalation (Amirah et al., 2013).

Most of health risk assessment has been taken by developed countries (Milacic and Kralj, 2003), but inadequacy for developing countries (Lock and de Zeeuw, 2001). For Thailand, The Eighth National Research Strategy (2012-2016) emphasizes researches about pollution, environmental impact assessment and human health for improving the quality of life. Meanwhile, Loei River is one of the tributaries of Mekong River. The river is 231 km long and originated from Phu Luang Wildlife Sanctuary, which covering four districts including Phu Luang District, Wangsaphung District, Mueang Loei District and Chiang Khan District, Loei Province. The dominant land use is agricultural land as 1,060,017 rai (Regional Environmental Office 9, 2007; Pongpetch and Amchuem, 2015). The previous studies about Loei River revealed that Regional Environmental Office 9 (2014) has been investigating physical, chemical and biological parameters in Loei River for a long time. The result reported that water quality has been degraded since 2010-2013 and heavy metals have not yet been studied. On the other hand, Pongpetch and Amchuem (2015) studied heavy metal in water and fish around cage fish culture. However, this research did not covered throughout the river and heavy metal accumulation in sediment and vegetables were not studied, which vegetables around Loei River are used in cooking. Moreover, Chauhan and Chauhan (2014) showed that one of food contaminants are heavy metals. Therefore, this study aims to investigate heavy metal accumulation in surface water, sediment and vegetables and to assess health risk through consumption of vegetables around Loei River.

2. Methodology

2.1 Study area

Loei River is one of the tributaries of Mekong River. The river is 231 km long and originated from Phu Luang Wildlife Sanctuary, which covering four districts including Phu Luang District, Wangsaphung District, Mueang Loei District and

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Chiang Khan District, Loei Province. The dominant land use is agricultural land as 1,060,017 rai (Regional Environmental Office 9, 2007; Pongpetch and Amchuem, 2015).

The study area was defined by using land use map of the Land Development Department in 2009 and preliminary survey about growing vegetables and community along Loei River. Therefore, the accumulation of heavy metals was determined in surface water, sediment and vegetables, which collected from 10 areas at Ban Khon Daeng (BKD), Mueang Loei Municipality Market (MLM), Ban Kam Nerd Pet (BKN), Ban Tha Manao (BTM), *Ban Tha Wang* Khaen (BTW), Ban That (BTH), *Ban* Hua Kaeng (BHK), *Ban* Kaeng *Mi* (BKM), *Ban Klang* (BKL) and *Ban* Khok Mat (BKH) (Figure 1).

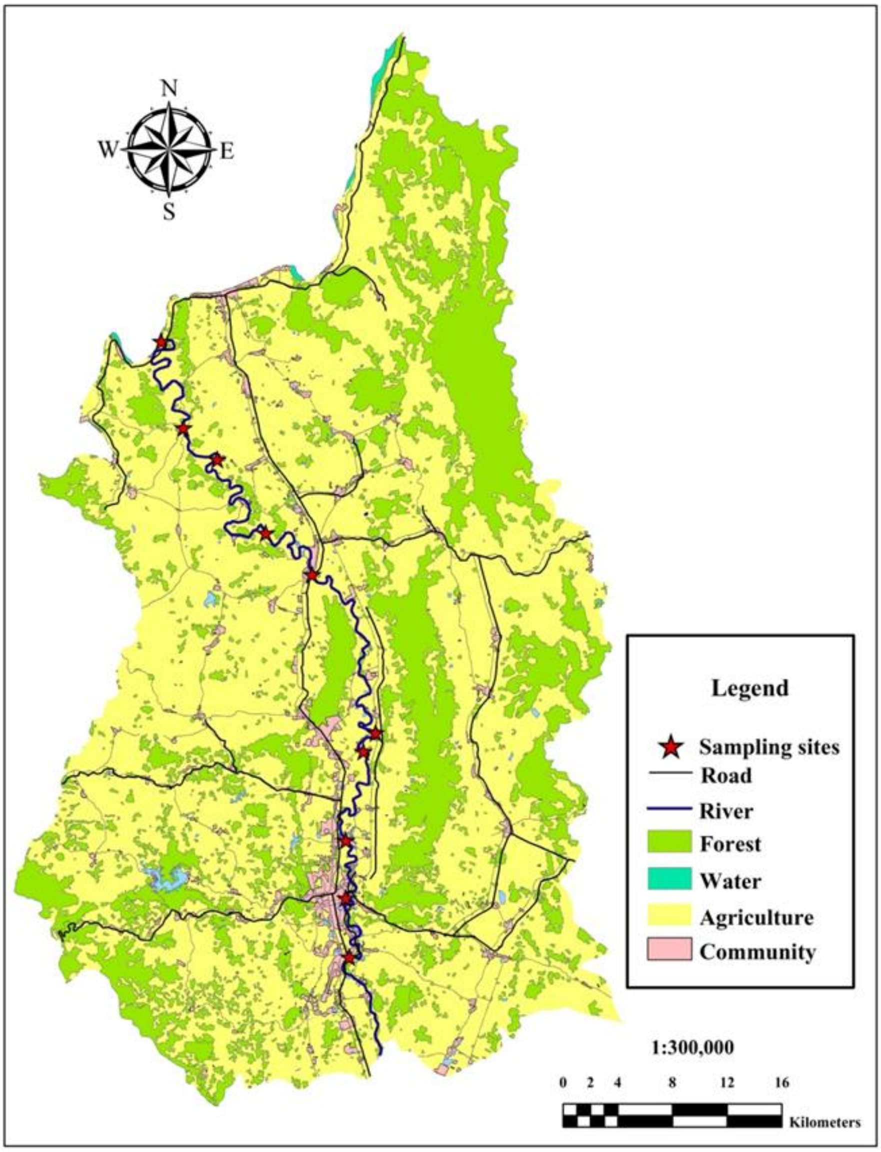

Figure 1. Sampling Sites of surface water, sediment and vegetables

2.2 Samples collection

Surface water, sediment and vegetables samples (Papaya, Lvy Gourd and Lead tree) were collected during August to December 2017. There were 180 samples including 30 samples of surface water (3 samples in each area), 30 samples of sediment (3 samples in each area) and 90 samples of vegetables (3 species X 3 samples in each area) were determined. Surface water samples were digested by using USEPA Method: 3005A (USEPA, 1992). Sediment and vegetables samples were digested by following method of Allen et al. (1986). Concentrations of arsenic (As), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr) and copper (Cu) were analyzed by using Inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3 Data analysis

2.3.1 Daily intake of metals (DIM)

DIM was calculated by the following equation:

$$DIM = C_{metal} \times C_{factor} \times D_{food\ intake} / B_{average\ weight}$$

where, C_{metal}, C_{factor}, $D_{food\ intake}$ and $B_{average\ weight}$ represent the heavy metal concentrations in plants (mg kg^{-1}), conversion factor (0.085), daily intake of vegetables and average body weight, respectively (Chary et al., 2008). Average body weight and daily intake of vegetables were calculated by surveying though 100 questionnaires (males and females) along Loei River having an average body weight of 61.2 kg. Moreover, daily intake of Papaya, Lvy Gourd and Lead tree were 0.5 kg/day, 0.2 kg/day and 0.18 kg/day, respectively.

2.3.2 Health Risk Index (HRI)

HRI is used to assess the human health risk of heavy metal. HRI for As, Cr, Cu, Pb, Cd, and Zn was calculated by the following equation:

$$HRI = DIM/R_{fd}$$

Where DIM and R_{fd} represent the daily intake of metals and reference oral dose (kg). R_{fd} value for As, Cr, Cu, Pb, Cd and Zn is 0.0003, 1.5, 0.04, 0.004, 0.001 and 0.30 mg/kg bw/day, respectively (USEPA, 2002; USEPA, 2005).

3. Results and discussion

3.1 Heavy metal accumulation in surface water, sediment and vegetables

The average concentrations could be presented in descending order as follows: Zn>Cr>Pb>Cu>As=Cd. The accumulation of heavy metal in surface water found that there was no accumulation of As and Cd. Meanwhile, Zn, Pb, Cr and Cu were found ranged from 0.027–0.132 mg/L, 0.001–0.019 mg/L, 0.023–0.041 mg/L and 0.001–0.006 mg/L, respectively (Table 1). However, all heavy metals in surface water were below the surface water quality standard of Thailand. Moreover, this result was accordant with the result of Pongpetch and Amchuem (2015) who studied water quality around cage fish at Mueang Loei and Chiang Khan Districts.

Heavy metal accumulation in sediment was presented in Table 2. The maximum and minimum values were found in Zn and Cd. Accumulation of As, Zn, Pb, Cd, Cr and Cu were in the range from 1.381–7.108 mg/kg, 6.373–59.270 mg/kg, 2.171–5.327 mg/kg, nd–0.129 mg/kg, 3.091–10.860 mg/kg and 3.192–15.880 mg/kg, respectively. The average concentrations could be presented in descending order as follows: Zn>Cu>Cr>Pb>As> Cd. However, all heavy metals were below soil quality standard of Thailand.

When comparing the average heavy metals in surface water with sediment, it was found that heavy metals in sediment were higher than surface water because heavy metals are non-degradable. When they are released into the water, they is absorbed and accumulated in sediments and aquatic organisms (Ruangsomboon, 2004).

Contamination of heavy metal in Papaya, Lvy Gourd and Lead tree found that As, Zn, Pb, Cd, Cr and Cu were found ranged from nd–2.710 mg/kg, nd–78.980 mg/kg, nd–0.947 mg/kg, nd–0.281 mg/kg, nd–2.167 mg/kg and nd–11.580 mg/kg (Table 3). The average concentrations could be presented in descending order as follows: Zn>Cu>As>Cr>Pb>Cd. When comparing heavy metals with standard of contaminants in food, it was found that most heavy metal was below standard. However, As in Lvy Gourd (3.33% of all samples), Cr in Papaya (1.11% of all samples), Cr in Lvy Gourd (21.11% of all samples) and Cr in Lead tree (100% of all samples) were exceed standard level. When comparing this study with other studies, they showed that most contamination of As was found in cereal, bean, green vegetable and tuber crops (Chandorkar and Deota, 2013; Song et al., 2015). Meanwhile, the highest Cr was found in Lead tree followed by Lvy Gourd and Papaya, respectively. Cr has acutely and chronic toxicity such as dizziness, diarrhea, thirst, abdominal pain, vomiting, shock, asthma and death (Kukusamude et al., 2003). Therefore, sources of Cr should be studied. However, heavy metal contamination in vegetables along Loei River was still satisfactory as safe because most samples were edible fruits, leafy crops, stem height and non-tuber crops. Rattan et al. (2005) demonstrated that contamination of heavy metal depends upon plant species, absorption efficiency of each plant and transfer factor from soil to plant.

Table 1. Heavy metal accumulation in surface water

Sampling sites	Heavy metal (mg/L)					
	As	Zn	Pb	Cd	Cr	Cu
BKD 1	nd	0.047	0.006	nd	0.038	0.004
BKD 2	nd	0.039	0.005	nd	0.038	0.003
BKD 3	nd	0.034	0.007	nd	0.038	0.002
MLM 1	nd	0.042	0.006	nd	0.037	0.002
MLM 2	nd	0.047	0.007	nd	0.035	0.003
MLM 3	nd	0.099	0.006	nd	0.037	0.004
BKN 1	nd	0.049	0.006	nd	0.040	0.006
BKN 2	nd	0.030	0.006	nd	0.039	0.003
BKN 3	nd	0.038	0.006	nd	0.038	0.004
BTM 1	nd	0.031	0.006	nd	0.038	0.004
BTM 2	nd	0.076	0.006	nd	0.039	0.005
BTM 3	nd	0.043	0.007	nd	0.041	0.005
BTW 1	nd	0.047	0.005	nd	0.030	0.003
BTW 2	nd	0.043	0.005	nd	0.038	0.003
BTW 3	nd	0.054	0.006	nd	0.038	0.004
BTH 1	nd	0.049	0.005	nd	0.038	0.004
BTH 2	nd	0.049	0.003	nd	0.038	0.003
BTH 3	nd	0.031	0.003	nd	0.037	0.002
BHK 1	nd	0.037	0.006	nd	0.039	0.002
BHK 2	nd	0.132	0.007	nd	0.037	0.004
BHK 3	nd	0.045	0.004	nd	0.038	0.003
BKM1	nd	0.067	0.004	nd	0.036	0.004
BKM2	nd	0.068	0.004	nd	0.035	0.002
BKM3	nd	0.070	0.004	nd	0.037	0.003
BKL 1	nd	0.027	0.005	nd	0.038	0.003
BKL 2	nd	0.042	0.006	nd	0.038	0.003
BKL 3	nd	0.042	0.019	nd	0.038	0.005
BKH 1	nd	0.059	0.001	nd	0.023	0.001
BKH 2	nd	0.057	0.004	nd	0.038	0.004
BKH 3	nd	0.030	0.004	nd	0.038	0.003
STD[a]	0.010	1.000	0.050	0.005	0.050	0.010

[a]Surface water quality standard of Thailand, Notification of the National Environmental Board, No. 8, B.E. 2537 (1994).

Table 2. Heavy metal accumulation in sediment

Sampling sites	Heavy metal (mg/kg)					
	As	Zn	Pb	Cd	Cr	Cu
BKD 1	3.045	59.270	3.254	0.091	5.403	5.343
BKD 2	3.102	50.270	3.280	0.129	5.980	5.398
BKD 1	3.045	59.270	3.254	0.091	5.403	5.343
BKD 2	3.102	50.270	3.280	0.129	5.980	5.398
BKD 3	2.967	52.369	3.007	0.123	5.578	5.387
MLM 1	4.206	19.640	3.638	nd	8.222	9.763
STD[b]	30.000	70.000	55.000	0.150	80.000	45.000

[b]Soil quality standard of Thailand B.E. 2548 (2005)

Table 2. Heavy metal accumulation in sediment (cont.)

Sampling sites	Heavy metal (mg/kg)					
	As	Zn	Pb	Cd	Cr	Cu
MLM 2	3.589	15.690	3.544	nd	7.313	9.254
MLM 3	3.466	11.220	2.922	nd	7.476	8.168
BKN 1	4.476	21.310	4.168	nd	8.195	11.490
BKN 2	4.132	21.500	4.211	nd	7.369	10.740
BKN 3	2.797	12.320	3.065	nd	5.178	6.889
BTM 1	1.519	6.900	2.673	nd	4.065	3.326
BTM 2	1.746	8.486	2.543	nd	4.967	4.484
BTM 3	2.291	9.037	3.346	nd	4.823	5.557
BTW 1	3.100	16.280	4.007	nd	6.747	7.719
BTW 2	2.502	12.570	2.709	nd	5.380	5.689
BTW 3	2.865	15.480	3.547	nd	5.779	7.480
BTH 1	1.653	6.847	2.187	nd	4.295	3.609
BTH 2	1.824	7.476	2.384	nd	4.295	3.987
BTH 3	3.088	12.750	2.536	nd	6.085	7.806
BHK 1	3.441	17.660	3.114	nd	6.650	8.808
BHK 2	3.188	12.260	3.684	nd	5.587	7.261
BHK 3	4.329	17.100	4.134	nd	7.376	10.770
BKM1	2.512	10.030	2.823	nd	4.840	6.728
BKM2	2.126	7.936	2.171	nd	4.582	4.317
BKM3	1.381	6.471	2.515	nd	3.091	4.068
BKL 1	1.524	6.373	2.252	nd	4.032	3.319
BKL 2	1.935	7.342	2.948	nd	4.260	3.192
BKL 3	1.922	7.832	2.213	nd	3.849	3.979
BKH 1	7.108	26.450	4.673	nd	10.860	15.880
BKH 2	5.550	20.450	4.029	nd	8.669	14.160
BKH 3	6.184	23.230	5.327	nd	9.072	13.930
STD[b]	30.000	70.000	55.000	0.150	80.000	45.000

[b]Soil quality standard of Thailand B.E. 2548 (2005)

Table 3. Heavy metal accumulation in vegetables

Samples	Sampling sites	Heavy metal (mg/kg)					
		As	Zn	Pb	Cd	Cr	Cu
Papaya	BKD 1	1.466	20.010	nd	nd	nd	3.396
	BKD 2	1.685	11.060	nd	nd	nd	1.905
	BKD 3	1.104	17.040	nd	nd	nd	1.425
Lvy Gourd	BKD 1	nd	nd	nd	nd	nd	nd
	BKD 2	1.644	54.780	0.078	nd	nd	5.402
	BKD 3	1.082	12.750	nd	0.054	nd	3.288
Lead tree	BKD 1	nd	28.830	0.112	nd	1.154	7.205
	BKD 2	nd	22.540	0.049	nd	0.900	3.193
	BKD 3	nd	23.990	0.182	nd	0.893	3.342
	STD[c]	2.000	100.000	1.000	0.300	0.500	20.000

[c]Standard of contaminants in food, Notification of the Ministry of Public Health (No. 273) B.E. 2546 (2003)

Table 3. Heavy metal accumulation in vegetables (cont.)

Samples	Sampling sites	Heavy metal (mg/kg)					
		As	Zn	Pb	Cd	Cr	Cu
Papaya	MLM 1	1.090	11.390	nd	nd	nd	0.850
	MLM 2	1.165	8.718	nd	nd	nd	0.816
	MLM 3	0.888	3.705	nd	nd	nd	nd
Lvy Gourd	MLM 1	2.086	30.660	0.067	nd	nd	9.042
	MLM 2	1.570	20.920	0.017	nd	nd	7.033
	MLM 3	1.634	19.540	0.079	0.007	nd	7.648
Lead tree	MLM 1	nd	25.910	0.173	nd	1.164	8.036
	MLM 2	nd	22.570	0.156	nd	1.322	5.850
	MLM 3	nd	22.280	0.091	nd	1.191	7.912
Papaya	BKN 1	1.258	8.874	nd	0.003	nd	2.148
	BKN 2	1.608	13.170	nd	0.011	nd	2.709
	BKN 3	1.523	9.056	nd	0.018	nd	1.427
Lvy Gourd	BKN 1	1.627	12.090	0.129	0.064	nd	4.542
	BKN 2	1.518	16.930	0.148	0.030	nd	5.311
	BKN 3	1.440	15.640	0.331	0.076	nd	4.582
Lead tree	BKN 1	nd	21.110	0.207	nd	0.970	6.044
	BKN 2	nd	19.700	0.221	nd	0.977	7.486
	BKN 3	nd	25.490	0.169	nd	1.231	6.082
Papaya	BTM 1	1.772	17.270	nd	nd	nd	1.505
	BTM 2	1.964	20.790	nd	nd	0.120	2.745
	BTM 3	1.528	24.680	nd	nd	nd	2.307
Lvy Gourd	BTM 1	2.190	17.120	0.630	0.281	0.647	6.610
	BTM 2	1.457	19.540	0.097	0.106	nd	5.741
	BTM 3	2.710	29.280	0.947	nd	nd	4.292
Lead tree	BTM 1	nd	22.710	0.121	nd	1.097	8.255
	BTM 2	nd	23.960	0.094	nd	0.996	6.160
	BTM 3	nd	14.710	0.108	nd	1.310	5.397
Papaya	BTW 1	1.375	11.590	nd	nd	nd	1.189
	BTW 2	1.703	13.530	nd	nd	nd	2.220
	BTW 3	1.227	23.940	nd	nd	nd	3.362
Lvy Gourd	BTW 1	0.720	20.630	0.433	0.045	1.275	3.879
	BTW 2	nd	78.980	0.863	0.042	1.371	4.952
	BTW 3	nd	32.820	0.154	nd	1.260	6.179
Lead tree	BTW 1	nd	14.510	nd	nd	0.957	6.996
	BTW 2	nd	16.270	0.059	nd	1.319	7.688
	BTW 3	nd	14.860	0.215	nd	0.964	9.490
Papaya	BTH 1	1.566	15.900	nd	nd	nd	1.095
	BTH 2	nd	13.360	nd	nd	0.618	2.149
	BTH 3	1.178	nd	nd	nd	nd	nd
Lvy Gourd	BTH 1	nd	25.000	0.760	nd	1.332	7.704
	BTH 2	nd	27.010	0.813	0.027	1.477	8.709
	BTH 3	nd	27.660	0.763	nd	1.465	8.661
	STD[c]	2.000	100.000	1.000	0.300	0.500	20.000

[c]Standard of contaminants in food, Notification of the Ministry of Public Health (No. 273) B.E. 2546 (2003)

Table 3. Heavy metal accumulation in vegetables (cont.)

Samples	Sampling sites	Heavy metal (mg/kg)					
		As	Zn	Pb	Cd	Cr	Cu
Lead tree	BTH 1	nd	15.250	0.340	nd	1.110	7.556
	BTH 2	nd	14.610	0.210	nd	1.096	7.485
	BTH 3	nd	12.570	0.159	nd	0.959	6.407
Papaya	BHK 1	1.240	9.064	nd	nd	nd	1.223
	BHK 2	1.569	11.150	nd	nd	nd	1.765
	BHK 3	1.671	22.070	nd	nd	nd	2.202
Lvy	BHK 1	nd	22.860	0.158	0.026	1.296	6.208
Gourd	BHK 2	nd	18.660	0.030	nd	0.943	3.750
	BHK 3	nd	21.860	0.242	0.037	1.543	5.363
Lead tree	BHK 1	nd	18.860	0.061	nd	0.951	6.032
	BHK 2	nd	14.530	0.004	nd	0.612	4.695
	BHK 3	nd	13.350	nd	nd	0.530	4.709
Papaya	BKM 1	1.200	14.620	nd	nd	nd	0.715
	BKM 2	1.396	18.090	nd	nd	nd	1.767
	BKM 3	1.521	18.120	nd	nd	nd	1.786
Lvy	BKM 1	nd	26.680	0.422	nd	1.908	6.612
Gourd	BKM 2	nd	23.220	0.334	nd	1.390	5.467
	BKM 3	nd	36.620	0.640	nd	2.167	8.224
Lead tree	BKM 1	nd	14.110	nd	nd	0.853	6.361
	BKM 2	nd	15.250	0.161	nd	0.947	6.081
	BKM 3	nd	15.620	0.065	nd	0.882	6.656
Papaya	BKL 1	1.429	7.825	nd	nd	nd	1.139
	BKL 2	1.508	5.563	nd	nd	nd	1.234
	BKL 3	1.379	6.054	nd	nd	nd	0.609
Lvy	BKL 1	nd	18.110	0.040	nd	1.204	2.263
Gourd	BKL 2	nd	21.780	0.161	nd	1.602	2.295
	BKL 3	nd	13.990	0.058	nd	1.001	1.651
Lead tree	BKL 1	nd	12.240	0.032	nd	1.864	3.832
	BKL 2	nd	13.090	0.081	nd	1.997	3.810
	BKL 3	nd	11.340	0.020	nd	1.640	3.208
Papaya	BKH 1	1.514	15.670	0.250	nd	nd	1.809
	BKH 2	1.187	15.860	0.269	nd	nd	1.779
	BKH 3	1.120	19.430	0.028	0.012	nd	2.446
Lvy	BKH 1	nd	22.330	0.113	nd	1.323	11.580
Gourd	BKH 2	nd	15.510	0.369	nd	1.276	8.383
	BKH 3	nd	23.030	0.108	nd	1.304	10.790
Lead tree	BKH 1	nd	14.670	0.151	nd	0.898	6.444
	BKH 2	nd	13.770	0.012	nd	0.899	6.038
	BKH 3	nd	11.830	0.102	nd	0.748	4.835
	STD[c]	2.000	100.000	1.000	0.300	0.500	20.000

[c]Standard of contaminants in food, Notification of the Ministry of Public Health (No. 273) B.E. 2546 (2003)

3.2 Health risk assessment

Health risk index (HRI) for heavy metals by consumption of vegetables is used for health risk assessment. The results showed that the maximum HRI was found for Papaya followed by Lvy Gourd. HRI values of As, Zn, Pb, Cd and Cu were found ranged from nd–4.546, nd–0.073, nd–0.066, nd–0.078, nd–0.080, respectively. The highest HRI value was found for as followed by Cu, Zn, Pb, Cd and Cr, respectively (Figure 2). HRI values of the study area suggested that Lead tree grown along Loei River was harmless for consumption (HRI<1) but Papaya and Lvy Gourd pose severe health risk with regard to As (HRI>1). This finding was consistent with previous studies, they illustrated that most health risk came from as contamination

(Chandorkar and Deota, 2013; Wachirawongsakorn et al., 2015; Mahmood and Malik, 2014). Song et al. (2015) reported that mining activities cause heavy metals accumulation such as As, Pb, Cu and Cd in soil. Moreover, Pholwean and Keithmaleesatti (2014) also revealed that as accumulation can be found high levels in gold mining areas and gold mining is also found in Loei Province. Therefore, heavy metal accumulation should be studied more deeply because concentration of some heavy metals is below standard, but they can also be harmful on human health.

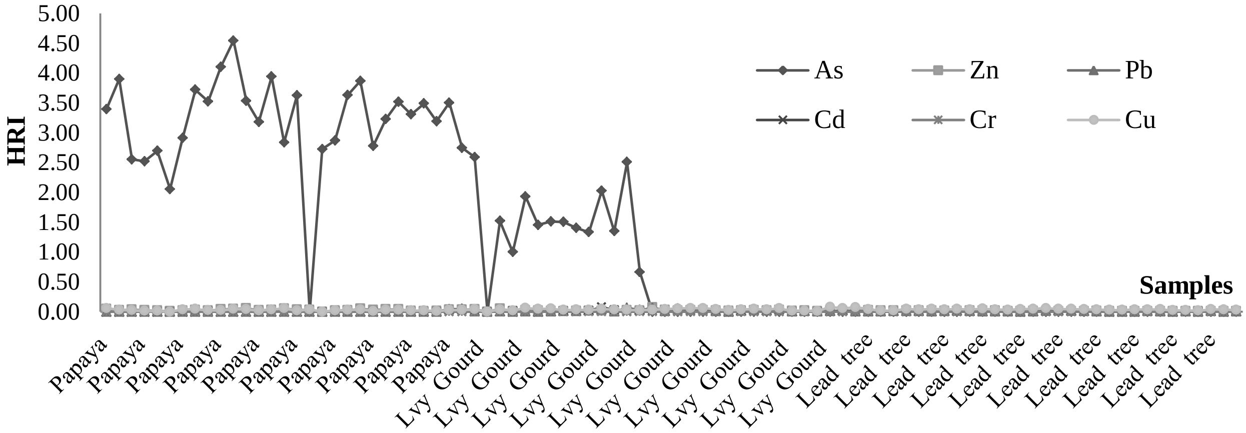

Figure 2. Health risk index (HRI)

4. Conclusions

Most heavy metals were below standard of Thailand. The maximum accumulation of heavy metal was found in sediment followed by vegetables and surface water, respectively. The maximum and minimum values were found Zn and Cd. The average concentrations of surface water, sediment and vegetables could be presented in descending order as follows: Zn>Cr>Pb>Cu>As=Cd, Zn>Cu>Cr>Pb>As>Cd and Zn>Cu>As>Cr>Pb>Cd, respectively. HRI indicated that most vegetables are less than 1, it still have some risk but lower than the risk criteria, except As in Papaya and Lvy Gourd (HRI>1). The highest HRI value was found for As followed by Cu, Zn, Pb, Cd and Cr, respectively. Therefore, sources for As should be studied to prevent and monitor heavy metal contamination on human health.

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Study on Production of Decomposing Cellulose Microbial Product from Microorganism Isolates from Nghi Yen Landfill

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Abstract

Farmers have adopted the strategy of increasing crop yields by applying large amounts of chemical fertilizers and pesticides. However, at present the negative effects of heavy applications of chemical inputs, in terms of production, environment, and quality deterioration are becoming apparent. Organic wastes are utilized in agriculture commonly used to improve soil quality and also to manage organic wastes for a sustainable land use. Using effective microorganism for bio-fertilizers and bio-fertilizer microbial product that have been encouraged to development by Vietnam's Agricultural Ministry and Researchers, improving soil properties, increasing soil fertility, maintain growth and crop yield and reducing environmental pollution. From Nghi Yen landfill, a few decomposing cellulose microorganisms was selected including: One strains of *Streptomyces*, other strain of Bacteria. The study has identified the appropriate specifications for each microorganism strain to creat biomass in level 2. The cell density after fermentation reached above 10^9 CFU/ml. Microbial product produced with mixing ratio of microorganisms of 1:1 and ratio of microorganisms and peat of 10/100, met Vietnam's standard of TCVN 6168-2002 ($\geq 10^8$ CFU/g) and ensured quality after 3 months of storage and stable biological activity of the microorganisms.

Keywords: Microorganism/ cellulose/ degradation

1. Introduction

Viet Nam is an agricultural country so the raw material (agricultural by-product residue) to serve the biological conversion by cellulase enzyme is very rich. About half of the carbon in the above-ground biomass is cellulose, accounting for 35-50% of plant biomass. Previously, in order to increase productivity and yields in crops, people often use chemical fertilizers and pesticides. However, this use is only of immediate benefit but does not guarantee the sustainable cultivation of crops, since the products derived from the chemical make the soil more and more degraded, nutrients lose weight. The ecosystem balance in the soil, the micro-organisms in the soil are destroyed, the residues of toxic substances in the soil more and more, leading to the unpredictable number of pests. to human health and environmental pollution [11]. The use of microbial cellulose degradation has been the subject of scientists in the world and in VietNam research. In India, Behera et al. (2014) isolates cellulose-degrading bacteria from mangroves and identified them as *Micrococcus spp., Baccilus spp.,* and *Pseudomonas spp.*[3]; in China, Yang Ling Liang et al. (2011) isolated 22 strains of cellulose isolate [16]. In Vietnam, Ha Thanh Toan et al. (2008) [13], Vo Van Phuoc Qui and Cao Ngoc Diep (2011), Le Pham Tuong Anh (2012) have also studied cellulose degradation microorganisms [15][2]. The use of microorganisms in agriculture, especially cellulose degradation products, is a trend of Vietnam in particular and the world in general to ensure biosafety, food safety and environmental safety. However, it still ensures productivity and quality for cultivation, towards a clean agriculture, sustainable development. Research to find the appropriate microbial product preparation for the treatment of agricultural by-product residues (straw, fungus, grass), contributing to the production of high quality and safe organic fertilizers crop.

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2. Methodology

2.1. Research on the production of microbial product from selected strains of cellulolytic microorganism

2.1.1. Isolation and selection of high cellulose degradable microorganisms

We used two strains of microorganisms isolated and selected from Nghi Yen landfill, Nghe An province: One strain of Streptomyces (B16) and other strain of Bacteria (A56) [11].

2.1.2. Proliferate and fixation microbial strains on suitable carriers to form microbiological product

Determination of microbial density base on Koch method [1].

The technique of producing microbiological products is inherited from scientific studies that have been successful in the past [1][4][5]. Factors affecting the multiplication process of level 1 and 2: Two microbial strains (isolated and selected from Nghi Yen landfill) were cultured on the specific culture medium, after testing of the density and purity, these microbial strains were transplanted into fermenter with rate of 5 percent.

Determination of biomass fermentation medium: Conduct test with specific medium for microorganisms, SX1 medium (Molasses 20g, yeast powder 10g, K_2HPO4 0.2g, distill water 1000ml) with temperatures in the biomass fermentation of 30^{o}C, fermentation time of 72 hours, air volume level of 0.7 dm^3 air/liter medium/minute. Biomass fermentation medium is suitable when the microbial density is highest and biological activity of microorganisms must be stable [4][5].

Methods of studying the effect of temperature: Optimum culture medium was selected from the above method; Microbial strains rate is 5 percent; pH is 7; The fermentation time is 48 hours. Temperatures in the biomass fermentation were adjusted at 20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C, 45^{o}C. The optimal biomass fermentation rate when the microbial population density is highest and the microbial activity of the microorganism must be stable [4][5].

Method of studying the effects of pH: Based on the optimal conditions of the two methods, the pH of the fermentation media was adjusted to 5.0, 5.5, 6.0, 7.0, 7.5, 8.0. The optimal pH when microbial population density is highest and biological activity of microorganisms must be stabilized [4][5].

Method of determining the rate of first microbial strains: With the optimum conditions selected, the percentage of first grade varieties is added at different levels from 0.5 percent, 1 percent, 2 percent, 3 percent, 4 percent, 5 percent. Level 1 was multiplied to the standard medium, tested for purity and cell density >= 10^8 CFU/ml. The optimum microbial strains rate when the density of the microbial cell is highest and that the microbial activity of the microorganism is stable [4][5].

Method of determining the rate of mixing of microorganisms: Microorganisms are mixed in a ratio of 1:1, 1:2, 2:1 mixture of microbial fluids mixed into the carrier at a rate of 10%. The ratio of suitable mixes when microbial cell density was highest and biological activity of microorganisms stable at 0 hour, 7 days, 15 days, 1 months, 2 months, 3 months [4][5].

Method of selecting carrier: Two microorganisms are cultured on three substrates (peat, rice bran, starch). Mix ratio of microbial strains:carrier is 1:10. The mixture is stored in a plastic bag at room temperature. Check for microbial cell density after 30 days of preservation [4][5].

Method of determining the mixing rate of biomass mixture of microorganisms and carriers: Mixing of biomass mixture to suit the carrier of 5/100; 10/100; 15/100 and 20/100. The appropriate mixing ratio when the microbial cell density is highest and microbial activity is stable at 0 hour and after 30 days [4].

Storage method: The product is then dried lightly at 40°C for 3-4 hours, then packed in dark plastic bags, stored under room conditions. Products are tested for micro-organism storage time 0 hours, 1 month, 2 months, 3 months and 4 months.

2.2. Evaluation of straw treatment efficiency of microbial product in field conditions

Field experiments were conducted to evaluate the straw treatment efficiency of the product. The experiment consisted of two treatments, four replications including inoculant treatment and no inoculant treatment. Weekly monitoring and after 4 weeks of decomposition of straw samples comparing the criteria such as color, temperature, length of straw.

3. Results

3.1. Production of microbial product from selected strains of cellulolytic microorganism

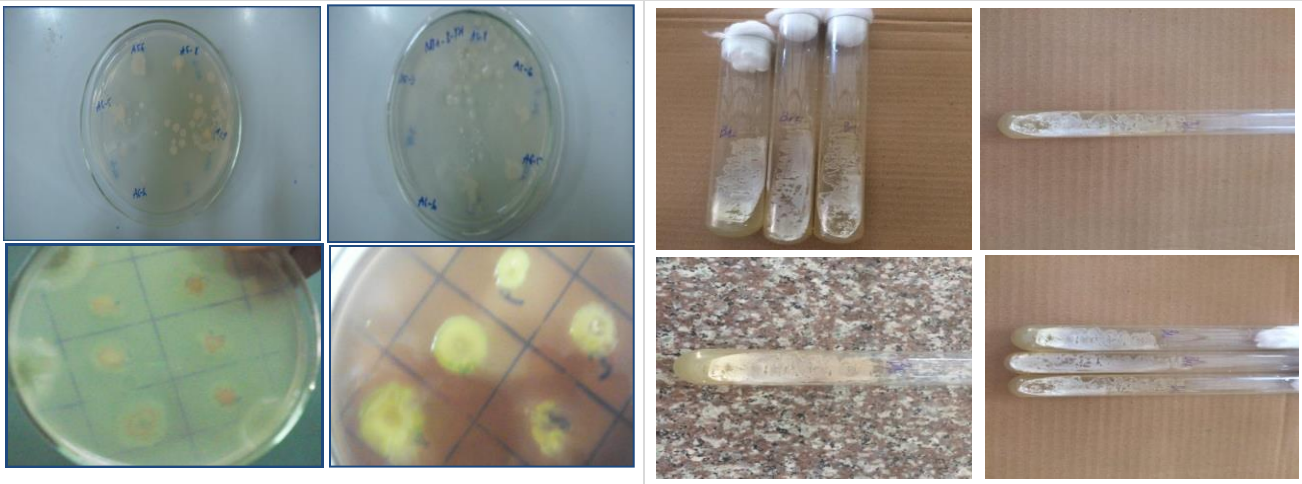
3.1.1. Selected highest cellulolytic microorganisms

From six strains of microorganism were isolated from Nghi Yen landfill [11] and stored in the laboratory - Nghe An Center of Applied Science and Technology, two strains with highest cellulose degradability were selected, including: One strain of *Streptomyces* (B16) and other strain of Bacteria (A56) (Figure 3.1).

3.1.2. Conditions for the multiplication of microbial strains

These two strains of microorganisms have been identified for their biological, safety and viability characteristics in the same condition. Several factors affecting the growth of microorganisms such as temperature, pH, air quality, medium and culture time have been identified to optimize for the secondary biomass process. The results are summarized in Table 3.1.

Results of optimum multiparametry parameters of B16 and A56 strain, basic parameters for *Bacillus* strains and *Streptomyces* sp. similar with Trinh Thanh Trung et al (2013) [14].



Bacteria strain | **Streptomyces strain**

Figure 3.1. Strains of cellulolytic microrganism

Table 3.1. Conditions for the multiplication of microbial strains

Specifications	Microorganism Strains	
	B16 (Streptomyces)	A56 (Bacteria)
pH fermentation medium	7.5	6.5
Temperature of biomass fermentation (^oC)	35	30
Time of biomass fermentation (hour)	72	48
Rate of microbial strains (%)	5	5
Biomass fermentation medium	SX1	SX1
Air supply (air dm^3/dm^3 medium/min)	0.7	0.65
Cell density (CFU/ml)	7.21×10^9	8.11×10^9
Diameter of cellulose ring	≥35mm	≥30mm

3.1.3. Selected of carrier

Result of testing microbial survival on different substrates after 30 days is presented in Table 3.2.

The results show that peat, rice bran and cassava can be used as carriers for microbial inoculants in subsequent studies. However, we compare the prices of three products to show that peat is the best choice (Peat price: 600 VND/kg, rice bran price: 7000 VND/kg, cassava price: 9000 VND/kg).

3.1.4. Rate of mixing of microorganisms

The results presented in Table 3.3 show that the rate of mixing the biomass of microbial strains affects their viability on peat carriers.

Table 3.2. Result of testing microbial strains survival on different substrates after 30 days

Microorganism Strains	Peat		Rice bran		Cassava	
	0 hour	After 30 days	0 hour	After 30 days	0 hour	After 30 days
B16 (Streptomyces)	4.31×10^8	3.98×10^8	4.98×10^8	6.22×10^8	4.61×10^8	6.32×10^8
A56 (Bacteria)	4.62×10^8	5.04×10^8	4.71×10^8	4.01×10^8	6.73×10^8	6.54×10^8

Table 3.3. Rate of mixing of microorganisms

Microorganism strains	Test time	Rate of mixing of microorganisms		
		1:1	1:2	2:1
B16 (Streptomyces)	0 hour	4.35×10^8	4.11×10^8	4.59×10^8
	After 30 days	4.61×10^8	8.97×10^7	3.13×10^8
A56 (Bacteria)	0 hour	4.68×10^8	5.28×10^8	5.31×10^8
	After 30 days	5.72×10^8	3.87×10^8	7.15×10^7

The results showed that at 1:1, after 30 days of storage, the density was stable compared to 0 hours. When the rate of microorganisms changes, cell density is unstable and tends to decrease. Thus, the ratio of 1:1 biomass is suitable for the production of microorganisms.

3.1.5. Mix rate of microbial strains with carrier

The results in Table 3.4 show that the biomass of microbial strains/carrier mix ratio is 5/100, the density of the microorganisms at 0 h is 10^7 CFU/g, after 30 days the microbial density on the the carrier has no change and still maintains 10^7 CFU/g. So at this rate, the density of useful microorganisms in the composition will not ensure quality according to Vietnam's standard of TCVN. At the same time, the rate of microbial/peat mixture was 10/100, 15/100, 20/100, the density of microorganism after 30 days preserved density > 10^8 CFU/g. The results showed that the ratio of 10/100 microalgae biomass/peat mix was suitable for inoculant production.

Table 3.4. Mix rate of microbial strains with carrier

Microorganism strains	Test time	Mix rate of microbial mix with carrier			
		5/100	10/100	15/100	20/100
B16 (Streptomyces)	0 hour	6.88×10^7	3.71×10^8	4.51×10^8	4.87×10^8
	After 30 days	6.63×10^7	4.55×10^8	3.99×10^8	5.02×10^8
A56 (Bacteria)	0 hour	5.98×10^7	3.37×10^8	6.02×10^8	6.01×10^8
	After 30 days	6.08×10^7	5.37×10^8	5.11×10^8	5.87×10^8

3.1.6. Quality and preservation time of microorganisms

Results of the study on the viability of microbial carriers on peat carrier are presented in Table 3.5. The results showed that at 0h, under the conditions of the microbial density mixture used in the study, > 10^8 CFU/g, after 7 days, the density of microbial cells was maintained at >10^8 CFU/g. At periods of 1, 2, 3 months of testing, the inoculant density of the inoculants remained stable (> 10^8 CFU/g). After 4 months, the microbial density decreased from the original. Therefore quality of microbial preparations to ensure quality according to Vietnam's standard of TCVN after production and quality assurance after 3 months of preservation [9][10].

3.1.7. Construction of production process of microbial product (NA1)

Based on the results of studies from 3.1.1 to 3.1.7, we developed the production process of cellulolytic microbial product (NA1) (Figure 3.2).

Table 3.5. Quality and preservation time of microorganisms

Microorganism strains	Test results									
	0 hour		After 1 month		After 2 months		After 3 months		After 4 months	
	D	BA	D	BA	D	BA	D	BA	D	BA
B16 (Streptomyces)	5.31×10^8	$+^{(*)}$	5.58×10^8	$+^{(*)}$	5.48×10^8	$+^{(*)}$	4.97×10^8	$+^{(*)}$	8.87×10^7	$-^{(*)}$
A56 (Bacteria)	5.01×10^8	$+^{(*)}$	5.75×10^8	$+^{(*)}$	5.04×10^8	$+^{(*)}$	4.11×10^8	$+^{(*)}$	8.63×10^7	$-^{(*)}$

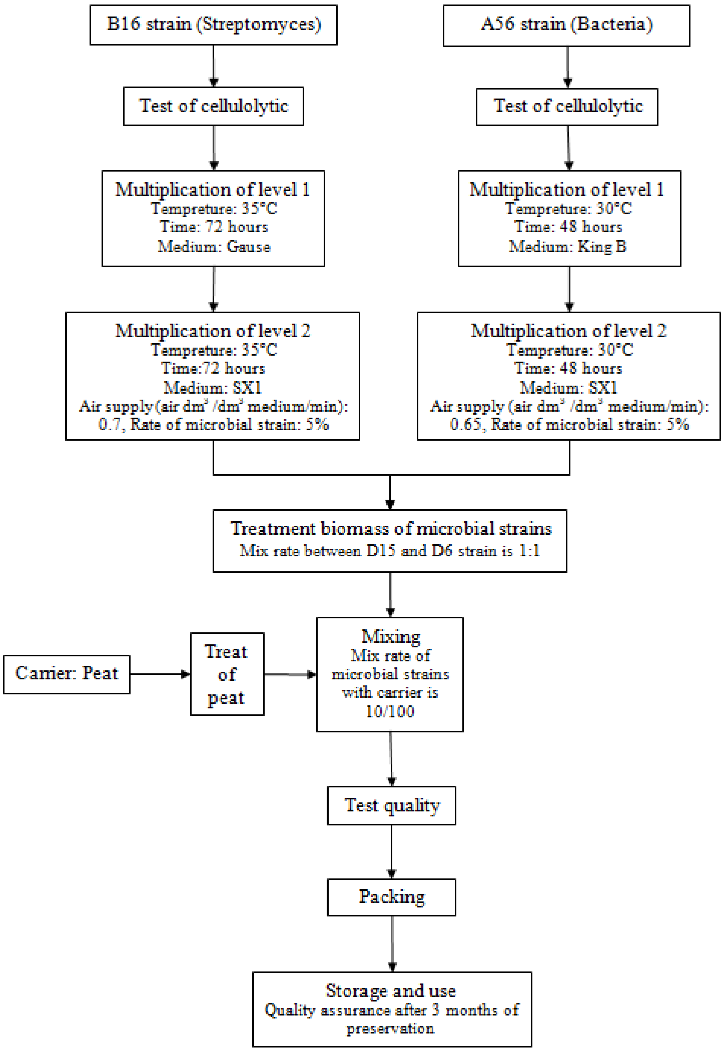


Figure 3.2. Process produce of cellulolytic microbial product (NA1)

3.2. Evaluation of straw treatment efficiency of microbial product in field conditions

The result show this cellulolytic microbial product (NA1) can efficiency treatment of straw in field conditions (Figure 3.3).

High temperatures during the 16 to 19 days of incubation have created the highest bacterial counts in these days. This is the stage with the most powerful and effective biodegradation process. According to Ryckeboer et al. (2003), the temperature of the incubation tank increased from day 6 and until day 22 then the temperature returned to its initial value, with the maximum temperature of the incubation unit over 70°C [8]. However, during the experiment, the temperature of the incubator was only 36.5^{o}C due to the fact that the experiment was arranged on a sunny and rainy day, which affected the ambient temperature.

In the experimental plot, the temperature increased higher than that of the control solution extracted from the cellulose extract of rice straw. By the last week, the process of decomposition of straw was completed, the temperature decreased. While the control samples started to separate straw fibers, the temperature was higher but lower than that of the control sample (32^{o}C).

As a result, in the experimental form, straw fibers have been decomposed into humus so that the dark, soft and soft colors can be used to fertilize crops. Also in the control sample straw is still light brown, not soft.

From the results, it was found that the addition of microorganism to the compost pile was more effective than that of natural microorganism in the compost pile, shortening the tempering time, reducing the environmental pollution caused by composting time long.

Experiment	Control

Figure 3.3. Strains of cellulolytic microorganism

Microbial product from B16 and A56 strains have good treatment efficiency, the degree of decomposition of the compost material of this composition is higher than that of other preparations isolated from waste of Nguyen Thi Thu Thuy et al,.2017 [12].

4. Discussion and Conclusions

The optimum biomass parameter for B16 is total bacterial culture, pH = 7, temperature 30°C, 5% feed rate, air flow rate 0.65 liters air/liter environmentally stirring speed 300 rpm, culture time 36 hours. The optimum biomass for the B16 strain is only A56 at the feed rate of 0.7 liters of air per liter of medium and the agitator speed is 250 rpm.

Microbial product (NA1) from B16 and A56 strains after production reached VietNam's standard of TCVN 6168: 2002, inoculant treatment of agricultural by-products [10].

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Radiological Hazard Assessment and Excess Lifetime Cancer Risk Evaluation in Surface Soil Samples Collected from Mueang District in Rayong Province, Thailand

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Abstract

Specific activities of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in 66 surface soil samples collected from Mueang district in Rayong province in eastern region of Thailand, have been studied and measured. A high-purity germanium (HPGe) detector and gamma spectrometry analysis system were employed to carry out all experimental results. The median values of the specific activities of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs were studied and calculated from the asymmetrical distribution in all surface soil samples and equal to 49.58 ± 1.38, 16.67 ± 0.65, 297.20 ± 8.50 and 1.41 ± 0.40 Bq/kg, respectively. Four radiological hazard indices which are gamma-absorbed dose rate (D), radium equivalent activity (Ra_{eq}), external hazard index (H_{ex}) and annual external effective dose rate (AED_{out}), were evaluated for the investigated area by using the median values of specific activities of ^{226}Ra, ^{232}Th and ^{40}K. Furthermore, the excess lifetime cancer risk evaluation (ELCR(outdoor)) could be calculated by using the AED_{out} value. Moreover, the results were also compared with the Office of Atoms for Peace (OAP) annual report data, Thailand and global radioactivity measurement and calculations. From this study, the corresponding annual external effective dose rate (AED_{out}) and the excess lifetime cancer risk evaluation (ELCR(outdoor)) were equal to 0.06 ± 0.002 mSv/y and $(0.21 \pm 0.01) \times 10^{-3}$, respectively. These two values were lower than the worldwide average. The meaning is that the surface soil in the investigated area are not harmful to human health, but continuous exposure can cause the long-term effects.

Keywords: Specific activity/ Natural and anthropogenic radionuclides/ Radiological hazard index/ Annual external effective dose rate/ Excess lifetime cancer risk/ Gamma spectrometry

1. Introduction

Radioactivity is naturally present in the environment. Some resource extraction activities accidentally concentrate radioactivity as an indirect result of processes to obtain other articles – examples include oil and gas extraction, phosphate production and the production of rare-earth elements. The industrial processes result in wastes and/or contamination that need to be managed carefully due to the levels of naturally occurring radioactive material (NORM) and technologically-enhanced NORM (TENORM) (https:// www. quintessa.org/sectors/nuclear/norm-and-tenorm).

Hence, awareness of exposure to natural radioactivity and its technological enhancement in both the industrial and environmental domains is growing. The increased awareness of the importance of natural radioactivity as a source of general everyday radiation exposure is reflected in the changing estimates by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Its estimates of the average natural dose to a member of the public from the natural decay series radionuclides have changed from about 50 $\mu Sv/y$ in 1962, to about 150 in 1972, to 1040 in 1977, to 1140 in 1982 and to about 1400 today (Baxter, 1993). According to this concern, many researches and projects have been carried out on activity concentration in natural samples such as rock, soil, beach sand and sediments in different areas around the world (Masok, et al., 2011; Al-Ghamdi, et al., 2016; Saini, et al., 2017).

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Furthermore, some researches have been studied and analyzed on activity concentration and some radiation parameters to evaluate the risks of radiations from NORM/TENORM wastes that produced from oil and gas industry (ALNabhani, et al., 2016; ALNabhani, et al., 2017; De-Paula-Costa, et al., 2018; Hilal, et al., 2014; Bakr, et al., 2010). Moreover, the radiological hazard indices and excess lifetime cancer risk in soil samples in many places around the world have been also evaluated and presented by many researchers(Oghenevovwero E, et al., 2017; Mohammed, et al., 2017; Taskin, et al., 2009; Dizman, et al., 2016).

In Thailand, Rayong province is located on the East Coast of Thailand, about 200 kilometers from Bangkok. It has 100 Kilometers of coastline, making Rayong province an important fishing and seafood area. This province is an interesting area of Eastern Thailand, with large areas of coast and beaches. Together with this, Rayong province has national parks with waterfalls. The beaches along the coast are varied, being pure leisure and working fishing beaches. All are interesting with individual characteristics. Many of the local villages carry out fishing and subsequently the drying, processing and packaging of their products. Hence, Rayong province is one of the most important suppliers of fish and seafood to the Eastern area of Thailand. Industrially, Rayong province is the home of the southeast asia industrial estate which is Map Ta Phut, a huge cluster of different industries. This place includes one of the largest Petrochemical plants in the world. For this reason, Rayong province is also known as an Industrial area, because there are many factories based there (http://www.smilingin thailand.com/east-coast-provinces/rayong/index.html).

Despite all these reports, it suffices to note that the Rayong province especially in Mueang district has never been studied before for natural and anthropogenic radioactivity levels. Furthermore, the Map-Ta-Phut sub-district which located in Mueang district, should be examined and measured the NORM/TENORM level in this environment.

The aim of this work is to measure and analyze the specific activities of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides from surface soil samples collected from Mueang district in Rayong province where is the area of oil and gas industry. Furthermore, radiological hazard indices and the excess lifetime cancer risk evaluation (ELCR(outdoor)) in the studied area were also evaluate for the risks of radiations. This would protect people in the investigated area of oil and natural gas production industry as well as our environment by reducing its activity concentration consequently and the health hazard potential.

2. Methodology

2.1 Sample collection and preparation

During the investigation, surface soil samples were collected from 66 predetermined sampling locations in Mueang district in Rayong province in eastern region of Thailand as shown in Fig. 1. An area approximately 1 m^2 was marked out at each sampling site. Then, about 1 kg of surface soil sample was collected at a depth of about 15 cm from the marked area after the soil inside were shoveled and piled together. The collected soil samples were mixed and taken in labeled plastic bags and sent back to Nuclear and Material Physics Research Unit (NUMPRU) at Thaksin University. All of the soil samples were dried up at room temperature and sieved through a 2 mm mesh-sized sieve to remove stone, grass and other macro-impurities. Before the measurement and analysis, the samples were oven dried at a temperature of 110 °C for 5 hours for removing moisture. The prepared and homogenized samples each of which is about 250 g, were placed in a PVC cylindrical container. The container was sealed hermitically and externally using a cellophane tape and kept aside for about a month to ensure equilibrium between ^{226}Ra and its daughters and ^{228}Ra and its daughters before being taken for measurement and analysis by using gamma spectrometry technique.

2.2 Measurement and analysis

Measurement and analysis of the surface soil samples were performed using a high-purity germanium detector (HPGe, EG&G ORTEC Model GEM) and gamma spectrometry analysis system at advanced laboratory, Thailand Institute of Nuclear Technology (Public Organization) (TINT). The detector was enclosed in a massive 10 cm thick lead shielding. The spectrum analyses were carried out by means of computer software obtained from ORTEC. Geometric efficiency for soil matrices in

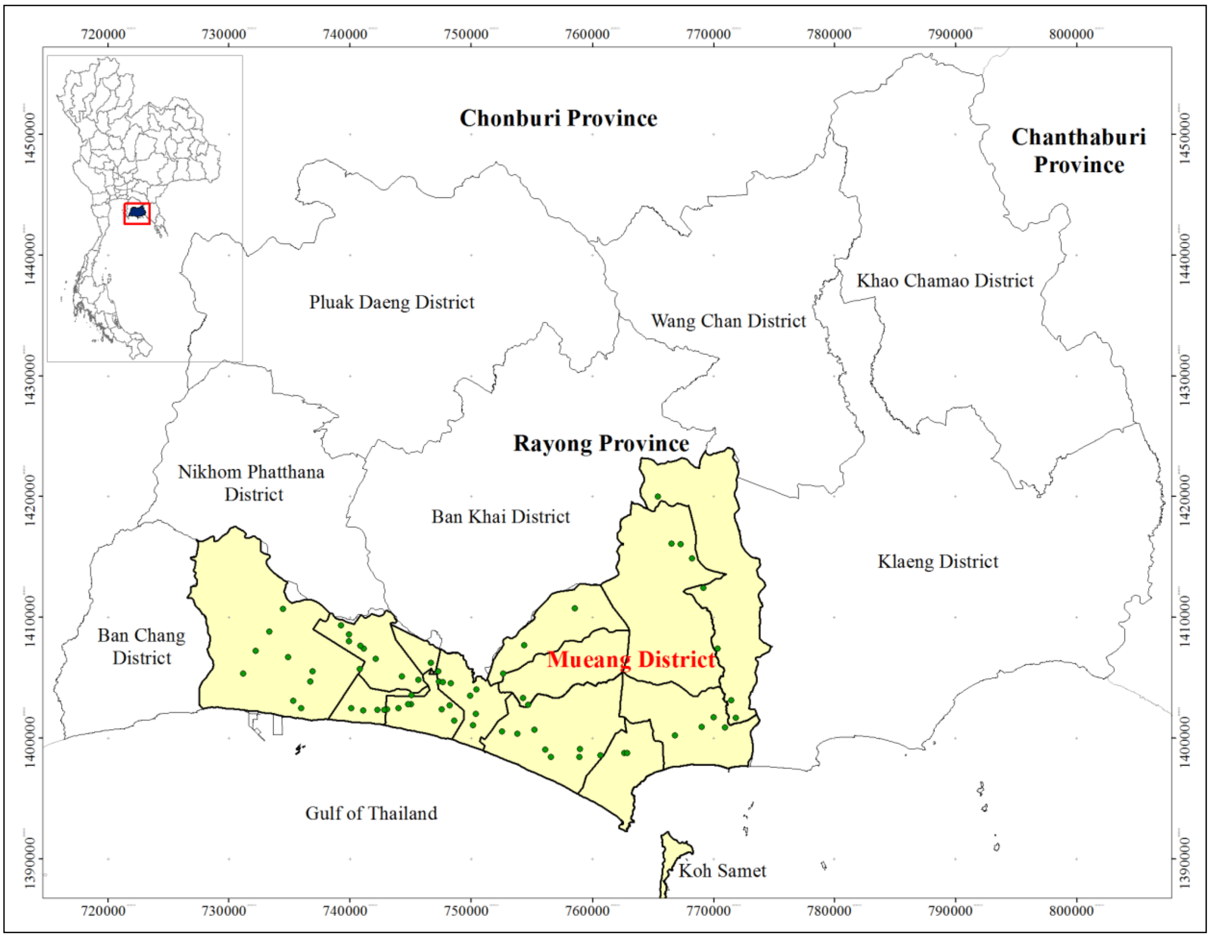


Figure 1. Map of Mueang district in Rayong province and sampling locations.

the container was determined by the IAEA Soil-375 reference materials (International Atomic Energy Agency IAEA, Vienna, Austria). Counting time or interval was 10800 s. The background spectrum was recorded immediately after before the sample counting. The frequency distribution of specific activities of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in the surface soil samples for the investigated area, were also studied and analyzed using the statistic computer program. Furthermore, the results were also compared with some research data in Thailand as well as global measurements and evaluations. Moreover, four radiological hazard indices and the excess lifetime cancer risk evaluation in the study area were also evaluated by using the appropriate medium values of the frequency distribution and the equations as shown in the following section.

2.3 Emission reporting

To determine the specific activity of the radionuclides activities in the surface soil samples were calculated from the following equation:

$$S.A. = \frac{C}{\varepsilon \times P \times m \times t}, \quad (1)$$

where S.A. is the specific activity of a radionuclide (Bq/kg), C is the count rate under the related full energy peak, ε is the detection efficiency at energy E, P is the abundance of the gamma line in a radionuclide, m is the soil sample mass (kg) and t is the counting time. Besides the minimum detectable activity (MDA) of each of the radionuclides was also evaluated at the 95% confidence level. The MDA for ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs were found to be 7.80, 3.36, 55.77 and 0.89 Bq/kg, respectively for a counting time of 10800 s and a 250 g of the surface soil sample.

The absorbed dose rate (D) in outdoor air at 1 m above the ground was calculated by using the conversion factors published in (Singh, et al., 2005) with all median values of specific activity of ^{226}Ra, ^{232}Th and ^{40}K from this study and is given below

$$D = 0.461C_{Ra} + 0.623C_{Th} + 0.0414C_{K}, \qquad (2)$$

where C_{Ra}, C_{Th} and C_K are the median values of specific activity of ^{226}Ra, ^{232}Th and ^{40}K in Bq/kg, respectively. Furthermore, the radium equivalent activity (Ra_{eq}) was calculated through the following relationship (Veiga, et al., 2006):

$$Ra_{eq} = C_{Ra} + 1.43C_{Th} + 0.077C_{K} \qquad (3)$$

Moreover, the external hazard index (H_{ex}) was also evaluated by using the equation which was defined as (Veiga, et al., 2006):

$$H_{ex} = C_{Ra}/370 + C_{Th}/259 + C_{K}/4810 \leq 1 \qquad (4)$$

The annual external effective dose rate (AED_{out}) were calculated using the absorbed dose rates in air (D) obtained from the median values of specific activity of natural (^{226}Ra, ^{232}Th and ^{40}K) radionuclides in surface soil samples, adopting the conversion factor of 0.7 Sv/Gy to convert from the absorbed dose in air to the effective dose received by adults and considering that people in Thailand, on average, spend approximately 20% of their time outdoors. The annual external effective dose rate (AED_{out}) were calculated using the following equation (Singh, et al., 2005; Mohanty, et al., 2004):

$$AED_{out}\ (mSv/y) = D\ (nGy/h) \times 8760\ h \times 0.2 \times 0.7\ (Sv/Gy) \times 10^{-6} \qquad (5)$$

All four radiological hazard indices and their average values were calculated and compared with the recommended values reported by United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR).

Furthermore, the excess lifetime cancer risk (ELCR(outdoor)) can be calculated from the relation (Qureshi, et al., 2014):

$$ELCR(outdoor) = AED_{out} \times LF \times RF \qquad (6)$$

Where AED_{out}, LF and RF are the annual effective dose rate, life expectancy of Thai people (75 years) and risk factor (0.05 Sv^{-1}), respectively.

3. Results

3.1 Mean values and ranges of specific activities of natural and anthropogenic radionuclides

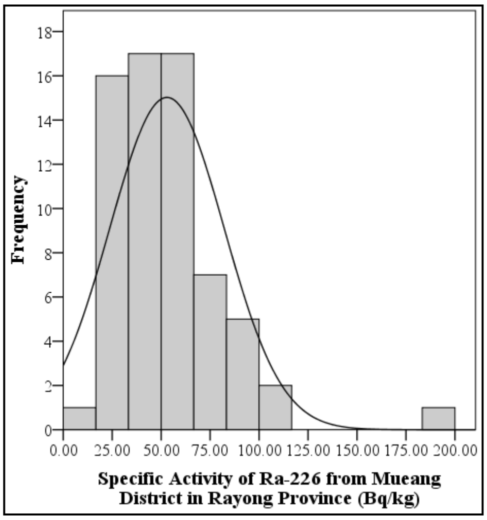
Specific activity mean values and ranges of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in 66 surface soil samples collected from Mueang district in Rayong province in eastern region of Thailand was measured and calculated by using HPGe detector and gamma spectrometry technique. All results were shown in Table 1.

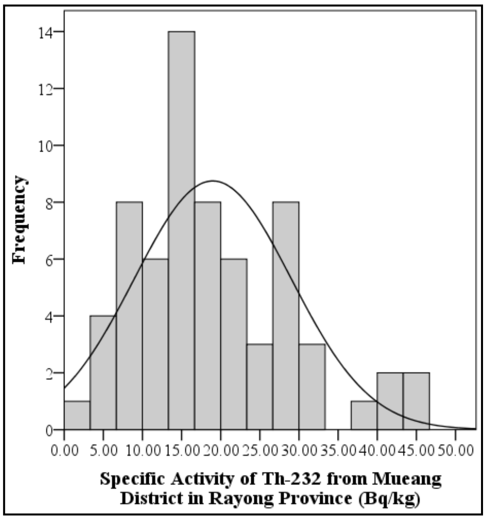
3.2 Frequency distribution of specific activities of natural and anthropogenic radionuclides

The frequency distribution of specific activities of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in 66 surface soil samples collected from Mueang district in Rayong province were studied and analyzed by using a statistic computer program and all results were presented in the following Fig. 2. Furthermore, all statistic values which were given from the studying were also shown in Table 2.

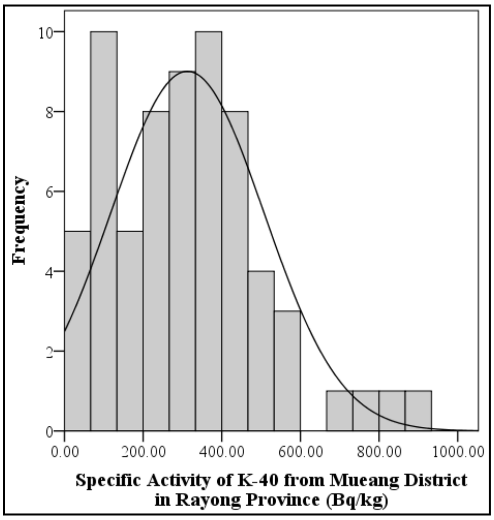
Table 1. Mean values and ranges of specific activity of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in Bq/kg which evaluated in 66 surface soil samples collected from Mueang district in Rayong province.

Surface soil samples collected from Mueang district in Rayong province (66 samples)	Specific Activities (Bq/kg)			
	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
Ranges	16.80 – 195.19	5.20 – 44.58	32.34 – 922.99	< 0.89 – 1.95
Mean values	52.64 ± 1.41	18.00 ± 0.64	319.69 ± 8.67	1.45 ± 0.40





(a) (b)



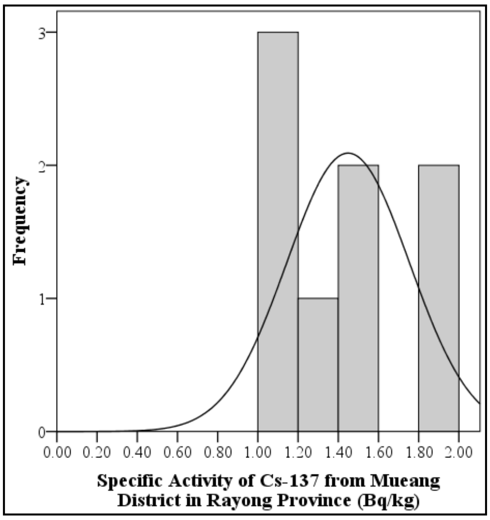


Figure 2. Frequency distribution of specific activities of (a) ^{226}Ra (b) ^{232}Th (c) ^{40}K and (d) ^{137}Cs in 66 surface soil samples collected from Mueang district in Rayong province.

Table 2. Statistic values of frequency distribution of specific activities of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in 66 surface soil samples collected from Mueang district in Rayong province (Thailand).

Statistic values	Analyzed values			
	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
Mean (Bq/kg)	52.96 ± 1.41	18.98 ± 0.65	312.56 ± 8.39	1.45 ± 0.40
Median (Bq/kg)	49.58 ± 1.38	16.67 ± 0.65	297.20 ± 8.50	1.41 ± 0.40
Mode (Bq/kg)	41.36	14.27	340.81	1.20
Skewness	1.98	0.85	0.90	0.62
Kurtosis	7.57	0.36	1.02	-0.70
Minimum value (Bq/kg)	11.44	2.89	32.34	< 0.89
Maximum value (Bq/kg)	195.19	45.17	922.99	1.95

From Fig. 2 and all calculated statistic values in Table 2, it was found that the frequency distribution of specific activities of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in 66 surface soil samples collected from Mueang district in Rayong province, were asymmetrical distribution with the skewness of 1.98, 0.85, 0.90 and 0.62, respectively. For this reason, the median values of ^{226}Ra, ^{232}Th, ^{40}K, and ^{137}Cs which were 49.58 ± 1.38, 16.67 ± 0.65, 297.20 ± 8.50 and 1.41 ± 0.40 Bq/kg, should be chosen for calculation the corresponding radiological hazard evaluation in the studied area. Moreover, the excess lifetime cancer risk ((ELCR(outdoor)) could be also evaluated by using the calculated value of the annual external effective dose rate (AED_{out}) from this study.

3.3 Comparison of the specific activities of natural and anthropogenic radionuclides to the south of Thailand and worldwide means

The median values of specific activities of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in 66 surface soil samples in the investigated area were also compared with the Office of Atoms for Peace (OAP) annual report data, Thailand and global radioactivity measurement and evaluations as shown in Table 3.

Table 3. Comparison of median values of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in 66 surface soil samples collected from Mueang district in Rayong province with some research data in Thailand, Office of Atoms for Peace (OAP) annual report data and worldwide mean.

Locations	Specific Activity (Bq/kg)			
	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
Songkhla (Ratanasumniang, et al.,2009)	107.28 ± 6.74	51.53 ± 5.02	3562.14 ± 223.56	3.05 ± 1.58
Nakhon Si Thammarat (Thongna, et al., 2009)	108.55 ± 31.19	73.95 ± 4.15	4313.18 ± 148.16	4.84 ± 2.59
Phatthalung (Daoh, et al., 2010)	135.89 ± 6.71	76.34 ± 5.32	3573.35 ± 203.89	4.00 ± 1.92
Chumphon (Chauymanee, et al., 2012)	57.32 ± 5.19	59.98 ± 4.68	2135.69 ± 168.87	2.30 ± 1.38
Surat Thani (Kessaratikoon, et al., 2013)	75.72 ± 5.75	47.39 ± 4.76	2119.10 ± 171.72	3.85 ± 1.67
Satun (Kessaratikoon, et al., 2015)	102.54 ± 7.05	124.46 ± 4.52	4146.73 ± 251.13	5.30 ± 2.30
Yala (Jahlee, et al., 2012.)	128.94 ± 7.42	85.93 ± 6.13	3607.70 ± 235.48	4.53 ± 2.24
Phuket (Kessaratikoon, et al., 2015)	240.11 ± 10.79	210.68 ± 9.89	4896.45 ± 288.74	5.91 ± 2.94
Phang-Nga (Kessaratikoon, et al., 2015)	165.44 ± 8.44	160.11 ± 7.92	2879.87 ± 225.50	5.76 ± 2.49
Krabi (Kessaratikoon, et al., 2015)	65.75 ± 4.95	40.69 ± 3.96	802.58 ± 115.25	2.96 ± 1.59
Mueang District (Rayong province)[a]	49.58 ± 1.38	16.67 ± 0.65	297.20 ± 8.50	1.41 ± 0.40
OAP (Southern region of Thailand) (OAP, 1994 – 2002)	171.55 ± 3.13	211.19 ± 1.98	511.04 ± 7.04	1.13 ± 0.49
Worldwide mean (UNSCEAR, 2000)	35	30	400	N.A.

[a] Present study

Table 4. Gamma-absorbed dose rate (D), radium equivalent activity (Ra_{eq}), external hazard index (H_{ex}), annual external effective dose rate (AED_{out}) and the excess lifetime cancer risk (ELCR(outdoor)) of 66 surface soil samples collected from Mueang district in Rayong province and compared to OAP data and UNSCEAR recommended values.

Locations	D (nGy/h)	Ra_{eq} (Bq/kg)	H_{ex}	AED_{out} (mSv/y)	ELCR ($\times 10^{-3}$)
Mueang District Rayong Province	45.54 ± 1.39	96.30 ± 2.95	0.26 ± 0.01	0.06 ± 0.01	0.21 ± 0.01
OAP Data	231.81 ± 2.97	512.90 ± 6.50	1.39 ± 0.02	0.28 ± 0.01	1.07 ± 0.01
UNSCEAR (UNSCEAR, 2000,1988,1993)	55	370	1	0.48	1.8

From the result in Table 3, the value of specific activities of ^{226}Ra in 66 surface soil samples collected from Mueang district in Rayong province was only higher than the worldwide mean value and the lowest value compared to every provinces and OAP data. The value of specific activities of ^{232}Th and ^{40}K in 66 surface soil samples collected from Mueang district in Rayong province were the lowest value compared to every provinces, OAP data and worldwide mean value. Furthermore, the value of specific activities of ^{137}Cs was only higher than the OAP data and the lowest value compared to every provinces. Moreover, we could also see that the value of specific activities of ^{226}Ra which might be directly caused from oil and gas industry in Mueang district in Rayong province, was comparable and close to the value in Chumporn, Surat Thani and Krabi provinces and about 1.42 time higher than the worldwide mean value.

3.4 Radiological hazard indices and excess lifetime cancer risk evaluation and comparison

By using the median values of specific activities of ^{226}Ra, ^{232}Th and ^{40}K as presented in Table 2 and Table 3, four radiological hazard indices which are the gamma-absorbed dose rate (D), the radium equivalent activity (Ra_{eq}), the external hazard index (H_{ex}), the annual external effective dose rate (AED_{out}) and the excess lifetime cancer risk (ELCR(outdoor)) were evaluated for the investigated area by using equations (1) to (5). Then, all of those calculated values were compared to OAP data and UNSCEAR data and presented in Table 4.

From Table 4, we can see that the average values of all four radiological hazard indices and the excess lifetime cancer risk (ELCR(outdoor)) in the investigated area were lower than the OAP data and UNSCEAR recommended values. Furthermore, the gamma-absorbed dose rate (D) in this area was close to theUNSCEAR recommended values (about 0.83 times) but the annual external effective dose rate (AED_{out}) was lower than the UNSCEAR recommended values (about 8.0 times). We can realize that the studied area is the safe place and have no danger to people or tourists who came to visit.

4. Conclusions

According to the asymmetrical distribution of the specific activities of natural (^{226}Ra, ^{232}Th and ^{40}K) and anthropogenic (^{137}Cs) radionuclides in 66 surface soil samples collected from Mueang district in Rayong province, the median values of those radionuclides were selected and used to be the study representative values. Hence, the specific activities of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs radionuclides in the investigated area were 49.58 ± 1.38, 16.67 ± 0.65, 297.20 ± 8.50 and 1.41 ± 0.40 Bq/kg, respectively. It was found that the values of the specific activities of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs radionuclides were mostly lower than Thailand, OAP annual report data and worldwide values. The specific activity of ^{226}Ra which created from the oil and gas industry in this area, was about the same value of the worldwide mean value. The ^{226}Ra concentrations in the surface soil samples collected from Mueang district in Rayong province, could be generated and enhanced during the oil and gas production in this area (Baxter, 1993; Zambelli, et al., 2014). For this reason, the monitoring plan on ^{226}Ra concentration in this area should be initiated and organized. The value of specific activities of natural and anthropogenic radionuclides that have been evaluated from the surface soil samples collected from Mueang district in Rayong province show that none of the studied samples is considered a radiological hazard. Furthermore, four radiological hazard indices which are the gamma-absorbed dose rate (D), the radium equivalent activity (Ra_{eq}), the external hazard index (H_{ex}), the annual external effective dose rate (AED_{out}) and the excess lifetime cancer risk (ELCR(outdoor)) in the investigated area were also calculated and equal to 45.54 ± 1.39 nGy/h, 96.30 ± 2.95 Bq/kg, 0.26 ± 0.01, 0.06 ± 0.01 mSv/y and $(0.21 \pm 0.01)\times 10^{-3}$, respectively. From this study, the annual external effective dose rate (AED_{out}) and the excess lifetime cancer risk (ELCR(outdoor)) values were equal to 0.06 ± 0.002 mSv/y and (0.21 ± 0.01), respectively. These two values were relatively lower than the worldwide recommended values. Therefore, people who usually live or spend time in the investigated area should not get the radiological hazard impact to their health. However, long-term exposure to radiation is assumed to have some risks of causing cancer. This means that all people have a risk of getting cancer. The excess lifetime cancer risk (ELCR(outdoor)) is additional risk that someone might have of getting cancer if that person is exposed to cancer-causing materials for a longer time (Qureshi, et al., 2014). For this reason, the results of this study could be considered and used to be the baseline reference of background radiations for the studied area in the future.

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Bacterial Growth Competition and Their Nitrate Reduction End-Products in the Steady State Chemostat of Nitrate Reducing Bacteria Isolated from Estuarine Sediments

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Abstract

Denitrification and nitrate-ammonification are the responsible processes for nitrate removal in the estuaries. Temperature, nitrate and organic carbon availability are key factors controlling rate of the processes. This mixed cultures chemostat study investigates the competition ability and their nitrate reduction end-products of the bacteria isolated from an estuary at different temperatures. This study will help us to understand the seasonal nitrate reduction processes in an estuary. The experiments showed that a nitrate-ammonifier was the predominant process in the steady state chemostat at high temperature. While, a facultative denitrifier-nitrate ammonifier was the predominant process at low temperature. However the main end products of nitrate reduction at high temperature were up to 61% N_2 indicating a denitrifier still had an important role in the end products of nitrate reduction in the estuary. The data also showed that a nitrite respiring bacterium reduced nitrite to N_2, that responsible for approximately 6-9% of total N_2 produced in the culture. This study confirmed that nitrate ammonifiers out compete denitrifiers at high temperature, however denitrifiers still had an important role in end products of nitrate reduction.

Keywords: Nitrate reduction/ Temperature/ Denitrification/ Nitrate-ammonification/ Nitrite respiring/ Estuary

1. Introduction

Denitrification and nitrate-ammonification are the competing processes of microbial nitrate-reduction processes in the estuaries. These processes are the responsible processes for nitrate removal in the estuarine. Nitrate and organic carbon availability are key factors controlling rates of theses processes (Dong et al., 2000; Fulweiler and Heiss, 2014). Denitrification is a reducing process of nitrate to N_2O or N_2 gas. And nitrate ammonification is a process of reducing nitrate to nitrite and subsequently to ammonium. This process accumulates high nitrite and also produces N_2O as a byproduct. Some fermenting bacteria such as *Bacillus licheniformis, Citrobacter freundii, Klebsiella oxytoca, K. pneumoniae,* and *Echerichia coli* have been reported having ability to perform this process. However some strains of *Shewanella sp*. and *Serratia* sp. can possess both the denitrification and nitrate ammonification (Yoon et al. 2014).

Temperature could also affect the competition ability of these bacterial processes. Denitrification is the dominant process at low temperature (winter), and nitrate reduction to ammonium at high temperature (summer). While in tropical estuaries, nitrate ammonification dominates over denitrification (Dong et al. 2011). In sandy tidal sediment increasing of carbon to nitrate ratio, the supply of nitrite relative to nitrate and the microbial generation time determine whether nitrate is reduced to gaseous end products or to ammonium (Kraft et al. (2014). Only when nitrate was added instead of nitrite at a relatively high generation time, nitrate ammonification availed; otherwise denitrification dominated. (Bu, et al. 2017). Relationship between nitrate ammonifiers and organic matter and NO2−, suggesting that these substrates stimulated the metabolism of nitrate ammonifying bacteria and most of the converted ammonium was retained in the estuarine ecosystem (Yin et al. 2017).

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Some denitrifiers and nitrate ammonifiers have been isolated from estuarine sediment i.e. a nitrate ammonifier (*K. pneumoniae)*, a facultative denitrifier-nitrate ammonifier (*S. liquefaciens)*, a nitrite-respiring bacterium (*Alcaligenes faecalis)* and a denitrifier (*Comamonas testosteroni)* (Rusmana, 2006; 2007). Therefore this pure culture study investigates the growth competition between the bacteria carrying out these processes. It could help us to understand the growth and end-products of nitrate reduction in the estuaries.

2. Methodology

2.1 Bacterial isolates

Four bacteria representing a denitrifier (*C. testosteroni*), nitrate ammonifier (*K. pneumoniae*), a facultative denitrifier-nitrate ammonifier (*S. liquefaciens,*), and a nitrite-respiring bacterium (*A. faecalis*) were used to determine their competition ability for nitrate in steady state at 5, 10, 15, and 20°C of nitrate-limiting chemostat with mixed cultures of (1) a denitrifier and a nitrate ammonifier, (2) a denitrifier, a nitrate ammonifier, and a facultative denitrifier-nitrate ammonifier (3) a denitrifier, a nitrate ammonifier, a facultative denitrifier-nitrate ammonifier, and a nitrite respiring bacterium.

2.2 Chemostat conditions

The chemostat experiments were conducted using nitrate-limiting medium. The culture was conducted at four different temperatures, 5, 10, 15, and 20°C, using a 500 ml glass vessel sealed with a rubber bung (Rusmana, 2006). A peristaltic pump (multiperpex; LKB, Broma, Sweden) was adjusted to pump a sterile medium with dilution rate 0.02 h^{-1} for incubation for temperatures of 10, 15 and 20°C, and 0.01 h^{-1} for temperature of 5°C. Anaerobic condition was maintained by gassing the vessel and medium with sterile oxygen-free nitrogen (OFN). The OFN flow rate was 6 ml min^{-1} and the OFN was passed through a chromous acid trap to remove out any trace amount of O_2. The temperature in the glass vessel was adjusted and maintained by circulating a coolant liquid passed through a glass jacket around the growth vessel that regulated by a thermo-circulator (Grant CFC25, Grant instruments, Cambridge Ltd.) attached to a FH15 flow heater (Grant instruments, Cambridge Ltd.).

The medium was used with addition of 5560 μM glycerol, as *K. pneumoniae* and *S. liquefaciens* could not use acetate as their carbon source. The microbial growth was monitored by measuring the OD with a spectrophotometer at 550 nm. Steady state was achieved when the standard deviation (SD) at least six OD readings was <2% of the mean OD value. The culture samples at steady state were collected aseptically for measuring the total number of bacteria, nitrate, nitrite, ammonium, glycerol, and acetate concentrations. Headspace gas samples were also taken for analyzing N_2O concentrations.

2.3 Counting of bacterial numbers

The total number of bacteria was determined using a plate counts technique on nutrient agar, supplemented with 10 mM KNO_3 and 15%o NaCl. The steady state samples were diluted serially with ¼ strength Ringer's solution, and 0.1 ml of suitable dilutions was plated in triplicate on the agar plates and incubated at 20°C for 3 days. The bacterial pure cultures of C. testosteroni, K. pneumoniae, S. liquefaciens, and A. faecalis, were also plated on the agar plates, and used as colony morphology references to determine the colonies for each species. Each bacterium could be distinguished from others; C. testosteroni developed cream, translucent, circular, pulvinate colonies; K. pneumonaie developed whitish cream, opalescent, circular, convex colonies; S. liquefaciens developed reddish cream, translucent, round, convex colonies; and A. faecalis developed whitish cream, circular, translucent pinhead colonies.

2.4 Chemical analyses

Nitrate, nitrite and ammonium were analysed colourimetrically (Parson et al., 1984). Nitrate was analyzed as nitrite after the samples were passed through a copper-cadmium reduction column (Parson et al, 1984). Ammonium was measured using a modified indophenol blue method with dichloroisocyanurate as chlorine donor and developed in the dark (Harwood and Kuhn, 1970; Gravitz and Gleye, 1975; Krom, 1980).

The N_2O concentration was analysed using a gas chromatograph (Shimazu GC 14A, Dyson, Instruments, Washington, U.K.) with a ^{36}Ni electron capture detector (ECD) at 340°C, with argon-methane carrier gas (50 ml min^{-1}, 95% Ar, 5% CH_4).

The temperature of the internal main stainless steel column (4 m x 2 mm i.d.) packed with Porapak Q (60-80 mesh, Millipore Corporate, Milliford, U.K.) was set up at 25ºC.

3. Results

3.1 Bacterial growth competition

Bacterial cell concentration in mixed cultures of nitrate limited chemostats showed that the nitrate amonifier (*K. pneumonia)* was always dominant at high temperature in all mixed cultures of nitrate limited chemostats and increased by increasing the temperatures (Figure 1). The percentages of the population dominance were also increased by increasing the temperatures. However the percentages of its dominance was decreased by increasing the number of bacterial types of nitrate methabolisms in the mixed culture chemostats. At the highest temperature 20ºC, its population percentage was 98% in the mixed culture of a nitrate amonifier and a denifier, decresing to 80% in the mixed culture of a nitrate amonifier, a denifier and a facultative denitrifier-nitrate ammonifier, the to 49% in the mixed culture of a nitrate amonifier, a denifier, and a facultative denitrifier-nitrate ammonifier (Figure 2; 3). In the contrary, the denifier (*C. testosteroni*), was always at the lowest bacterial population (for about 1-3% in the population) in all conditions of temperatures and bacterial types number of of nitrate methabolisms in the mixed culture chemostats.

While the facultative denitrifier-nitrate ammonifier (*S. liquefaciens*) was dominant at low temperature. Its cell density and percentage of bacterial dominance was decreased by increasing the temperatures. At the lowest temperature (5ºC). its population percentage was 89% in the mixed cultures of a nitrate amonifier, a denifier and a facultative denitrifier-nitrate ammonifier and 61.9% in the mixed culture of a nitrate amonifier, a denifier, a facultative denitrifier-nitrate ammonifier and a nitrite respiring bacterium (Figure 2; 3).

Moreover, bacterial cell density and population percentage of an obligate nitrite utiliser (*A. faecalis*) was increased by increasing the temperatures. It was a similar tend with that of the nitrate ammonifier, although with lower bacterial cell density and population percentage (Figure 3).

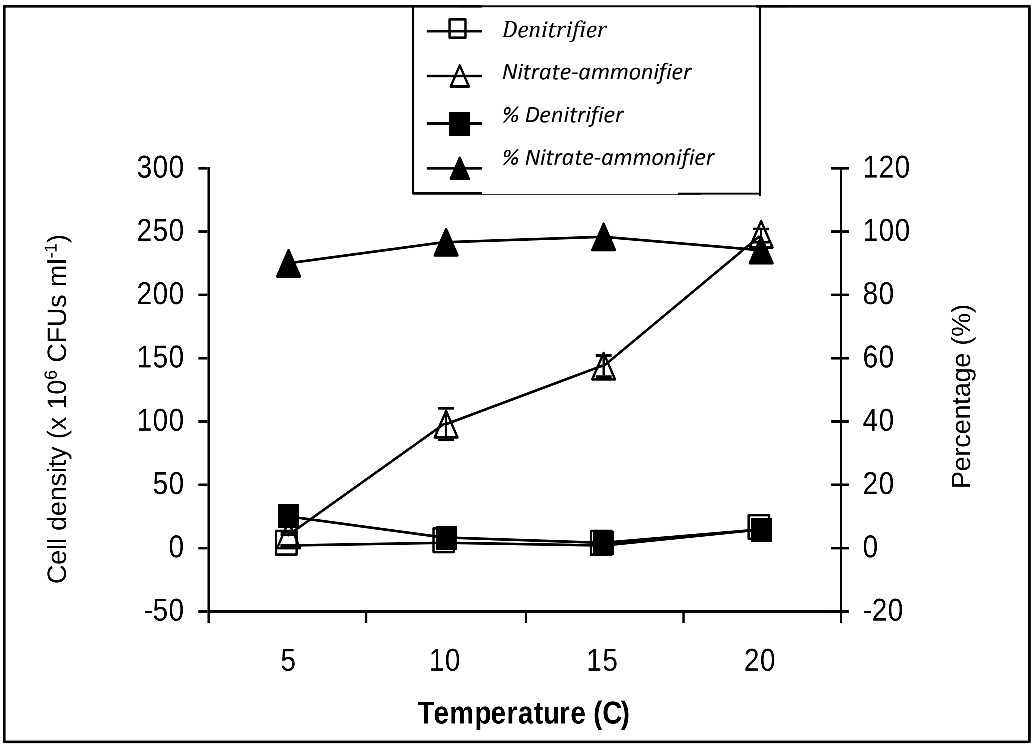


Figure 1. Cell density and percentage of a denitrifier and a nitrate-ammonifier, from the steady state of mixed culture chemostats at 5, 10, 15, and 20ºC. Bars indicate standard errors

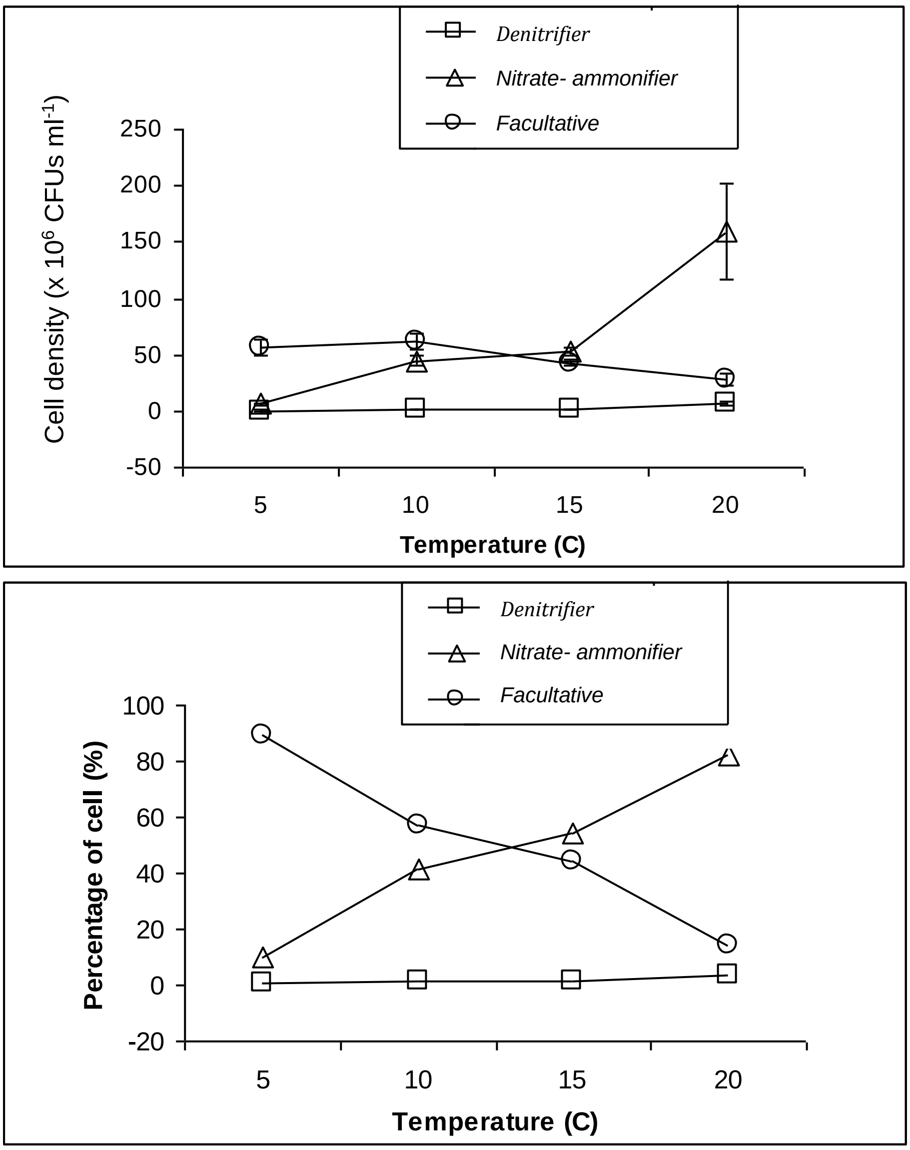


Figure 2. Cell density and percentage of a denitrifier, a nitrate-ammonifier, and a facultative denitrifier-nitrate ammonifier from the steady state of mixed culture chemostats at 5, 10, 15, and 20°C. Bars indicate standard errors.

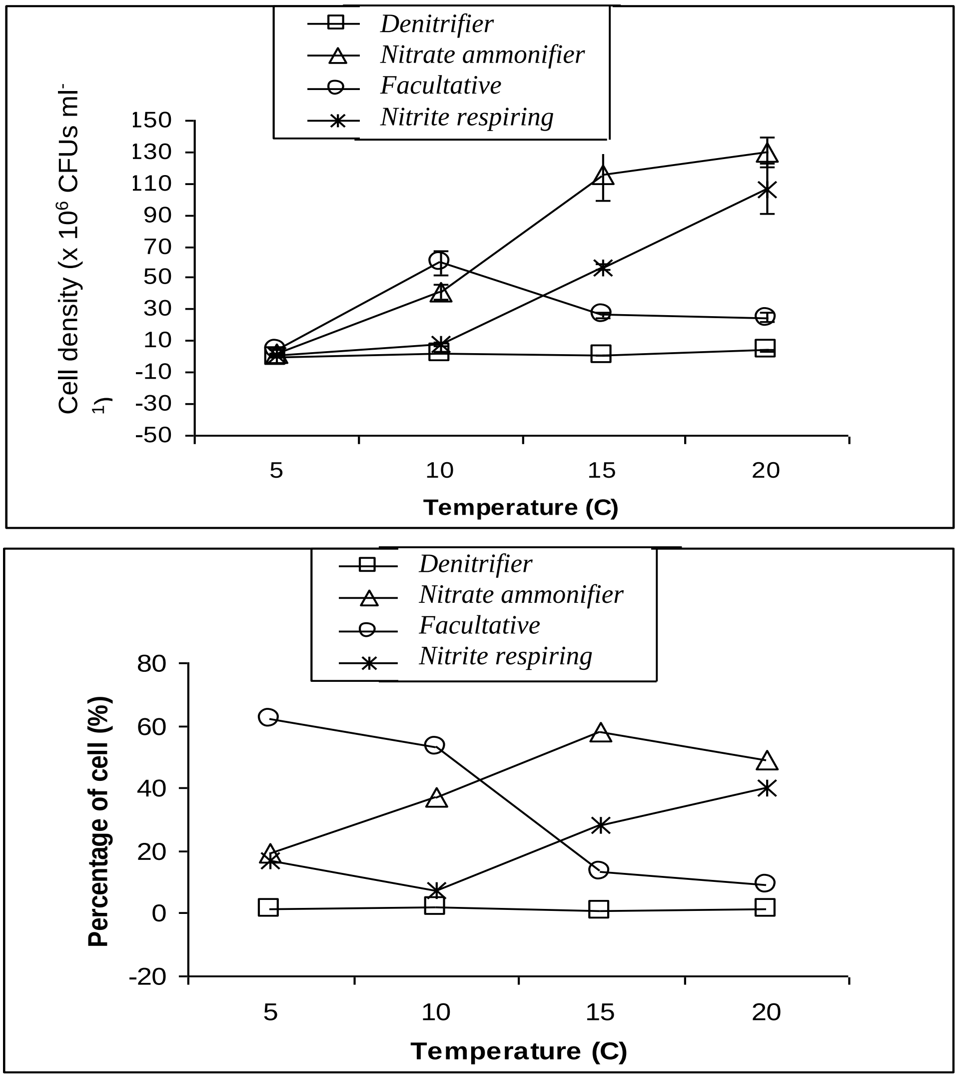


Figure 3. Cell density and percentage of a denitrifier, a nitrate-ammonifier, a facultative denitrifier-nitrate ammonifier and a nitrite respiring bacterium from the steady state of mixed culture chemostats at 5, 10, 15, and 20°C. Bars indicate standard errors.

3.2 End-products of nitrate reduction

The all mixed cultures utilized all nitrate (100%) in the steady state chemostat at the temperature of 10, 15, and 20°C. However at low temperature (5°C) , 88% nitrate was utilized in the mixed culture of f a nitrate amonifier and a denifier, increasing to 91.5% in the mixed culture of a nitrate amonifier, a denifier and a facultative denitrifier-nitrate ammonifier, then to 97.2% in the mixed culture of a nitrate amonifier, a denifier, a facultative denitrifier-nitrate ammonifier and a nitrite respiring bacterium.

Nitrite accumulation was high at low temperature (5°C), and the highest nitrite accumulation (5.9% of reduced nitrate) in the mixed culture of a nitrate ammonifier. However nitrite accumulation at the other temperatures was low (0.01-0.022% of reduced nitrate) in all mixed cultures (Table 1, 2, 3).

Similarly, ammonium production was high at low temperature (5°C) and the highest ammonium production (51.2% of reduced nitrate) was found in the mixed culture of a nitrate amonifier, a denifier and a facultative denitrifier-nitrate ammonifier. The trend of ammonium production was decreased by increasing temperature in all mixed cultures. The nitrogen assimilated to be organic compounds of bacterial cells was increased by increasing the temperatures. Percentages of assimilated nitrogen were 3-6% of reduced nitrate at 5°C, and 39-49%, 44-62% and 38-48% at 10°C, 15°C and 20°C respectively (Table 1, 2, 3).

Table 1. Concentrations of nitrate reduced and percentage recovery of nitrate reduction end-products during mixed cultures of a denitrifier,and a nitrate-ammonifier in steady state nitrate-limited chemostats at 5, 10, 15, and 20°C

Temperature (°C)	Steady state concentrations of								NO_3^- recovery (%)	Cellular N (µM)	N_2 (%)
	NO_3^- reduced		NO_2^- produced		NH_4^+ produced		N_2O Produced				
	(µM)	(%)	(µM)	(%)	(µM)	(%)	(µM)	(%)			
5	968.25 ± 78.4	88.02	57.30 ± 16.68	5.92	361.53 ± 59.32	37.34	0.10 ± 0.005	0.01	47.67	42.65 ± 4.48	52.33
10	1100 ± 0	100	0 ± 0	0.00	319.09 ± 17.08	29.00	0.25 ± 0.06	0.02	39.66	17.08 ± 6.89	60.34
15	1100 ± 0	100	1.93 ± 0.04	0.17	301.97 ± 43.46	27.45	0.19 ± 0.005	0.02	44.11	181.18 ± 2.21	55.89
20	1100 ± 0	100	1.47 ± 0.65	0.13	344.92 ± 28.61	31.35	1.41 ± 0.03	0.13	48.36	184.28 ± 4.78	51.64

Table 2. Concentrations of nitrate reduced and percentage recovery of nitrate reduction end-products during mixed culture of a denitrifier, a nitrate-ammonifier, and a facultative denitrifier-nitrate ammonifier in steady state nitrate-limited chemostats at 5, 10, 15, and 20°C

Temperature (°C)	Steady state concentrations of								NO_3^- recovery (%)	Cellular N (µM)	N_2 (%)
	NO_3^- reduced		NO_2^- produced		NH_4^+ produced		N_2O Produced				
	(µM)	(%)	(µM)	(%)	(µM)	(%)	(µM)	(%)			
5	1006.5 ± 65.6	91.50	2.25 ± 0.72	0.22	515.36 ± 96.07	51.20	0.24 ± 0.01	0.02	51.84	64.53 ± 3.80	42.16
10	1100 ± 0	100	0 ± 0	0.00	319.72 ± 22.33	29.06	0.23 ± 0.01	0.02	48.87	217.74 ± 18.77	51.13
15	1100 ± 0	100	2.07 ± 0.16	0.19	428.02 ± 49.34	38.91	0.31 ± 0.003	0.03	62.16	253.30 ± 9.01	37.84
20	1100 ± 0	100	1.85 ± 0.34	0.17	274.54 ± 23.80	24.96	1.35 ± 0.02	0.12	44.64	213.35 ± 6.76	55.36

Table 3. Concentrations of nitrate reduced and percentage recovery of nitrate reduction end- products during mixed cultures of a denitrifier, a nitrate-ammonifier, a facultative denitrifier-nitrate ammonifier and a nitrite respiring bacterium in steady state nitrate-limited chemostats at 5, 10, 15, and 20°C

Temperature (°C)	Steady state concentrations of								NO_3^- recovery (%)	Cellular N (µM)	N_2 (%)
	NO_3^- reduced		NO_2^- produced		NH_4^+ produced		N_2O Produced				
	(µM)	(%)	(µM)	(%)	(µM)	(%)	(µM)	(%)			
5	1069.3 ± 36.8	97.21	14.16 ± 4.85	1.32	513.38 ± 41.33	48.01	0.54 ± 0.02	0.05	52.68	35.32 ± 5.28	47.32
10	1100 ± 0	100	0 ± 0	0.00	273.50 ± 18.74	24.86	0.65 ± 0.02	0.06	39.03	155.22 ± 18.43	60.97
15	1100 ± 0	100	1.78 ± 0.08	0.16	380.00 ± 30.69	34.54	0.17 ± 0.005	0.01	53.84	210.53 ± 0.94	46.16
20	1100 ± 0	100	1.35 ± 0.11	0.12	239.22 ± 20.05	21.74	0.29 ± 0.005	0.02	38.55	183.41 ± 1.99	61.45

N_2O gas was produced in a small amount, 0.01 – 0.13% of utilized nitrate. However there was indication that N_2O gas production was increased by increasing temperatures in the mixed cultures without a nitrite respiring bacterium, but the opposite trend was found in the mixed culture with a nitrite respiring bacterium (Table 1, 2, 3). By calculation, the nitrogen recovery from the reduced nitrate was 38 up to 62% that estimation of the nitrate reduced into N_2 was 38 up to 62%. There was no trend indication of percentage of the nitrate reduced into N_2 regarding temperature changes. The average of the estimated percentage for all temperatures in each mixed culture was 46.6-55.0%.

4. Discussion

The competition experiments between a denitrifier and a nitrate ammonifier showed that nitrate ammonifier was the dominant species in the steady state chemostats at all temperatures. In substrate limited condition the ability of one species to compete to others depend on the ability of the species to sequester a substrate/nutrient. The indicator of species ability to sequester a substrate is the specific affinity (a_A), termed as μ_{max}/K_s (Nedwell, 1999a; 1999b). The specific affinity for nitrate of *Klebsiella pneumoniae* (a nitrate ammonifier) with glycerol as a substrate is more than 10 times that of *Comamonas testosteroni* (a denitrifier) with acetate. Specific affinity for nitrate is affected by the environmental temperature and the type of carbon sources used (Nedwell, 1999b). The big differences of the a_A values of the nitrate ammonifier made it out compete the denitrifier. However, the nitrate amminifier did not completely exclude the denifier. The main end products from nitrate reduction in the chemostat mixed cultures of a denitrifier and a nitrate-ammonifier were average 55% N_2, 30% ammonium and 0.01% nitrite at 10–20°C; and 52% N_2, 37% ammonium, and 6% nitrite at 5°C. Ammonium production in the mixed culture chemostat at 10, 15, and 20°C was lower than that of a culture of a nitrate-ammonifier alone (Lloyd, 2000) indicating that the denitrifier has an effect in the balance of end products when in co-culture. One possible way was that the denitrifer denitrified nitrite to N_2 (Rusmana, 2006). The same indication was also shown in nitrite production at 5°C. Nitrite production in mixed culture chemostat was decreased up to 77% of than that of a culture of a nitrate-ammonifier alone (Lloyd, 2000). These results indicated that the denitrifier still has an important role in influencing the end product of nitrate reduction, although it was not a dominant species in the community. The denitrifier could maintain in the steady state probably by using nitrite accumulated from nitrate reduction by the nitrate-ammonifier. Nitrate acculation by the nitrate ammonifier due to thermodynamically, nitrate reduction to nitrite is more efficient to gain energy than to ammonium. The ΔG^o per mole of H_2 at pH 7 of nitrate reduction to nitrite is more negative than nitrate reduction to ammonium or nitrite reduction to ammonium as shown below:

$$NO_3^- + H_2 \rightarrow NO_2^- + H_2O$$
$$(\Delta G^o = -163.03 \text{ KJ per mole } H_2)$$

$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$$
$$(\Delta G^o = -149.97 \text{ KJ per mole } H_2)$$

$$NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$$
$$(\Delta G^o = -145.95 \text{ KJ per mole } H_2)$$

Table 4. Specific affinity values for nitrate of *Klebsiella pneumonaie* with glycerol as a substrate and *Comamonas testosteroni* with acetate as a substrate.

Species	Specific affinity for nitrate (l µmol^{-1} h^{-1}) at			Reference
	10°C	15°C	20°C	
Klebsiella pneumonaie	0.00114	0.00177	0.00221	Lloyd, 2000
Comamonas testosterone	0.00006	0.00009	0.00021	Rusmana, 2007

Nitrite, on the other hand, is also more favorable as an electron acceptor for denitrification than nitrate as shown on the following free energy (ΔG^o) values per mole H_2 at pH 7 as shown below:

$$2NO_2^- + 3H_2 + 2H^+ \rightarrow N_2 + 4H_2O$$
$$(\Delta G^o = -265.21 \text{ KJ per mol } H_2)$$

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$$
$$(\Delta G^o = -224.33 \text{ KJ per mol } H_2)$$

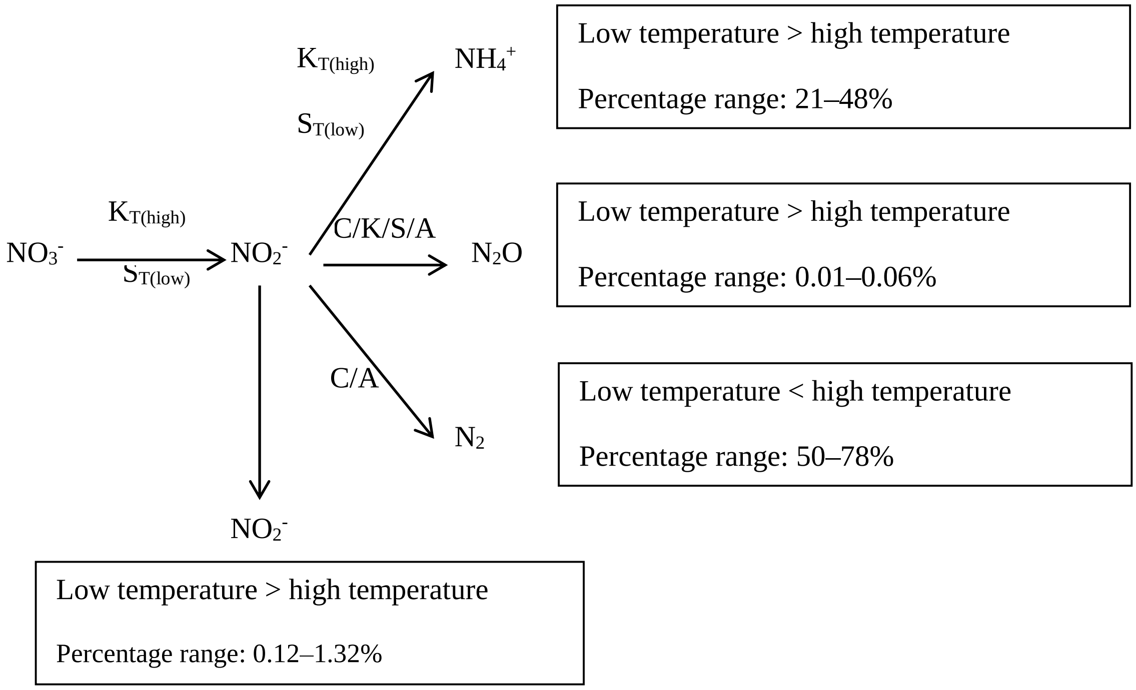
N_2O production at low temperature was higher than that of high temperature. The production was only a small proportion of the nitate reduced, between 0.01-0.13%.

The next experiment competing a denitrifier, nitrate-ammonifier and facultative denitrifier-nitrate ammonifier (*S. liquefaciens*) showed that the nitrate-ammonifier was again the dominant species in the steady state chemostat at high temperature (20°) but acultative denitrifier-nitrate ammonifier was the dominant species at low temperature (5°C). While the denitrifier was still out competed, but it was not excluded completely from the chemostat. Probably the denitrifier could maintain its presence in the chemostat by using nitrite with similar indication as described above. The specific affinity for nitrate of the facultative denitrifier-nitrate ammonifier at low temperature was higher (10°C, $a_{A(NO3^-)}$=0.00265 l μmol^{-1} h^{-1}) than that of the nitrate ammonifier (10°C, $a_{A(NO3^-)}$=0.00114 l μmol^{-1} h^{-1}) (Nedwell, 1999b). At high temperature, on the contrary, the nitrate ammonifier had higher specific affinity for nitrate (20°C, $a_{A(NO3^-)}$=0.00221 l μmol^{-1} h^{-1}) than the facultative denitrifier-nitrate ammonifier (20°C, $a_{A(NO3^-)}$=0.00005 l μmol^{-1} h^{-1}) (Nedwell, 1999b). The environmental temperature affects the specific affinity for substrate due to its effect on active transport of substrates (Nedwell 1999). Nitrate is taken into cells by active transports whether using nitrate/nitrite antiporter mechanism via NarK2 or nitrate/proton symporter mechanism via NarK1 (Wood et al., 2002). Temperature affects these transporters probably on a charge energetic state for powering conformational change, and the fluidity of the membrane (Nedwell, 1999b). Therefore the specific affinity for nitrate in bacteria decreases along with decreasing environmental temperature (Nedwell 1999). The present data support that hypothesis as $a_{A(NO3-)}$ generally decreases with temperature. However, the facultative denitrifier-nitrate ammonifier has a high specific affinity for nitrate than the other bacteria at low temperature, possibly because the bacterium has two different nitrate reductase enzymes; Nar and Nap, that were determined the presence of *nar*G and *nap*A genes (unpublished data). The activity of Nar depends on nitrate transport by NarK1 or NarK2 because the active site of this enzyme is in the cytoplasm (Moreno-Vivian et al. 1999; Richardson, 2000). The repression by low temperature on nitrate uptake is probably due to this transport enzyme activity, similar to that inhibition shown in the nitrate mmonifier. Molecular detection of the nitrate reductase gene presence showed that the bacterium has only *nar*G (unpublished data). Nap, in contrast, does not need nitrate transporters for its activity, because the active site of this enzyme is in the periplasm (Moreno-Vivian et al., 1999; Richardson, 2000). Nap activity is more dominant in low nitrate conditions than that of Nar (Potter et al., 1999); Richardson, 2000). Therefore the high specific affinity for nitrate of the facultative denitrifier-nitrate ammonifier at low temperature than that of the nitrate ammonifier may due to this Nap activity.

The main end products from nitrate reduction in this chemostat mixed culture were approximately 37-55% N_2, 35-49% ammonium and low nitrite 0.0-0.19% at 10-20°C, and 42% N_2, 51% ammonium and 0.22% nitrite at 5°C. Ammonium production in this mixed culture chemostat at 10, 15, and 20°C was lower than in the mixed culture of the nitrate ammonifier and the facultative denitrifier-nitrate ammonifier (Lloyd, 2000) indicating that the denitrifier denitrified nitrite to N_2 (Rusmana, 2006; 2007). At low temperature, nitrite productions in the three bacterial components mixed culture chemostats were decreased by between 40-90% of that that of in the mixed cultures of the nitrate ammonifier and the facultative denitrifier-nitrate ammonifier (Lloyd, 2000) at 5 and 10°C respectively. These results indicated that the denitrifier still had an important role influencing the end product of nitrate reduction although it was not a dominant species in the community, as described previously. N_2O production was only in a small amount, 0.02% for all temperature.

Similar trends population dominance for the denitrifier, the nitrate ammonifier and the facultative denitrifier-nitrate ammonifier were found in the in the steady state chemostat mixed culture of the denitrifier, nitrate ammonifier, the facultative denitrifier-nitrate ammonifier and the nitrite respiring bacterium (*A. faecalis*). while the the nitrite resipiring bacterium had similar trends with the nitrate ammonifier that the bacterial cells were increased by increasing temperature indicating that there was a commensally interaction between the nitrate ammonifier and the nitrite resipiring bacterium. The the nitrite resipiring bacterium used nitrite produced by the nitrate ammonifier.

The main end products from nitrate reduction in this chemostat mixed culture were approximately 46-61% N_2 and 22-34% ammonium at 10-20°C, and 47% N_2 and 48% ammonium at 5°C. Compared with previous the mixed culture experiment without the nitrite resipiring bacterium, N_2 production was increased by approximately 6-9% at 10-20°C. In contrast, ammonium production in the mixed culture chemostats at 10, 15, and 20°C was decreased approximately 13-15% compared to mixed cultures without the nitrite resipiring bacterium. These results again suggested that the nitrite resipiring bacterium reduced nitrite to N_2. Molecular analyses of the nitrate reduction genes showed that the nitrite resipiring bacterium (*A. faecalis)* has NapA, NirK, and NosZ (unpublished data) indicating that this nitrite-respiring bacterium can reduce nitrite into N_2. Nitrite productions at low temperature was higher that that of high temperature. The nitrite production in steady state chemostats was between 0.12-1.32%. N_2O production at low temperature was higher than that of high temperature. The production was only in a small amount, between 0.01-0.06%. So that the nitrate reduction mechanisms and end products of this mixed culture are probably as a following scheme:



Where K is a reduction by the nitrate ammonifier, C is a reduction by the denitrifier, S is a reduction by the facultative denitrifier-nitrate ammonifier, A is a reduction by the nitrite respiring bacterium, $K_{T(high)}$ is a reduction by the nitrate ammonifier at high temperatures, and $S_{T(low)}$ is a reduction by the facultative denitrifier-nitrate ammonifier at low temperatures.

Predictions of seasonal dissimilative nitrate reduction process outcome that occur in natural environments are important to anticipate future implications of global warming as denitrification and nitrate ammonification can produce N_2O. This competition experiments for nitrate by pure cultures demostrated that the nitrate ammonifier was a predominant species in the steady state chemostat at high temperature (20°C). In contrast, the facultative denitrifier-nitrate ammonifier was the predominant species at low temperature (5°C). Specific affinity for nitrate ($a_{A(NO3-)}$) is a suitable parameter to predict the competition ability of one species to others. The specific affinity for nitrate of the nitrate ammonifier was higher at high temperature than that of the facultative denitrifier-nitrate ammonifier. On the contrary, the facultative denitrifier-nitrate ammonifier had a higher $a_{A(NO3^-)}$ value at low temperature. Environmental temperature can affect the specific affinity for nitrate due to its effect on active transport of nitrate probably on a charge energetic state for powering conformational change, and the fluidity of the membrane (Nedwell, 1999b), so that nitrate transport into cytoplasmic cells via NarK1, a nitrate/proton symporter, or NarK2, a nitrate/nitrite antiporter, (Wood et al., 2002) is repressed. As a result, the specific affinity values decrease along with decreasing environmental temperature.

Interestingly, the facultative denitrifier-nitrate ammonifier had a higher specific affinity for nitrate than the other bacteria at low temperature. Molecular study of dissimilative nitrate reductase genes of the bacteria (unpublished data) demonstrated that this bacterium had *nar*G and *nap*A genes which encode membrane-bound nitrate reductase (Nar) and periplasmic nitrate reductase (Nap) respectively, meanwhile the nitrate ammonifier had only *nar*G gene. Smith et al. (2015) suggested that nitrate reduction might correlate with genetic potentials. Correspondence between rate processes and gene abundances and expression is likely to be close. Furthermore other physical-chemical factors such as temperature may control key enzyme activity, and in-situ rates, without necessarily directly affecting the genes expression (Smith et al. 2015), especially *nap* and *nar* genes.

The active site of Nar is in the cytoplasm, therefore Nar activity depends on nitrate transport by NarK1 or NarK2 (Moreno-Vivian et al., 1999; Richardson, 2000; Wood et al., 2002). The low temperature repression of nitrate uptake is probably due to this Nar activity, similar to that repression in the nitrate ammonifier. However, Nap does not need nitrate transporters for its activity, because the active site of this enzyme is in the periplasm (Moreno-Vivian et al., 1999; Richardson, 2000), so that the high specific affinity for nitrate at low temperature in the facultative denitrifier-nitrate ammonifier might be due to this Nap activity. This result indicated that during the winter Nap activity may be higher than Nar, and it can be expected that bacteria carrying out Nap such as *S. liquefaciens* and *Shewanella* will be the predominant species in the winter season. It also becomes understandable why N_2O production was higher in the winter, probably because bacteria like *Shewanella* which produced N_2O as the end product of nitrate or nitrite reduction (Rusmana, 2006) was dominant in the community. However, it is still unclear whether there is any physiological correlation between Nap activity and N_2O production in the winter (low temperature).

Some researchers (Richardson et al., 1988; Berk et al., 1995; Sear et al., 1997; Richardson, 2000; Richardson et al., 2001) believe that Nap is a dissimilatory enzyme to regulate redox balancing, so that the bacteria can maintain and support optimal growth under some physiological conditions, such as during fermentative process in enteric bacteria and oxidative metabolism of highly reduced carbon substrates (Zumft, 1997; Richardson, 2000). Other proposed roles of Nap are for a transition adaptation to anaerobic metabolism from aerobic conditions (Richardson et al., 2001), or for a self-defense mechanism by forming high nitrite levels to inhibit the growth of potential competing bacteria (Richardson, 2000; Richardson et al., 2001). In the estuaries, the organic carbon content of the sediment was higher in winter than summer (Dong et al., 2000). This may suggest that in the winter Nap acts to control redox balancing, and the reduction step of N_2O to N_2 will be repressed in order to dissipate excess reducing power and control the cyclic electron-transport system in balance. The reduction of N_2O to N_2 yields more standard free energy compared to the reduction of nitrate or nitrite to N_2O. This strategy can also reduce a potential toxicity of high accumulation of nitrite during the winter, because winter temperatures might stimulate nitrite production. High concentrations of nitrite have been reported to inhibit N_2O reductase activity (Firestone and Tiedje, 1979; Kaldorf et al., 1993), and can lead to high production of N_2O during the winter. Moreover Yon et al. (2014) suggested that denitrification dominated at low carbon-to-nitrogen (C/N) ratios (that is, electron donor-limiting growth conditions), whereas nitrate ammonification was predominant at high C/N ratios (that is, electron acceptor-limiting growth conditions), and at intermediate C/N ratios, denitrification and nitrate-ammonification occurred concomitantly. Nitrate-ammonifying bacteria with high nitrate concentration, produced ammonium and nitrite simultaneously during anaerobic growth on nitrate (Streminska et al. 2012). However, the nitrite to ammonium ratio was dependent on the bacterial species and the electron donor. The ratio of electron donor to acceptor can influence the pathway and fate of nitrate. Nitrate ammonification has a higher affinity for nitrate than denitrification and may be favored in nitrate-limited, carbon-rich environments (King and Nedwell, 1985; Burgin and Hamilton, 2007; Kraft et al., 2014). This is due to the requirement of only 5 electrons to reduce nitrate in denitrification verses the eight required for nitrate ammonification (Tiedje, 1988). Nitrate ammonification may therefore outcompete denitrification in nitrate-limited environments where these organisms gain more energy from nitrate ammonification than denitrifiers can from denitrification. Many studies in the estuaries have shown that nitrate ammonification is the dominant process of nitrate reduction. Nitrate ammonification was greater than denitrificationin 30% (Giblin et al., 2013) and responsible for 44–74% of nitrate reduction in the estuarine sediments (Song et al. 2014). Therefore this pure culture study confirmed the importance of nitrate ammonification as a significant process of nitrate reduction in the estuaries, however denitrifiers still had an important role in end products of nitrate reduction in the estuaries.

5. Conclusions

Competing of denitrification and nitrate-ammonification for nitrate removal in the estuary is affected by temperature. The experiments showed that the nitrate-ammonifier *(K. pneumonia*) was the predominant species at high temperature. In contrast, a facultative denitrifier-nitrate ammonifier (*S. liquefaciens*) was the predominant species at low temperature. There was also a commensally interaction between nitrate-ammonifier (*K. pneumonia)* and a nitrite-respiring bacterium (*A. faecalis*) at high temperature. However the main end products of nitrate reduction at high temperature were 65-78% N_2 indicated that a denitrifier (*C. testosteroni*) still had an important role in end products of nitrate reduction, although it was not a dominant species in the estuary. The data also confirmed that a nitrite respiring bacterium (*A. faecalis*) reduce nitrite to N_2, that responsible for approximately 6-9% of total N_2 produced in the cultures. This study shows that nitrate ammonifiers out compete denitrifiers at high temperature, however denitrifiers still had an important role in end products of nitrate reduction in the estuaries.

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Environmental Pollution of Solid Waste Burial Sites in Nghe An Province, Vietnam

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Abstract

The total amount of solid waste in Nghe An, a province in the North Central region of Vietnam, is about 2.443,8 tons/ day, of which only approximately 50% of the waste is collected and treated at solid waste burial sites. Out of 21 districts and towns in Nghe An, 16 have invested or are investing in the construction of solid waste burial sites. However, only 33% of solid waste burial sites have built enough items according to regulations. The management and treatment of solid waste in Nghe An still has many problems, including the lack of compliance with national regulations, leading to environmental pollution. This report assesses the current state of environmental pollution from solid waste burial sites in Nghe An and suggests efficient solutions for treatment and management.

Keywords: Solid waste/ Solid waste burial sites

1. Introduction

Nghe An currently has 16 large-scale solid waste burial sites in 16 districts and towns. There are also many small landfills located near markets and residential areas. Household solid wastes and wastes from production activities, small-scale agricultural production and craft villages are collected and gathered in landfills. However, the process is still in its infancy. In these landfills, leachate is not collected and treated thoroughly. It frequently drains to the ground or overflows into surrounding areas. In addition, when the waste is dry, in order to reduce the volume, trash is burned and smoke from burning is not yet processed and controlled. This status has caused heavy air, soil and water pollution in these areas, especially during rainy season.

We conducted this study to evaluate the current status and level of environmental pollution caused by solid waste burial sites in Nghe An, thereby proposing treatment and management solutions to minimize environmental pollution.

2. Methodology

- Collect data to assess the current state of environmental pollution by conducting surveys, samples of environmental indicators of the leachate and the surrounding air.
- Collect data on solid waste management and treatment through observation and interviewing key players.
- Data on environmental status are analysed and compared with Vietnam's environmental standards.
- The data is analysed and shown by charts on Excel.

3. Results

3.1 The arising and management status of waste from solid waste burial sites

3.1.1. Type of waste and management status

4 out of 19 residential solid waste burial sites and mixed-landfill sites were shut down by the end of 2017, namely the Cua Lo, Dien Chau, Thai Hoa and Dong Vinh. All the solid waste burial sites including 15 active sites and 04 closed sites were in violation of environmental and technical regulations.

Figure 1 shows the proportion of solid waste burial sites that violate government regulations. It can be seen from the chart that none of the waste burial sites implement environmental monitoring, no

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leachate and landfill gas collection and treatment; very few solid waste burial sites have sprayed waste treatment preparations, and no walls or tree fence for shielding. Most of these burial sites are arbitrary, operated without local government's management. 4 out of 19 burial sites have been closed because of heavy pollution, being expired and in violation of national regulations, in which 3 sites have no plan for treatment of remaining waste.

3.1.2. Environmental pollution from leachate

Most leachate in the solid waste burial sites exceeds Vietnam's Environmental Standards for organic matter, suspended solids and sulphides. Some samples exceed the standards on microorganisms, heavy metals and phenol.

Figure 2 shows serious pollution level of leachate from solid waste burial sites in Nghe An, especially Con Cuong, Tan Ky and Thai Hoa sites, which are located in high mountains areas, the effect of leachate on receiving environment is increased. The indicators of pH, organic matter, microorganism, turbidity and sulphide exceed the permitted standards the most.

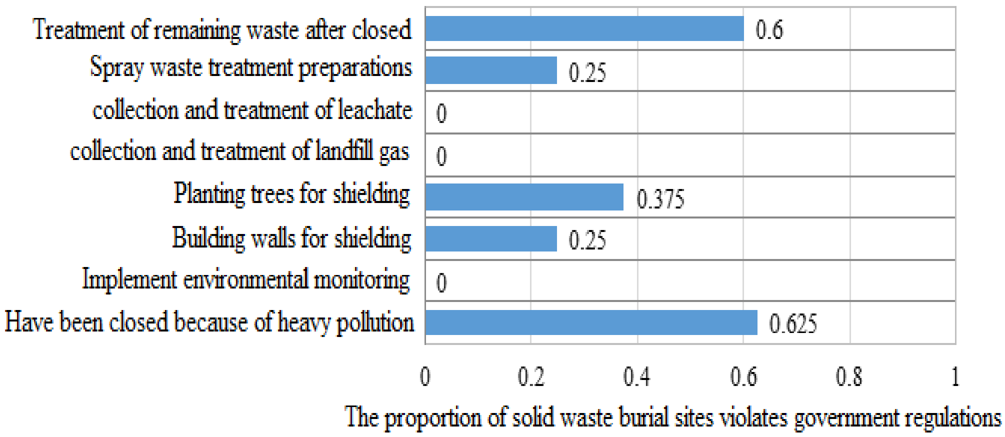

Figure 1. Proportion of solid waste burial sites implemented national regulations

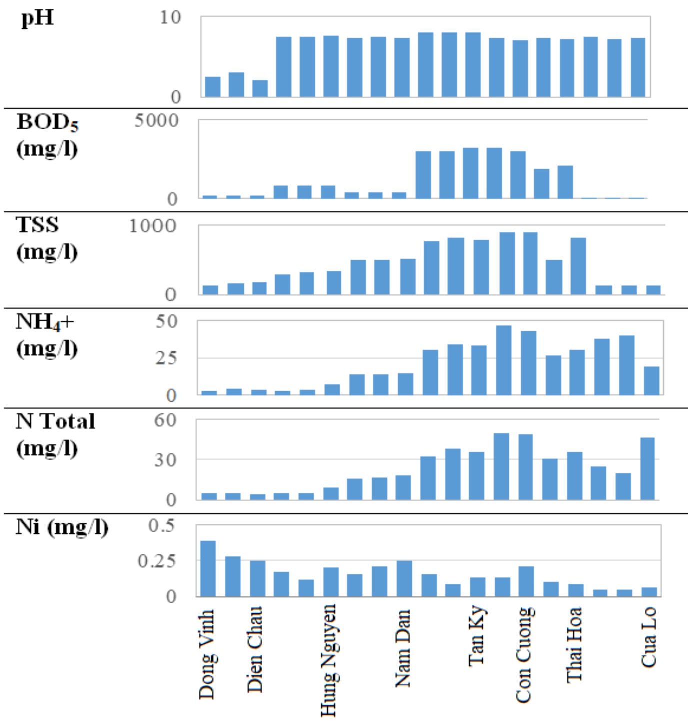

Figure 2. Some environmental parameters of landfill leachate assessment

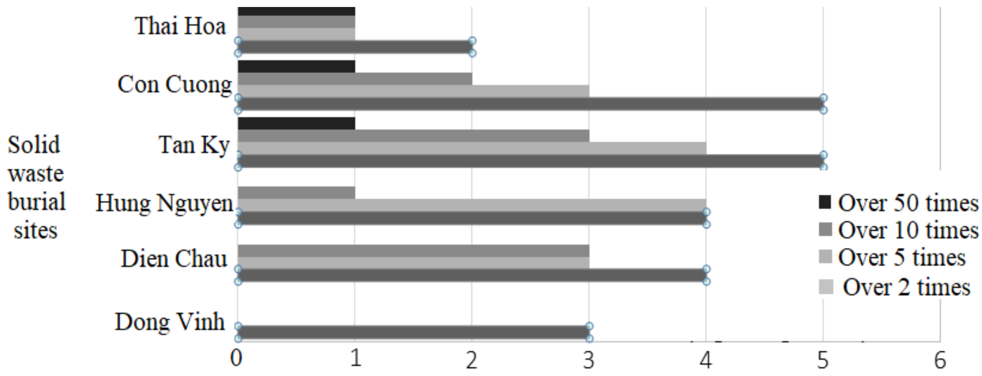

Figure 3. The number of indicators exceeds Vietnam's Environmental Standards

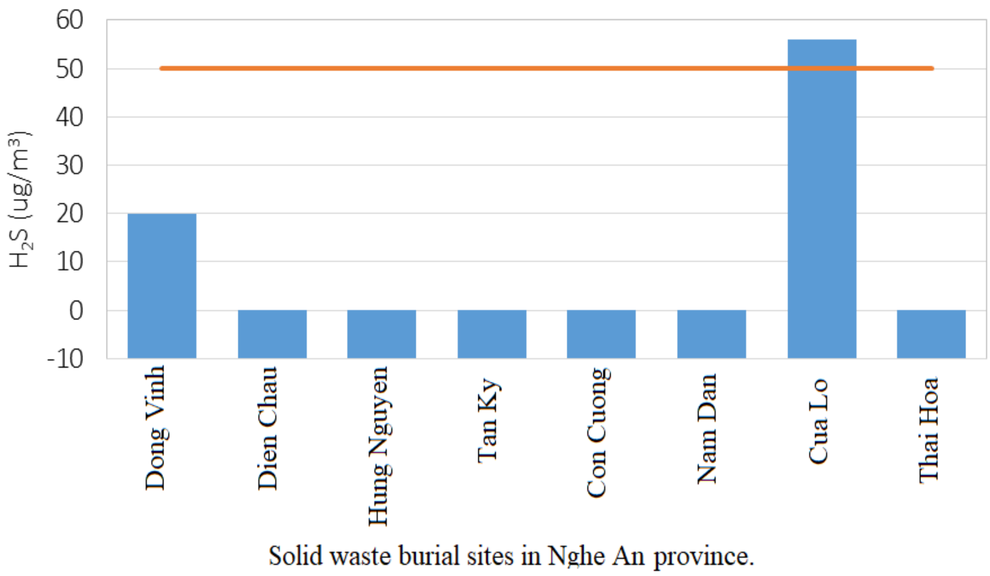

Figure 4. H_2S concentration in the surrounding air around burial sites

All of the solid waste burial sites have from 2 to 5 parameters that exceed Vietnam's Environmental Standards over 2 times, 1 to 4 parameters exceed over 5 times, 1 to 3 parameters exceed over 10 times and 3 sites have one parameter exceeds over 50 times (Figure 3).

3.1.3. Air environment status at solid waste burial sites

Environmental air quality at solid waste burial sites is generally poor; however, most of the sites are located in vacant terrain, away from residential areas, so the impact of air pollution from landfill sites on local environment is not serious. H_2S concentrations were investigated to assess the effect of odours from the decomposition of organic metabolites on the surrounding environment near the waste burial sites in Nghe An. The results shows H_2S content in Cua Lo site is beyond the Vietnam's Environmental Standards. However, due to limited sample size, this result cannot fully reflect the impact of solid waste burial sites on human health, environment and ecosystems.

3.2 Proposing solutions for solid waste management and pollution treatment

3.2.1 Strengthening the management of solid waste at source

- To strengthen the management and classification of solid wastes at the source, avoiding production solid waste mixed with hazardous solid waste, domestic solid waste and solid waste from craft villages.

- To strengthen the collection, classification, packaging, recycling and reuse of valuable solid waste

- To strictly managing the collection, packing, statistics and temporary storage of solid waste in a safe and proper manner

- To compile registration dossiers of hazardous waste management for waste source owners, then transfer to agencies that have the handling function in accordance with national and local regulations.

- To collect waste tax and waste fees properly and sufficiently, which helps to increase investment in solid waste treatment.

- To apply the waste exchange market model among stakeholders to minimize waste disposal that can be recycled in order to save processing costs.

3.2.2 Strengthening treatment activities at solid waste burial sites

- At present, the environmental assessment for closed solid waste burial sites should be carried out, find suitable measures to treat remaining waste in a hygienic and thorough manner

- To develop a transparent and flexible finance mechanism to attract reputable solid waste management and treatment companies, mobilize financial resources from various sources

- To plan and build large-scale, safe and efficient waste treatment system in a large area where they can be operated continuously from collection, classifications to recycle processing and treatment in order to reduce the number of small-scale solid waste burial sites that lack of treatment technology

- To learn and apply advanced solid waste management and treatment models, which have similar conditions and have been successful in other areas

- To stop burying waste, and to use advanced technologies in solid waste treatment such as pyrolysis of reusing heat or electricity, PJM plasma burning, plastic waste recycling technology, rubber waste into diesel oil, etc.

4. Conclusions

- Most of the solid waste burial sites in Nghe An do not comply with national and local technical and environmental regulations. Leachate in all burial sites exceeds Vietnam's Environmental Standards by at least one indicator, especially BOD, COD, TSS, coliform and ammonium. H_2S and NH_3 emissions from burial sites are approximate or beyond the standard at some locations and periods, but do not cause serious pollution.

- To recommend Nghe An authorities to issue more appropriate policies and financial mechanisms in planning, managing and treating solid waste and pollution from waste burial sites. Thoroughly treat remaining waste in all closed solid waste burial sites; apply sanitary and recycling methods to current active solid waste burial sites; work towards eliminating solid waste burial, use more advanced and efficient methods and technologies for waste management and treatment.

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A Simple Method for Synthesis of Triamine-SiO_2 Material toward Aqueous Nitrate Adsorption

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Abstract

In this study, we report a new and simple method for preparation of mesoporous silica at ambient condition using cheap and available commercial SiO_2 precursor. The mesoporous material was then loaded with amine (Amine-P-SiO_2) and applied for nitrate removal in aqueous solution. The materials were characterized by XRD, TGA, FTIR, and SEM to explore the properties. Effects of pH, nitrate concentration, adsorbent dosage, and temperature on the nitrate adsorption capacity were investigated. Amine-P-SiO_2 material was superior to commercial adsorbent (Akualite A420) for nitrate adsorption with capacity reached 32.5 mg/g and relative stable after 10 cycles of adsorption–desorption. Moreover, the adsorption follows Langmuir model, proving that this chemical adsorption could effective remove nitrate from aqueous solution for water and advanced wastewater treatment applications.

Keywords: Triamine/ Mesoporous silica/ Amine-P-SiO_2/ Aqueous nitrate adsorption

1. Introduction

The increase of domestic water use causes a pressure on water treatment and the shortage of clean water due to climate change and water pollution from anthropogenic sources. Nitrate ion exists naturally in the nitrogen biogeochemical cycle and serves as an essential nutrient for plants on Earth (Rivett et al., 2008). However, the increase of nitrate pollution causes many issues for both environment and human health (Ward and Brender, 2018; Fan, 2011). Nitrate concentration in drinking water is limited by WHO guideline at 45 ppm and by every countries (Fan, 2011). Therefore, the removal of nitrate contaminant from water has been attracted many attentions and proposed with various methods. Conventional approaches such as biological treatment, ion exchange, adsorption, reverse osmosis, electrochemical, and chemical methods show several limitations (Tyagi et al., 2018) while advanced methods using nanotechnology such as reduction by zero valent iron (Araújo et al., 2016), catalytic and electrocatalytic reduction (Martínez et al., 2017; Garcia-Segura et al., 2018), photocatalytic reduction (Tugaoen et al., 2017; Bahadori et al., 2018), adsorption (Bhatnagar and Sillanpää, 2011; Loganathan et al., 2013; Singh et al., 2018) emerge as potential technologies. Among these methods, adsorption using new and effective nanomaterials could be a very promising technology, which can remove nitrate from water and wastewater. Therefore, the development of simple and cheap adsorbents is necessary for effective and efficient nitrate removal and recovery in water environment.

In this study, we proposed a simple process for synthesis of a new and cheap mesoporous silica from available commercial SiO_2. The materials was then grafted with amine for nitrate removal from aqueous solution. The effect of environmental conditions on the nitrate removal efficiency and adsorption capacity was investigated, including solution pH, initial nitrate concentration, adsorbent dosage, and adsorption temperature. The adsorption capacity of the synthesized materials was compared with commercial ion exchange resin (Akualite A420) and the durability test was also conducted.

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2. Methodology

2.1 Material synthesis and characterization

Chemicals such as SiO_2, NaOH, HF, HCl – 36 vol.% (made in China); N^1 - (3 - trimethoxysilylpropyl) dimethylene triamine (named to be triaminesilane), toluene, pentane (Merck), DI (deionized) water were used for the adsorbent synthesis procedure. KBr and stock nitrate solution (Merck) with concentration of 1000 mg/L were used for determining FTIR characterization and nitrate adsorption experiments, respectively. Commercial anion exchange resin (Akualite A420, China) with size of 0.5 mm was used as a comparison to as-synthesized materials.

Commercial silica (industrial grade) was purchased from Ho Chi Minh City, Vietnam. It was firstly washed with DI water and dried at 100°C in an oven. The dried material was then sieved with mesh size from 20 to 50 μm and named as SiO_2 in this study. The mesoporous structure of silica was created by using chemical reaction of HF and SiO_2 (Reaction (1)). In a typical procedure, 20 g of SiO_2 powder was added into 1 L of 5% (vol/vol) HF solution under continuous stirring at ambient condition. After 30 min of reaction, the remained solid SiO_2 powder was filtered and washed with DI water. The mesoporous silica (named as P-SiO_2) was finally obtained by drying at 100°C for 4 h.

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O \qquad (1)$$

The amine-loaded materials (i.e. Amine-P-SiO_2 and Amine-SiO_2) were synthesized by grafting of triaminesilane onto P-SiO_2 and SiO_2 materials, respectively, followed a synthesis procedure described in our previous work (Thanh, 2016). In a typical experiment, P-SiO_2 (or SiO_2) was loaded into a multi-neck glass flask containing 150 mL of toluene and DI water at ratio of 0.3 mL DI water per 1 gram of P-SiO_2 (or SiO_2). After that, the glass flask was submerged in a silicon oil bath set at 85°C using a temperature controlled stirring hotplate with an external temperature probe. Triaminesilane (3 mL per gram of silica) was subsequently added to the mixture and left stirring for 16 h. The material was then filtered and washed with copious amounts of toluene, then pentane. Finally, the obtained solid material was dried at 100°C in a natural convection oven for 1 h and named as Amine-P-SiO_2 (or Amine-SiO_2).

The surface chemical characterization was performed using a Bruker FTIR (Fourier transform infrared spectroscopy) with spectrum scanned from 400 to 4000 cm^{-1}. The thermogravimetric (TGA) pattern was obtained using a TGA Q500 machine and carried out with air (100 mL/min) at temperature rate of 10°C/min and range of 30-1000°C. Crystalline structure of materials was analyzed by wide angle X-ray diffraction (WAXRD), using a D2 Phaser XRD 300 W diffractometer equipped with CuKα radiation (λ=1.5406 Å) at step size of 0.01° and step time of 30 s. The morphology and particle size of Amine-P-SiO_2 were evaluated by scanning electron microscopy (SEM). Additionally, the surface chemical composition of Amine-P-SiO_2 were determined by energy-dispersive X-ray spectroscopy (EDS). Brunauer–Emmett–Teller (BET) surface area and pore size of P-SiO_2 support and Amine-P-SiO_2 sample were determined from N_2 adsorption/desorption isotherms at 77 K (Porous Materials, BET-202A). Before BET measurement, the samples were degassed at 250°C for 3 h and the BET data corresponded to the annealed samples.

2.2 Nitrate adsorption experiment

The nitrate removal ability of amine-grafted materials was evaluated using batch nitrate adsorption test. The adsorbent (amount of 0.005-0.10 g) was added into 50 mL of aqueous nitrate solution (pH 2–10, nitrate concentration of 5–100 mg/L) and shaken in a thermostatic water-bath shaker operated at 120 rpm and temperature of 25±0.5°C. After reaching the equilibrium, the adsorbent was isolated by centrifuging at 10,000 rpm. All experiments were replicated three times and nitrate in the supernatant was analyzed by brucine-sulfanil colorimetric method using a UV-visible spectrophotometer (SPECORD 210 Plus, Analytik Jena, Germany). Nitrate adsorption capacity (defined as amount of nitrate adsorbed per mass unit of adsorbent) of the material is calculated by Eq. (2).

$$q_e = \frac{C_0 - C_e}{m} \times V \qquad (2)$$

Where q_e (mg/g) is the adsorption capacity, C_o and C_e (mg/L) are the initial and equilibrium nitrate concentrations in the aqueous solution; V (mL) is the volume of the experimental solution (50 mL) and m (mg) is the adsorbent weight.

The regeneration of adsorbents was carried out by brine desorption technique. Typically, 1000 mL of 0.1 M HCl solution was used to regenerate these adsorbent (1 g) for 3 h at ambient condition. After washed with distilled water, the regenerated adsorbent was reused in the subsequent experiments.

3. Results and discussion

3.1 Characterization of Amine-P-SiO_2

FTIR was used for identification of surface amine group in the structure of amine-P-SiO_2 and ion exchange resin (Akualite A420). The FTIR spectra of amine-P-SiO_2 and Akualite A420 samples are shown in Figure. 1. The peak around 3442 cm^{-1} for strong band of the -OH stretching vibration was observed for both anion resin Akualite A420 and Amine-P-SiO_2 (silanol groups on P-SiO_2 surface). For Akualite A420 resin, peaks at wavenumbers of 3018 cm^{-1} and 2922 cm^{-1} related to the stretching vibrations of the ring C-H bonds and –CH_2 groups of matrix for cross-linked polystyrene structure (Sowmya and Meenakshi, 2013). At 1601 cm^{-1}, the presence of C-C bonds of styrene ring was found (Lazar et al., 2014). Peak at wave number of 1481 cm^{-1} was the symmetric and asymmetric vibrations of methyl group of quaternary nitrogen (Wołowicz and Hubicki, 2009; Gandhi et al., 2011) and at wavenumbers of 1039 and 1128 cm^{-1} were identified for the stretching vibrations of benzene ring from the linked matrix of styrene-divinylbenzene in the resin structure (Lee et al., 2003). For Amine-P-SiO_2 material, the stretching vibration of amine group was found at wavenumber of 1480 cm^{-1}, also observed for Akualite A420. The FTIR spectrum of Amine-P-SiO_2 material was identified with the presence of vibrations of silica and organic chemicals such as Si-H (650-840 cm^{-1}), Si-O-Si (1030-1130 cm^{-1}), C=C (1650 cm^{-1}), C-H (2930 cm^{-1}), and -OH (3420 cm^{-1}) (Ibrahim et al., 1980). This is an evidence for the presence of amine groups on the porous silica surface of Amine-P-SiO_2 material.

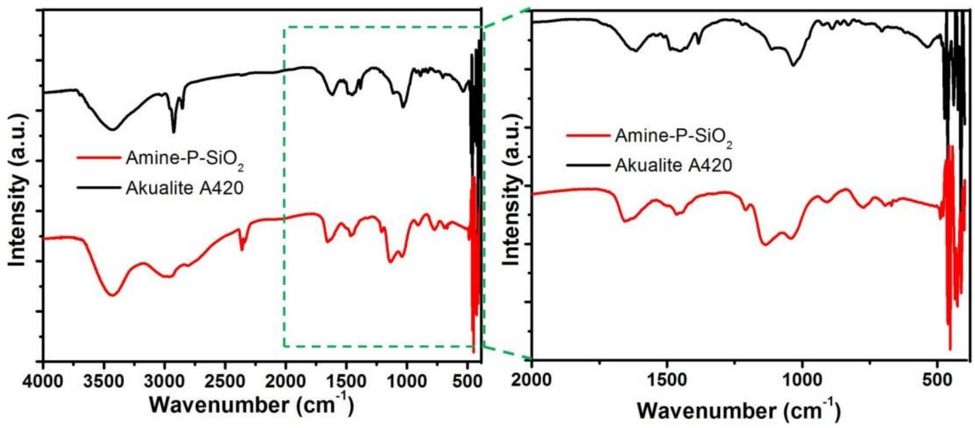

Figure 1. FTIR spectra of Amine-P-SiO_2 and ion exchange resin (Akualite A420)

Figure 2 shows the adsorption – desorption isotherms of nitrogen at 77 K for P-SiO_2 support and Amine-P-SiO_2 material, which follows type IV IUPAC isotherm for mesoporous materials. The BET surface area of supports amine-loaded materials is summarized in Table 1. There was a remarkable increase of 20 times in surface area of silica material after treated with HF (i.e., from 32.5 (SiO_2) to 675.8 m^2/g (P-SiO_2)) proven that HF treatment is a very effective method for generation of mesoporous silica from commercial silica. After loaded with amine, surface area of Amine-P-SiO_2 slightly decreased to 625.7 m^2/g; however, it was still 18 times higher than that of Amine-SiO_2.

Thermogravimetric analysis result is shown in Figure 2 over a wide temperature ranging from 30 to 1000°C. It is obvious that the mass of sample reduced with the increased temperature and three mass loss stages can be typically divided. The initial period ranged in 30-200°C with endothermic peak at 50-100°C and mass loss of ~16%, which was attributed to the release of moisture and low weight volatile compounds. Next, the mass loss of the second stage ranged from 200 to 430°C was mainly due to the dehydration of hydroxyl group at high pyrolysis temperature. The main of pyrolysis process occurred in the third stage as the temperature increased from 450 to 620°C and the weight loss of sample reached to ~56% after this period. The second and third stages were used as the baseline amount for the organic moiety of sample. As summarized in Table 1, the amine loaded on the surface of P-SiO_2 and SiO_2 was calculated to be 6.4% and 1.6%, respectively. This was due to the higher surface area of P-SiO_2 support or silicon density on silica support surface as compared to SiO_2 material.

Table 1. Surface area and amine loading of silica aterials

Sample	Surface area (m^2/g)	Amine loading (%)
SiO_2	32.5	---
P-SiO_2	675.8	---
Amine-SiO_2	34.6	~ 1.6
Amine-P-SiO_2	625.7	~ 6.4

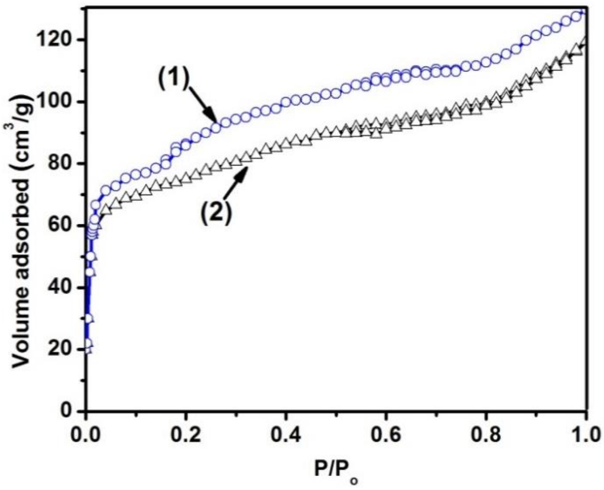

Figure 2. Nitrogen adsorption – desorption isotherm of (1) P-SiO_2 and (2) Amine-P-SiO_2.

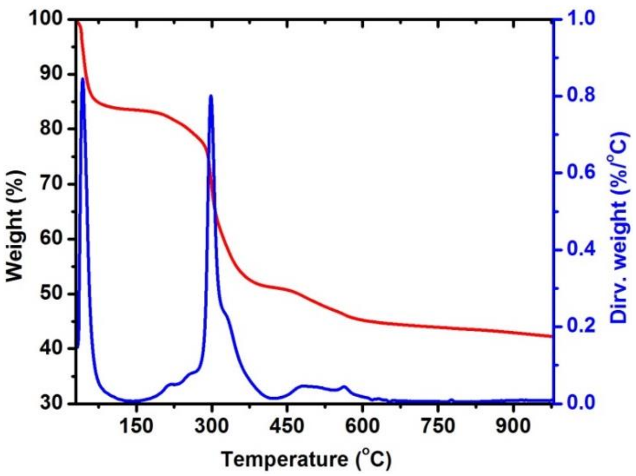

Figure 3. Thermogravimetric curves of Amine-P-SiO_2

Figure 4 displays SEM results of P-SiO_2 and Amine-P-SiO_2. It was observed that P-SiO_2 possessed highly porous structure, which was formed from the reaction of HF and SiO_2 during the synthesis of P-SiO_2. SEM-mapping results showed a relative homogeneous dispersion of amine on the surface of support and EDS results gave elemental ratios of O, C, Si, and N were 36.8, 31.6, 26.0, and 5.6%, respectively. There was not significantly changed in microstructure of P-SiO_2 after grafting with amine, as proven by XRD patterns in Figure 5 with only characteristic peaks of silica observed for both materials. The disappearance of some peaks at 73° and 77.5° and the lower intensity for other peaks of Amine-P-SiO_2 also proved that the successful grafting of amine on the surface of support material.

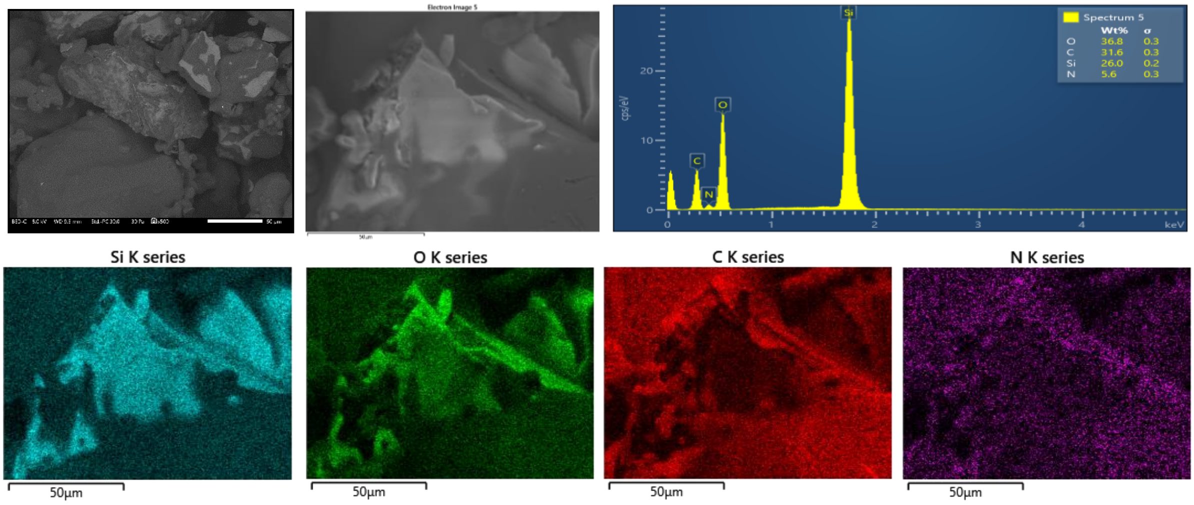

Figure 4. SEM images of P-SiO_2 and Amine-P-SiO_2, EDS spectrum, and SEM mapping images of Amine-P-SiO_2

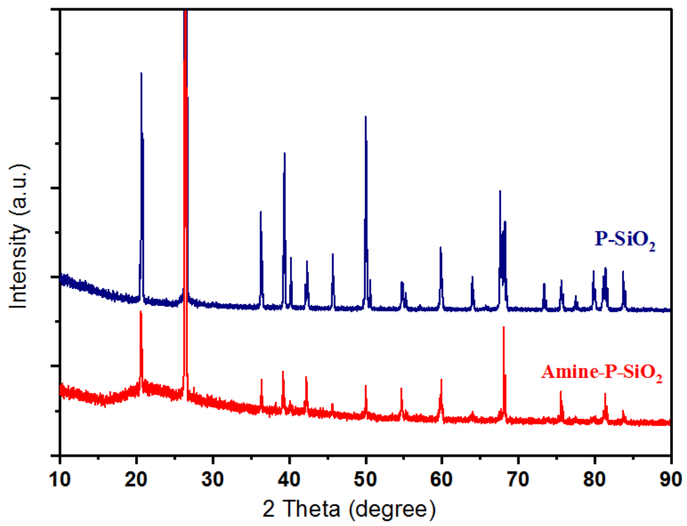

Figure 5. XRD pattern of P-SiO_2 and amine-P-SiO_2 materials

3.2 Adsorption tests of nitrate removal

The nitrate removal test using amine-loaded silica materials was evaluated using batch adsorption experiment. The effect of environmental conditions such as pH, concentration, dosage, and temperature on the adsorption capacity was investigated in this study. The influence of initial solution pH was examined in the range of 2–10 using an initial concentration of 10 mg/L of nitrate. As shown in Figure 6, the capacity of nitrate removal was higher than 18 mg/g as pH increased from 2 to 8 and reached maximum capacity of 19 mg/g at pH 5. However, the capacity of nitrate removal decreased significantly to 15 mg/g at pH 10. This could be explained by the effect of solution pH value on the nature of adsorbent surface and nitrate ions. Under acidic condition, the electrostatic interaction between the protonated nitrate and adsorbents (positively charged functional groups) might be weakened (Milmile et al., 2011) while the poor adsorption capacity of Amine-P-SiO_2 under basic condition may be attributed to the increasing competition for sites between OH^- ions and nitrate ions.

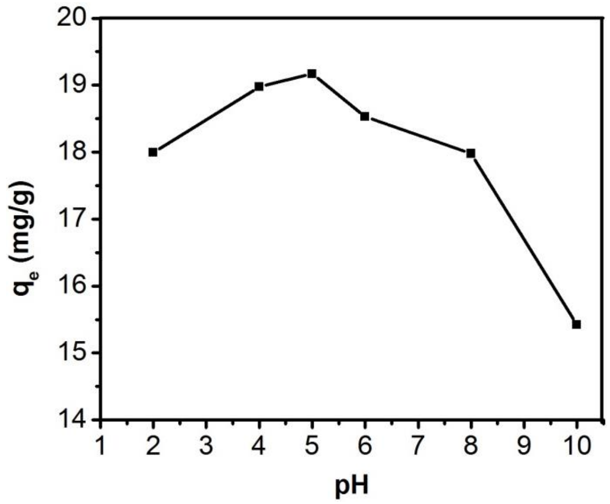

Figure 6. Effect of initial solution pH on nitrate removal of Amine-P-SiO_2 (Condition: temperature: 25±0.5°C, dosage: 0.6 g/L, nitrate concentration: 10 mg/L)

Effect of Amine-P-SiO_2 dosage on nitrate adsorption is shown in Figure 7. The adsorbent capacity of Amine-P-SiO_2 reached highest value of 66 mg/g at the lowest dosage of 0.1 g/L, but decreased to 10 mg/g at the highest dosage of 2.0 g/L. This proved the effectiveness of Amine-P-SiO_2 material for nitrate removal and adding more amount of adsorbent would not result in increase of adsorption capacity.

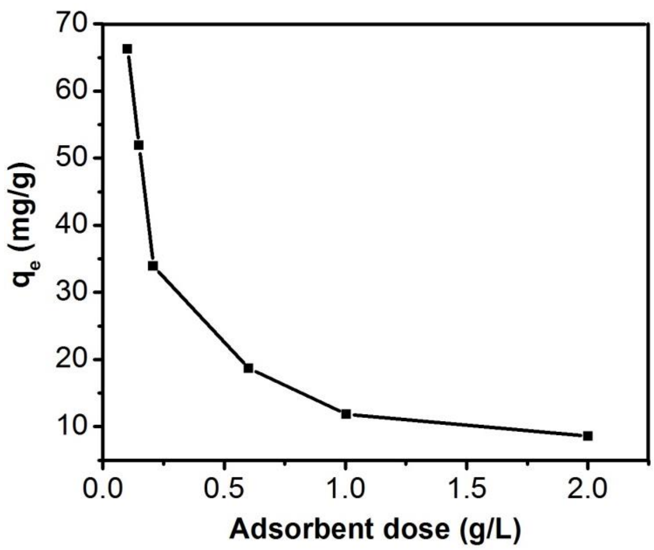

Figure 7. The effect of adsorbent dosage on nitrate removal by Amine-P-SiO_2 (Condition: temperature: 25±0.5°C, pH 5, nitrate concentration: 10 mg/L)

The comparison on nitrate removal of Amine-P-SiO_2, Amine-SiO_2, and anion exchange resin (Akualite A420) is presented in Figure 8(a). Amine-P-SiO_2 showed a superior adsorption capacity of 32.5 mg/g, which was 1.71 and 1.31 times higher than that of Akualite A420 and Amine-SiO_2, respectively. This confirmed the successful and effective modification of SiO_2 surface by HF solution. Moreover, both amine-loaded silica materials had higher adsorption capacity than Akualite A420, proving the strong affinity of amine on the surface of silica materials. The used Amine-P-SiO_2 and Akualite A420 were then regenerated using 0.1 M HCl solution and applied for durability test. As demonstrated in Figure 8(b), Amine-P-SiO_2 was more stable than Akualite A420 and the decline in adsorption of these materials was 20% and 50%, respectively, after 10 cycles of adsorption–desorption. This is a notorious advantage of Amine-P-SiO_2 as compared to other ion exchange resin for commercialization of the product in the future.

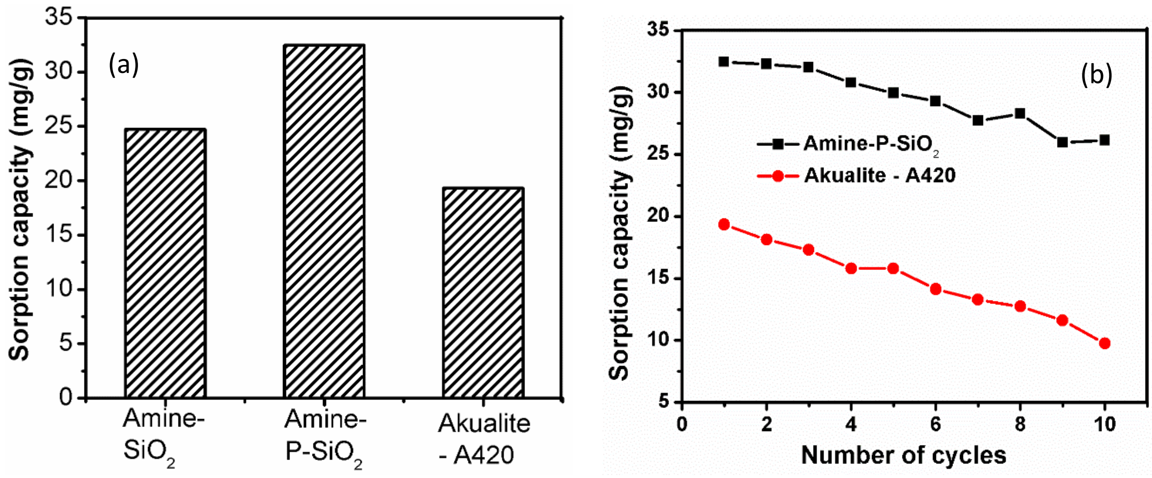

Figure 8. Nitrate sorption capacities of Amine-SiO_2, Amine-P-SiO_2, and Akualite-A420 materials (Condition: temperature: 25±0.5°C, pH 5, dosage: 0.6 g/L, nitrate concentration: 50 mg/L)

3.3 Adsorption isotherms, thermodynamics, and kinetics

The adsorption isotherms of nitrate by Amine-P-SiO_2 are illustrated in Figure 9. Langmuir and Freundlich models were used to analyze equilibrium adsorption isotherm data and related parameters were summarized in Table 2. Langmuir model assumes that a monomolecular layer is formed when adsorption occurs without any interaction between adsorbed molecules. The model is presented as follows:

$$q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \qquad (3)$$

$$\frac{C_e}{q_e} = \frac{1}{K_L Q_{max}} + \frac{C_e}{Q_{max}} \qquad (4)$$

Where C_e (mg/L) is the equilibrium concentration of nitrate solution and q_e (mg/g) is the amount of nitrate adsorbed. Q_{max} (mg/g) is the maximum capacity of the adsorbent and K_L (L/mg) is the equilibrium constant related to energy of adsorption.

The Freundlich adsorption isotherm model is expressed by Eq (5).

$$q_e = K_f C_e^{1/n} \qquad (5)$$

$$ln q_e = ln K_f + \left(\frac{1}{n}\right) ln C_e \qquad (6)$$

Where K_f ((mg/g)(L/mg)^n) and n are Freundlich temperature dependent constants.

Meanwhile, to conduct the grand total of deviations under different isotherm models, chi-square analyses were carried out and the mathematical expression was represented by Eq. (7). The purpose of the chi-square test is to compare the model with the experiment results. Small value of χ^2 would be turned up when the $Q_{max,cal.}$ values calculated from the models are similar to the obtained experimental values. When the values of $Q_{max,cal.}$ and $Q_{max,exp}$ are different, the value of χ^2 would be larger.

$$\chi^2 = \sum \frac{(Q_{max,exp} - Q_{max,cal.})^2}{Q_{max,cal.}} \qquad (7)$$

Where $Q_{max,cal.}$ (mg/g) was the maximum capacity of adsorbent obtained from the isotherm models and $Q_{max,exp}$ (mg/g) was the maximum experimental capacity of adsorbent.

Compared to the Freundlich model, the Langmuir model with the correlation coefficients between 0.9849 and 0.9992 represented a better fit of experimental data at different temperatures (Figure 9). Furthermore, the smaller values of χ^2 also indicated that the Langmuir isotherm is more fitted with the data of the 2 isotherms for Amine-P-SiO_2 adsorbent. Based on the Langmuir isotherm, the maximum capacities (Q_{max}) for nitrate ion were calculated to be 45.2-99.0 mg/g in temperature range of 288-318 K, respectively. These results demonstrated that the surface site saturation limited the adsorption process and the adsorption was homogeneous and monolayer. As also seen in Table 2, the Freundlich constant n for all the temperatures was found to be greater than 1, suggesting the favorable condition of adsorption. Thus, it can be concluded that monolayer adsorption was predominant in the adsorption of nitrate ions using Amine-P-SiO_2 adsorbent (Yousef et al., 2011).

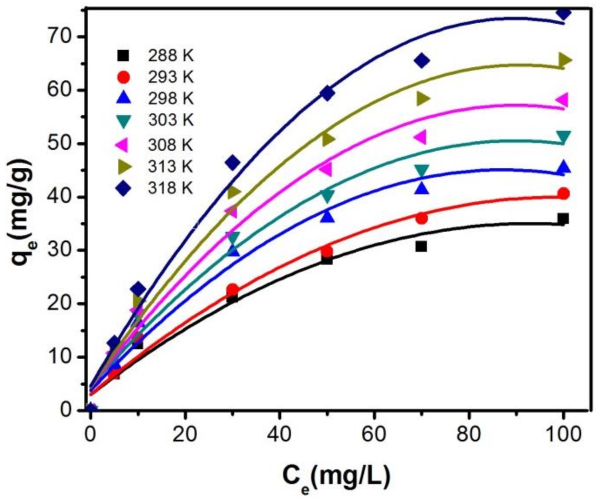

Figure 9. Adsorption isotherms of nitrate onto Amine-P-SiO_2 at different temperature (Condition: pH 5, dosage: 0.6 g/L)

Table 2. Adsorption isotherm parameters

Isotherms	Parameters	Temperature (K)						
		288	293	298	303	308	313	318
Langmuir model	Q_{max} (mg/g)	45.249	53.476	57.471	70.922	74.627	86.957	99.010
	K_L (L/mg)	0.034	0.029	0.036	0.028	0.033	0.0300	0.029
	R^2	0.9911	0.9849	0.9979	0.9905	0.998	0.999	0.9992
	χ^2	10.215	15.918	11.289	12.206	16.067	20.244	23.280
Freundlich model	K_f ((mg/g)(L/mg)^n)	3.215	3.181	4.146	3.806	4.876	4.999	5.442
	n	1.853	1.759	1.837	1.693	1.786	1.716	1.685
	R^2	0.980	0.980	0.968	0.989	0.979	0.981	0.979
	χ^2	5.149	4.851	15.499	38.635	40.808	75.475	212.39

The thermodynamic parameters, including Gibbs free energy change (ΔG^o), entropy change (ΔS^o), and enthalpy change (ΔH^o) were obtained using equilibrium constants at different temperatures (293–318 K). These thermodynamic parameters could be calculated by the following equations and presented in Table 3 (Duranoğlu et al., 2012).

$$\Delta G^o = -RTlnK_L \quad (8)$$

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad (9)$$

$$K = \frac{C_{Ae}}{C_e} \quad (10)$$

$$lnK = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \quad (11)$$

Where C_{Ae} (mg/g) and C_e (mg/g) are the nitrate amount on adsorbent phase and adsorbate phase, respectively. Values of ΔH^o (kJ/mol), ΔS^o (kJ/mol), and ΔG^o (kJ/mol) could be calculated from a plot of lnK versus 1/T.

As obviously seen from Figure 9 and Table 2, more nitrate ions was removed from the solution as temperature increased. The positive values of ΔH^o revealed that nitrate adsorption is an endothermic process, and the negative values of ΔG^o implied the feasibility and spontaneous nature of the process

(Milmile et al., 2011). Furthermore, the more negative values of ΔG^o with raising temperature inferred that the adsorption process was more favorable and relatively easier at higher temperatures (Katal et al., 2012). In addition, the positive values of ΔS^o indicated an increase in disorder and randomness during the adsorption of nitrate by Amine-P-SiO_2.

Table 3. Thermodynamic parameters of nitrate adsorption on Amine-P-SiO_2 at different temperatures (nitrate concentration: 10 mg/L)

ΔH^o (kJ/mol)	ΔS^o (J. $mol^{-1}K^{-1}$)	ΔG^o (kJ/mol)				R^2
		293	303	308	318	
18.852	124.5188	-17.632	-18.877	-19.500	-20.745	0.8717

4. Conclusions

A new Amine-P-SiO_2 material was successfully synthesized using a simple and low-cost route. The surface modification of silica precursor by HF reaction and amine loading was demonstrated by material characterization using TGA, XRD, FT-IR, BET, SEM and EDS-mapping. Results showed that Amine-P-SiO_2 had high nitrate adsorption of 32.5 mg/g, which was 1.71 times higher than Akualite A420 and followed Langmuir isotherm as chemical adsorption. Amine-P-SiO_2 could be a new and very potential material for nitrate removal in water and advanced wastewater treatments.

5. Acknowledgements

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Cancer Risk of 1,3-Butadiene Exposure to Various Receptors Living Near Heavy Traffic Area in Bangkok, Thailand

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Abstract

This study aims to estimate cancer risk of 1,3-butadiene exposure to receptors living or having activities in or near heavy traffic areas in Bangkok. Ambient air samples were actively collected at Ko Phaya Thai next to the Victory Monument area on an hourly basis for 15 consecutive hours on Sunday and Monday. The number of vehicles was simultaneously monitored for investigation of its relationship with atmospheric 1,3-butadiene concentration. In addition, separated air samples were continuously collected for 24 hours at the same time, for estimation of cancer risks. The collected samples were analyzed by GC-MS. The hourly concentrations of 1,3-butadiene were in ranges of 0.17-3.33 μ g/m^3 on weekends and 0.11 – 2.01 μ g/m^3 on weekdays. The vehicles were classified into 7 types, the passenger cars and Taxi cars were the biggest portions on weekends, motorcycles and passenger cars were the most numbered on weekdays. During daytime both on weekends and weekdays, the variations of 1,3-butadiene and number of vehicles tended to have a relationship whereas during nighttime they seemed to have weak relationship. This implied that there were other sources besides traffic. On weekdays the concentrations of average hourly 1,3-butadiene in the night-time were greater than that of the day time. This finding confirms previous arguments that rapid destruction of 1,3-butadiene during the day-time was stimulated by photoinitiated reaction. Estimated cancer risk using inhalation unit risk of 3 x 10^{-5} per µg m^{-3} and was found that adult people who have been working near this roadside for 8 to 12 hour per day during Monday to Friday and Monday to Saturday for 54 years will experience the lifetime cancer risk of 6.4-9.7 and 6.8-10.4 times higher than the USEPA benchmark of one in a million. Adult vendors working for 3 – 12 hours per day for 5 weekdays for 54 years will have lifetime cancer risk of 2.4x10^{-6}-9.7x10^{-6}. Bus passengers aged 3-21 years who spend 1 to 2 hours per day waiting for a bus will get lifetime cancer risks of 0.73x10^{-6} to 1.4x10^{-6}**,** and for residents, lifetime risks was 0.58x10^{-6}-1.2x10^{-6}.

Keywords: 1,3-butadiene/ Cancer risks/ Inhalation unit risk

1. Introduction

1,3-butadiene is defined in the third hazardous substance category (Hazardous production, import, export or possession must be licensed) under the Thai's Hazardous Substances Act B.E. 2535. Source of 1,3-butadiene are petroleum refining, production of synthetic rubber and tire manufacturers. 1,3-butadiene is a liquefied gas, mild oily odor, colorless, easy to volatile and degraded faster in the air with sunlight. In the presence of heat and sunlight, half of the amount emitted can be disintegrated within two hours. However, without sunlight, it can be maintained up to 2 - 3 days. The Institute of International Cancer (IARC, 2012) identified 1,3-butadiene as a class 1 carcinogen in humans (Confirmed Human Carcinogen). For toxicity, 1,3-butadiene is toxic to the nervous system, respiratory tract and mucous membranes. It also found that this compound is tumorigenic in vivo, psychedelic affecting blood vessels, lung, breast (PCD, 1999).

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1,3-butadiene has two major sources which are industry and vehicle fuel combustion. 1,3-butadiene are formed in vehicle exhaust and emitted to the atmosphere. Based on the available data, Increase of 1,3-butadiene emissions seems to be approximately in proportion to the increase of hydrocarbon emissions. 1,3-butadiene from freight traffic can affect people who live or have activities on roadside such as street vendors, traffic police, street sweepers, adjacent residential streets, including those who use mass transit.

This study will focus on assessing the risks of cancer from 1,3-butadiene to the occupants or occupations or any other activity that requires a roadside in the city. Being classified as confirmed carcinogen in humans by IARC, hence the study on how much 1,3-butadiene inhalation is risk to human health is very important. This finding will urge the stakeholder to drive some more effort on this health risk. Risk assessment refers to the process of evaluating the likelihood and severity will happen to humans or the environment by exposure/risk exposure depending on the environmental issue, including identifying uncertainties that follows. The process often relies on experts from various fields of public health, toxicology, epidemiology, statistics, engineering and environment. The assessment is divided into two groups of quantitative risk assessment and qualitative risk assessment.

In this study choose quantitative risk assessment for estimated cancer risk exposure inhalation from 1,3-butadiene because quantitative risk assessment used the scientific process to determine the parameters of the scientific instruments in laboratory analysis. So, the simple models and equations and standardized methods for analysis concentration of 1,3-butadiene in ambient air were applied for this study.

2. Methodology

2.1 Air sampling and sampling site

The samples were collected at Ko Phaya Thai Victory monument, Phayathai District. Figure 1 show top view of sampling site. by active sampling method following the Method TO1 7 (U.S. EPA, 2009) continuously in hourly basis during 07.00 AM – 10.00 PM for four weeks on Monday as representative of working day and Sunday as representative of holiday from 2 2 January to 20 February 2017. During the sampling period, 1,3-butadiene was collected in thermal desorption tube which contain Carbopack B and Carbosive SIII for adsorption 1,3-butadiene.

2.2 Analytical procedure

Thermal desorption is the process applied for extraction and desorption of 1,3-butadiene from a sorbent tube using heat and flow of inert gas . The sample was heated in thermal desorption unit to extract compounds from sorbent tube and transfer to column in gas chromatograph so as to separate volatile organic compounds and detect by mass spectrometer.

In this study the concentration of 1,3 butadiene in the air samples were calculated by the following equation.

$$A = \frac{C\ (\text{nmol}) \text{x MW } (\frac{\text{g}}{\text{mol}})}{1000\ (\text{nmol})/\mu\text{mol}\ ) \text{ x v } (\text{m}^3)} \quad (1)$$

where:

A = 1,3 butadiene concentration in the air ($\mu g/m^3$)
MW = Molecular weight of 1,3 butadiene (g/mol)
V = Volume of air sampling (m^3)

2.3 Estimation of cancer risks

Exposure concentration (EC) of 1,3 – butadiene was used to estimate the cancer risks from chronic exposure by using time-weighted average measurement concentration associated with exposure scenarios of subject. Exposure concentration was estimated from Equation 3.3 (US.EPA, 2009). In this study, the measurements of the average 24-hour concentrations of 1,3 – butadiene for 24 hour on weekends and weekdays were used as the contaminant concentration (CA) while other exposure factors including exposure time (ET), exposure frequency (EF) and exposure duration (ED) and averaging time (AT) for receptors in various scenarios of exposure were set as the default values as listed in Table 1.

Table 1. Exposure parameters for receptors in various scenarios of exposure

Receptor	ET (h/d)	EF (d/y)		ED (y)	AT (h)
		Weekday	Weekend		
Resident (child)					
0 to < 2 years	24	250	100	2	17520
2 to < 3 years	24	250	100	1	8760
3 to < 6 years	24	250	100	3	26280
6 to < 11 years	24	250	100	5	43800
11 to < 16 years	24	250	100	5	43800
Adult (16 – 70 years)	24	250	100	54	473040
Worker (16 – 70 years)					
Mon-Fri	8	250		54	473040
	12	250		54	473040
Mon-Sat	8	250	50	54	473040
	12	250	50	54	473040
Vendor	3	250	100	54	473040
	4	250	100	54	473040
	12	250	100	54	473040
Bus passenger (1 hour exposure)					
3-5 years	1	250	100	3	26280
6-11 years	1	250	100	6	52560
12-17 years	1	250	100	6	52560
18-21 years	1	250	100	4	35040
22-60 years	1	250	100	39	341640
61-70 years	1	250			
Bus passenger (2 hour exposure)					
3-5 years	2	250	100	3	26280
6-11 years	2	250	100	6	52560
12-17 years	2	250	100	6	52560
18-21 years	2	250	100	4	35040
22-60 years	2	250	100	39	341640
61-70 years	2	250	100	10	87600

For an air pollutant, cancer risk from exposure to this pollutant is characterized by its inhalation unit risk (IUR) and can be estimated from IUR and EC using the following equation. Inhalation unit risk (IUR) of 3 x 10-5 per µg m-3 recommended by USEPA (2012) for 1,3-butadiene was used to estimate cancer risks in this study.

$$\mathrm{EC} = \frac{\mathrm{CA\ x\ ET\ x\ EF\ x\ ED}}{\mathrm{AT}} \qquad (2)$$

$$\mathrm{Risk} = \mathrm{EC} \times \mathrm{IUR} \qquad (3)$$

Where:

EC = concentration of exposure ($\mu g/m^3$)
IUR = Inhalation Unit Risk $(\mu g/m^3)^{-1}$

USEPA (2005c) recommends to apply the following age-dependent adjustment factors (ADAF) to the lifetime cancer risk estimation when exposing to a chemical having mutagenic mode of action for carcinogenesis. This is the case for 1,3-butadiene which has been identified by USEPA as a mutagenic carcinogen (USEPA 2002). The adjustment factors are as follows.

Risk for birth to 2 yr
$$= \mathrm{EC\ x\ IUR} \times 10 \times 2\ \mathrm{yr}/70\ \mathrm{yr} \qquad (4)$$

Risk for age 2 yr to <16 yr
$$= \mathrm{EC\ x\ IUR} \times 3 \times 14\ \mathrm{yr}/70\ \mathrm{yr} \qquad (5)$$

Risk for age 16 until 70 yr

$$= EC \times IUR \times 54\ yr/70\ yr \quad (6)$$

Lifetime Risk

$$= EC \times IUR \times [(10 \times 2\ yr) + (3 \times 14\ yr) + 54\ yr]/70\ yr \quad (7)$$

3. Results and Discussion

3.1 Concentration of 1,3-butadiene

The hourly concentrations of 1,3-butadiene on weekends at week 1,2,3 and 4 were in ranges of 0.45-2.35, 0.09-1.71, 0.70-3.33 and 0.17-1.85 $\mu g/m^3$ respectively. On weekdays, the hourly 1,3-butadiene concentrations at week1, 2, 3 and 4 were in ranges of 0.35-1.77, 0.54-1.48, 0.53-2.01 and 0.11-1.15 $\mu g/m^3$ respectively. Most of hourly concentrations measured exceeded Thailand's national ambient air quality standard for 1,3-butadiene (annual 24-hr average) which is set to be less than 0.33 $\mu g/m^3$, however they were lower than the guideline value (24 hr) which is set to be less than 5.8 $\mu g/m^3$. The average hourly concentrations for 4 weeks were also confirmed that the concentration of 1,3-butadiene during 06.00-07.00 PM on weekday was higher than that of on weekend. In the study of Yurdakul et.al. (2014), ratio of weekday to weekend concentration of 1,3-butadiene were >1 but the difference was not statistically significant. They implied that no statistically significant difference between weekday and weekend concentrations may either from no difference between traffic counts in weekdays and weekends or implied that there was contribution of non-traffic sources. In addition other factors affecting 1,3-butadiene concentrations on weekdays and weekends, such as traffic characteristics, quality of fuel, vehicle ages, meteorological conditions, and building characteristics of the area, may contribute to the difference of concentrations of ambient air pollutants between weekdays and weekends (Arayasiri et al., 2010; Yurdakul et al., 2014; Yurdakul et al., 2017; McGaughey et al., 2010).

In this study, the vehicle was classified into 7 types. It was found that the passenger car and Taxi car were the biggest volumes on weekend. The ranking from maximum volume to minimum volume were PC > Taxi > MC >VP >Bus> PMC > Truck respectively. On weekday it was found that motorcycle and passenger car are the most vehicles and sort by maximum to minimum as follow MC > PC > VP, Taxi > PMC > Bus > Truck respectively. It can be seen in Figure 1 that the percentages of each type of vehicles on weekend and weekday were not significantly different

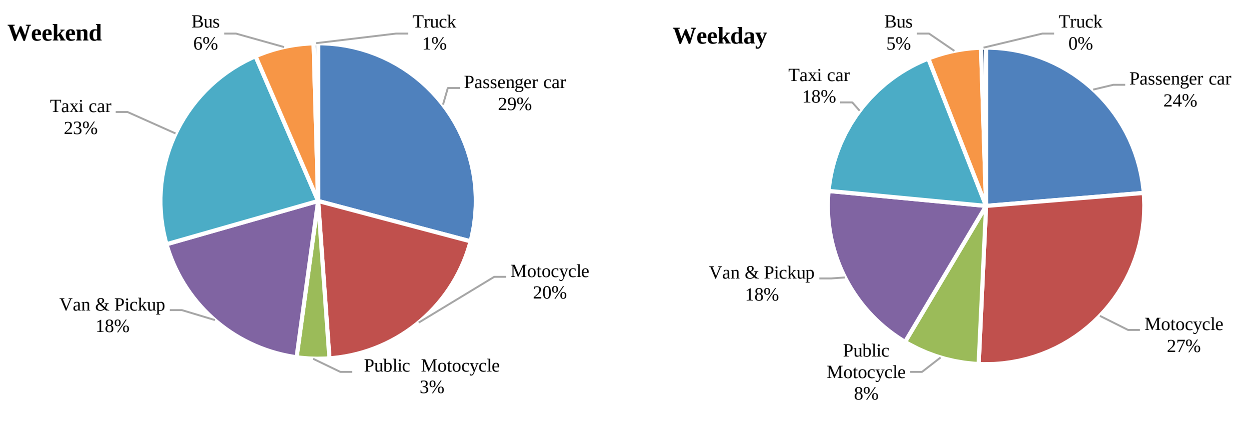

Figure 1. Comparison between types and the number of vehicles

The figure 2 shows that there might be some correlation between 1,3 – butadiene concentration and traffic volume. When the number of vehicle goes high, the concentration was also high at the same time. However it was found that in some periods, such as 11.00 – 12.00 am on both weekend and weekday, the number of vehicle travelled pass the monument was low but the concentration of 1,3 – butadiene was high. During such the periods, it was observed that there were heavy traffic jam, hence the vehicles travelled in very slow speed and/or brief stop and/or idling stop, which led the vehicle's engine to emit, including 1,3 – butadiene.

When considering the trend of average hourly concentrations of 1,3-butadiene and average hourly number of vehicles (Figure 2), the average hourly concentrations tended to be related to the average hourly number of vehicles during daytime (07.00

AM – 05.00 PM) both on weekends and weekdays, while during nighttime they seems to be unrelated. This implied that other factors such as sources other than traffic volume, meteorological factors may affect the concentrations of 1,3-butadiene during nighttime.

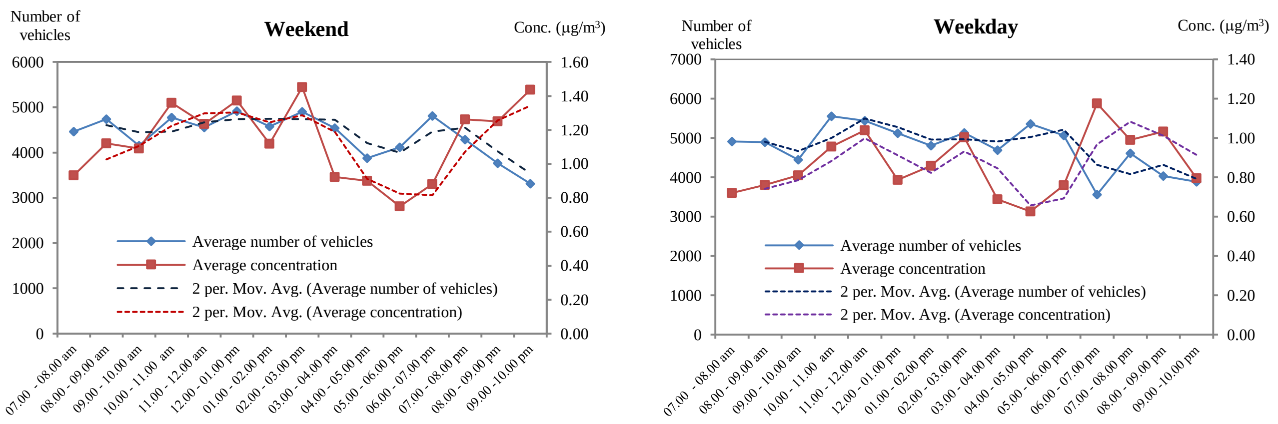

Figure 2. Comparison of average hourly concentrations of 1,3-butadiene and average hourly number of vehicles on weekend and weekday.

The average concentrations of 1,3-butadiene on weekdays tended to be higher during the nighttime than during the daytime but no trend was observed on weekend. However, when considering the average hourly concentrations on weekend shown in Figure 1, their concentrations during nighttime continued increasing from lowest concentration at 5.00-6.00PM. These results may indicate the rapid destruction of 1,3-butadiene by photoinitiated reaction during daytime (U.S. DHHS, 1992), while during nighttime when there is no sunlight this reaction does not occur. (Figure 3)

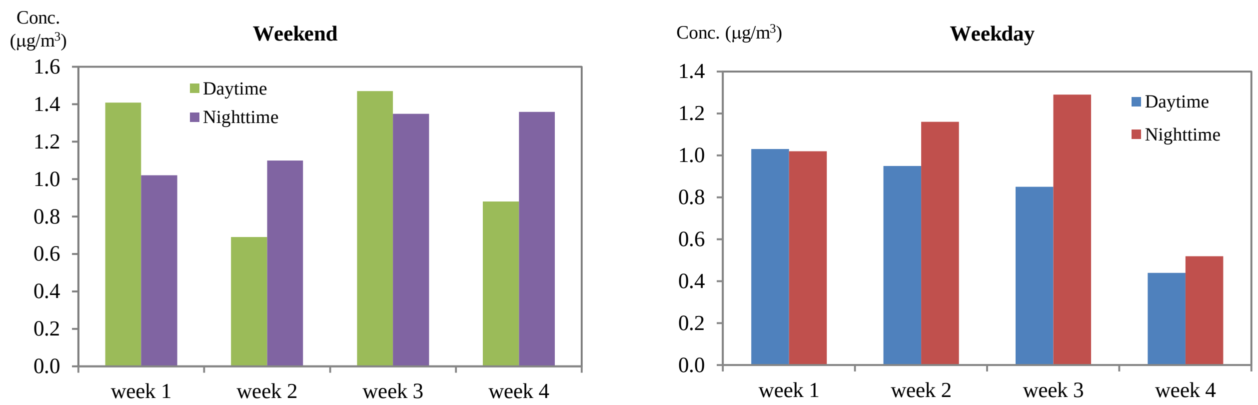

Figure 3. The average concentrations of 1,3-butadiene during daytime and nighttime on weekends and weekdays

3.1 Estimation of cancer risks from 1,3-butadiene exposure

The average 24-hour concentrations of 1,3- butadiene on weekends and weekdays are shown in table 2 The 24-hr concentrations of 1,3-butadiene were in the range of 1.07-2.08 µg/m^3 on weekend and 0.69-1.59 µg/m^3 on weekday which were lower than the national guideline value of less than 5.8 µg/m^3 but higher than the national standard annual average of less than 0.33 µg/m^3. (Laowagul et al., 2008) found that annual average concentration of 1,3-butadiene at roadside in Bankok in 2006 were in a range of 0.24 - 094 µg/m^3. The results from Environmental Research and Training Center, Thailand showed that 24-hr concentrations during November 2006-September 2007 at roadside sites in Bangkok were in a range of <0.12-1.8 µg/m^3. Their results were lower than what found in this study presumably due to lower emission sources in those sampling areas. However, other variables affecting 1,3-butadiene distribution must be considered.

Table 2. The average concentrations of 1,3-butadiene collected for 24 hours

Day / week	Concentration (μg/m³)				
	week 1	week 2	week 3	week 4	Average±SD
weekend	1.77	1.07	2.09	1.13	1.52±0.50
weekday	1.30	1.29	1.59	0.69	1.22±0.38

The average 24-hour concentrations of 1,3-butadiene measured in Table 2 were used as contaminant concentration in air or CA in μg/m³. In this study, Inhalation Unit Risk (IUR) of 3×10^{-5} per μg m^{-3} which is recommended by USEPA for 1,3-butadiene was used for estimation of cancer risks. In addition, age-dependent adjustment factors (ADAE) were applied to calculate lifetime cancer risk for lifetime expectancy of 70 years for various receptor-specific exposures. The results are summarized in Table 3-Table 6

Table 3. Minimum and Maximum lifetime cancer risks from exposure to 1,3-butadiene for age-specific residents

Receptor	Minimum risks (1×10^{-6})	Maximum risks (1×10^{-6})
Resident (child)		
0 to < 2 years	6.6	14.2
2 to < 3 years	1.0	2.1
3 to < 6 years	3.0	6.4
6 to < 11 years	4.9	10.7
11 to < 16 years	4.9	10.7
Adult (16-70 years)	17.7	38.5
Total risk (70 years exposure)	38.1	82.6

Table 4. Lifetime cancer risks from exposure to 1,3-butadiene for workers working from Monday to Friday and Monday to Saturday

Receptor	Mon-Fri (1×10^{-6})	Mon-Sat (1×10^{-6})
Workers (16-70 years)		
8 Hours/day exposure	6.4	6.8
12 Hours/day exposure	9.7	10.4

Table 5. Lifetime cancer risks from exposure to 1,3 – butadiene for vendors working during weekends and weekdays

Receptor	Weekday (1×10^{-6})	Weekend (1×10^{-6})
Vendors (16-70 years)		
2 Hours/day exposure	1.6	0.04
3 Hours/day exposure	2.4	0.10
4 Hours/day exposure	3.2	0.17
12 Hours/day exposure	9.7	1.6

Table 6. Lifetime cancer risks from exposure to 1,3- butadiene for bus passengers during weekends and weekdays

Receptor	Weekday (1×10^{-6})	Weekend (1×10^{-6})
Bus passenger (1h/d exposure)		
3-5 years	0.13	0.07
6-11 years	0.27	0.13
12-16 years	0.27	0.13

Table 6. Lifetime cancer risks from exposure to 1,3- butadiene for bus passengers during weekends and weekdays (cont.)

Receptor	Weekday (1×10^{-6})	Weekend (1×10^{-6})
Bus passenger (1h/d exposure)		
17-21 years	0.06	0.03
22-60 years	0.58	0.29
61-70 years	0.15	0.07
Total risk (70 years exposure)	1.5	0.73
Bus passenger (2h/d exposure)		
3-5 years	0.27	0.13
6-11 years	0.54	0.27
12-16 years	0.54	0.27
17-21 years	0.12	0.06
22-60 years	1.2	0.58
61-70 years	0.30	0.15
Total risk (70 years exposure)	2.9	1.5

It is not surprising that people who live near sampling site in this study and are exposed to 1,3-butadiene at the concentrations measured in this study from their birth through age 70 will have the highest lifetime cancer risks which are in a range of 38-83 times higher than the U.S.EPA benchmark of one in a million. However, in fact, people do not stay at home all the time, for example, they may be at work or school for approximately 8 hr/day. In this case, they will get exposed for approximately 16 hr/day respectively and have lifetime cancer risks lower to 2.54×10^{-5}- 5.5×10^{-5}. This indicates that lifetime cancer risks of residents significantly depend on their exposure time. For people who do not permanently live near this site such as workers, vendors and bus passengers, their lifetime cancer risks depends on their ages, exposure time, duration and frequency that they will be exposed to 1,3-butadiene. Adult people who have been working near this roadside for 8 to 12 hour per day during Monday to Friday and Monday to Saturday for 54 years will experience the lifetime cancer risk of 6.4-9.7 and 6.8-10.4 times higher than the USEPA benchmark of one in a million. Adult vendors will have lifetime cancer risk of 2.4×10^{-6}-9.7×10^{-6} when they work for 3-12 hour per day for 5 weekdays for 54 years. For bus passengers who spend approximately 1 or 2 hour per day waiting for their commutes, their lifetime risks will depend on their ages, exposure frequency and duration. Bus passengers in these scenarios can represent people who have been exposed to 1,3-butadiene while waiting for a bus during a trip for their study and working time period of their life. In case of students who spend 1 to 2 hour per day waiting for a bus from kindergarten level to undergraduate level (age 3-21), they will get lifetime cancer risks of 0.73×10^{-6} to 1.4×10^{-6}. Compared to this early-life exposure for 19 years (age 3- 21), later-life exposure for 39 years (age 22-60) gets lower lifetime risks of 0.58×10^{-6}-1.2×10^{-6} even through exposure duration is longer. This is also the case for the residents.

In conclusion, in order to decrease the lifetime cancer risks from exposure to 1,3-butadiene, the exposure concentration which is the results of the exposure time, frequency, duration and contaminant concentration must be reduced. In case that contaminant concentration cannot be easily reduced, the best way is to reduce exposure time, frequency and duration. If not possible, protect ourselves with suitable personal protective equipment such as wearing mask. In addition, early-life exposure to 1,3-butadiene for children must be avoided. However, according to the study of Thepanondh and Lertchaianon (2013), they considered VOCs species emitted at roadside stations that should give priority for management by considering concentrations and health impacts of VOCs and found that 1,3-butadiene should be given priority for the management even though it presented in much lower concentration than toluene or benzene. Therefore, 1,3-butadiene emission sources must be properly managed to reduce 1,3-butadiene emission into the atmosphere.

4. Conclusions

The hourly concentrations of 1,3-butadiene tended to increase from morning to early afternoon, then decreased from early afternoon to late afternoon, and increased again in the evening. During daytime both on weekends and weekdays, the variations of 1,3-butadiene and number of vehicles tended to have a relationship whereas during nighttime they seems to have weak relationship. This implied that there were the sources other than traffic.

Cancer risk of 1,3-butadiene was depend of the exposure time, frequency, duration and contaminant concentration.

5. Acknowledgements

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The Self-Heat Two-Stage Biomass Gasification Facility-Benefits, Questions and Prospects-

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Abstract

We developed the self-heat two-stage biomass gasification facility with automatic control system that makes it possible to constant the gasification conditions by keeping the temperature of each stage. One-week continuous trial using coniferous tree chip of every 200 kg per hour is carried out. Safety and stability of the automatic control system, and the homogenization of the quality has been demonstrated. We also present benefits, questions and prospects of this facility.

Keywords: Carbonization/ Hydrogen economy-society/ Renewable energy/ Reuse of waste

1. Introduction

While gasification technology using conventional woody biomass can easily obtain water gas by its combustion, it is essential to remove a large amount of tar that occurs at the same time, which is very heavy in terms of maintenance of the plant (Abdoulmoumine et al., 2015; Taniguchi et al., 2012). Therefore, unless it is a concentrated/ large-scale plant based on the boiler-steam turbine system, profit as a wood biomass power generation facility cannot be anticipated, which is a hindrance to the spread of distributed and small plants. In the most recent study, a large-scale (1.5 MWth) biomass gasification plant is already developed in China (Pei et al., 2018). This is a demonstration plant and continuous supply of huge amount of woody materials is necessary in practical use. Our concept is the spread of the compact gasification system (less than 0.2 MWth) in local area as Japan had done the charcoal in various places and is an application of Japanese traditional charming technology.

This study presents the original carbonization furnace systems driven by the self-heat of combustion. This has the same principle as the Japanese traditional flat kettle for "Bin-cho charcoal" or "Bin-cho-tan", which is produced by refining process under 1,100°C (e.g., Kishimoto, 1971; Kishimoto, 1998). It is higher temperature treatment than many other furnace systems (e.g., Sikarwar et al., 2016). Its structure of heat storage and air insulation achieves fully spontaneous combustion (Figure 1).

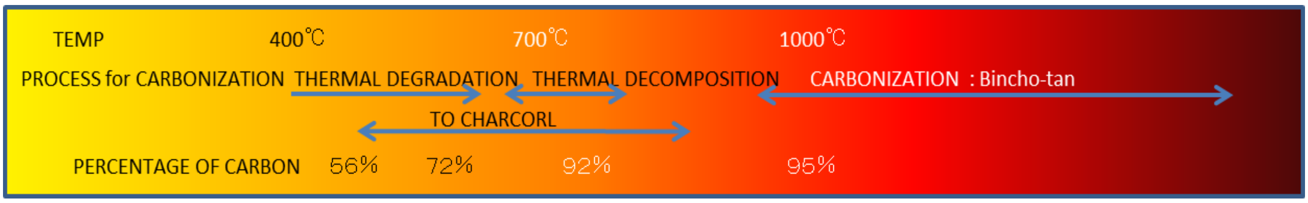

Figure 1. Process for carbonization and temperature of "Bincho charcoal"

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2. Methodology

2.1 System

The self-heat two-stage biomass gasification facility system is shown in Figure 2. This is also a demonstration plant (0.1 MWth) which supply 110 m^3/h of aqueous gas by putting 0.2 ton/h of wood chip with 15% of water content. Waste wood biomass resources, such as scrap wood cut below 35 mm size, wood shavings, woody roots, wood debris, dieback and wood chips, could be inputted into a carbonization furnace automatically and continuously in the first stage. The purified charcoal is transported to the gasification react furnace by conveyer, and stable supply of the aqueous gas, H_2 and CO are performed using liquid water and waste heat form the carbonization furnace in the second stage.

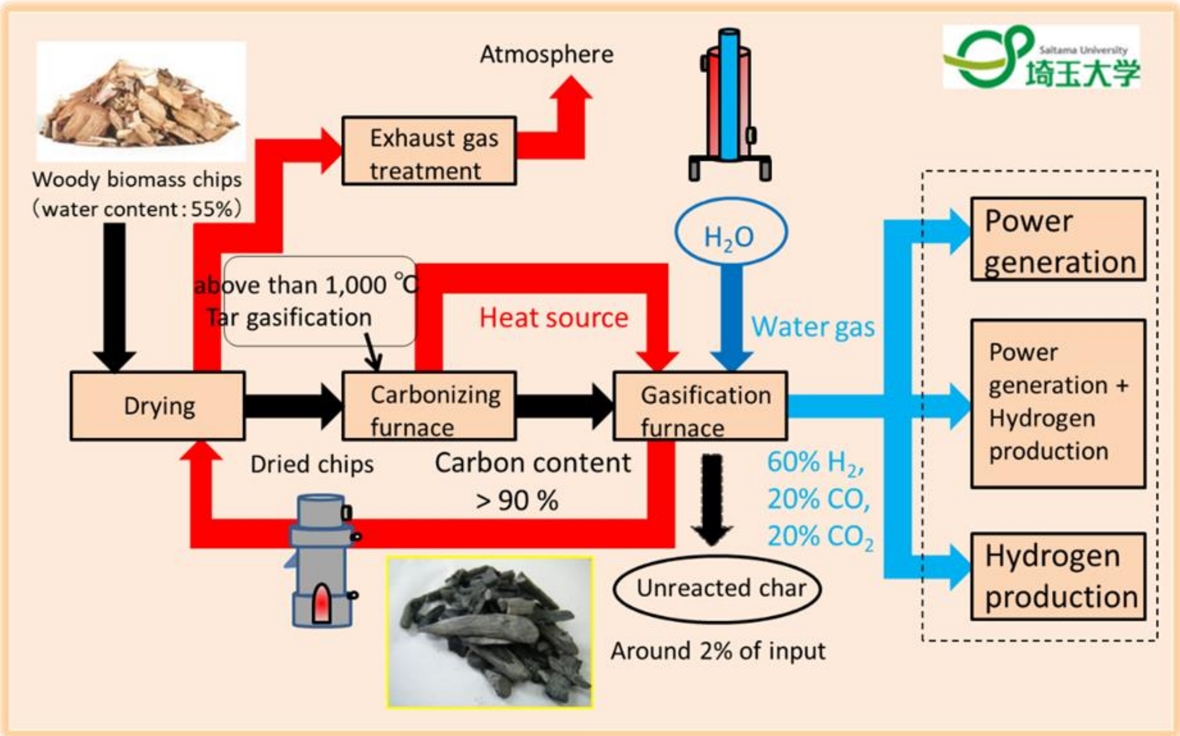


Figure 2. System configuration and material flow of the self-heat two-stage biomass gasification facility

2.2. Materials

In Japan, about 40% of the forest covered land is planted forest that had been used as energy until it has been replaced in fossil fuel. More than 90% of the planted trees are coniferous trees such as Japanese cedar and Japanese cypress (Statistics Bureau, Ministry of Internal Affairs and Communications, 2012), and tree thinning has been carried out for proper management. Recently, a large amount of thinning residues is abandoned and its effective use has been expected. Japanese cedar is commonly used for char producing and is applied for the plant, because it is porous rich and contains calcium and potassium which has a role of combustion improver (Walker et al., 1953; Kishimoto, 1979).

2.3 The automatic monitoring system

Collected Japanese cedar which has about 55% of the moisture content was cut below 35 mm size by woody chip product machine, and the cedar was naturally dried until 15% of the moisture content. However, woody material has some variations in the moisture content and shape. The automatic monitoring system which is PID (proportional-integral-derivative) controller regulate each the temperatures in tar combustion zone, T1, and material combustion/carbonization zone, T2, by controlling the air amount from blowers, and uniform quality of char is achieved (Araki, 2016). After char formation, crude materials are removed by refining process as “Smelting/extinction” (Kishimoto, 1979). The temperature decreases to

400ºC at the bottom of furnace, T3, and char is released by a turntable. The char temperature immediately decreases to the normal due to very low heat capacity of carbon with low crude material (Figure 3). Char is transported to gasification furnace by conveyor and is transferred to aqueous gases such as H_2, CO, and CO_2 by an endothermic reaction (Eq. 1-3).

Aqueous gas primary reaction:

$$C + H_2O \rightarrow CO + H_2 \quad (1)$$

Aqueous gas secondary reaction:

$$C + 2H_2O \rightarrow CO_2 + 2H_2 \quad (2)$$

Aqueous gas shift reaction:

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (3)$$

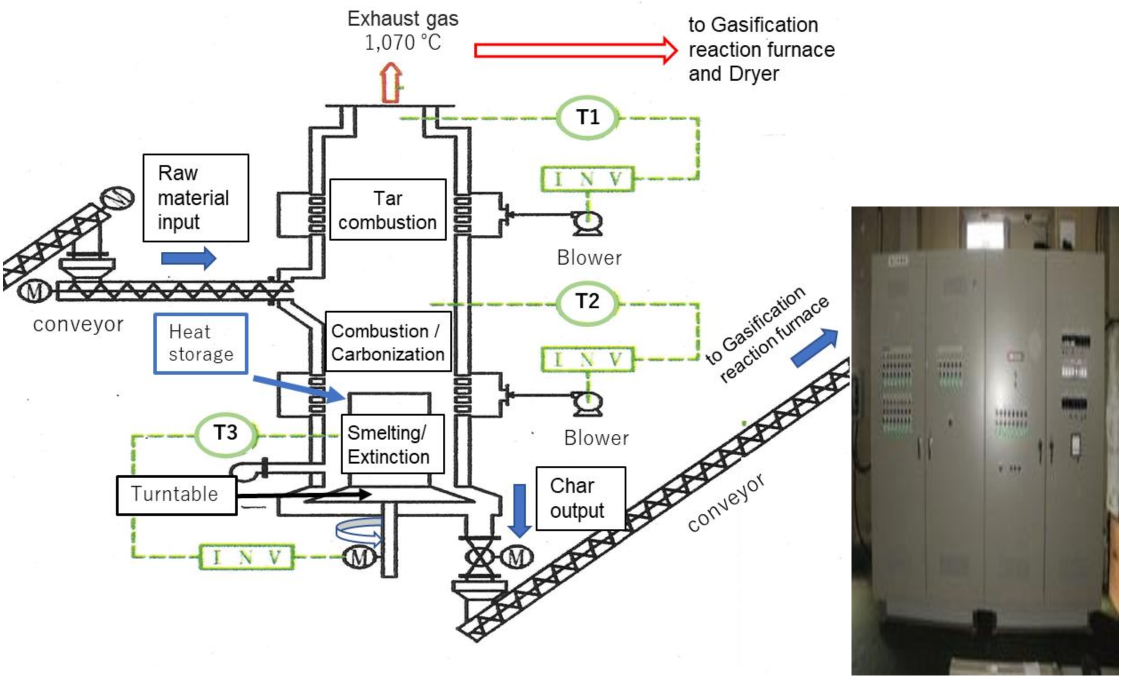


Figure 3. Material flow in carbonizing furnace and tar combustion by PID controller

3. Results

3.1 Temperature in carbonizing furnace

Temperatures at tar combustion zone, T1, material combustion/carbonization zone, T2, and smelting/extinction zone, T3 regulated by PID controller for 7 days test are shown in Figure 4. After the reaching 1,200ºC at tar combustion zone, stable temperatures were confirmed for T1 and T2. Although a spike-like change in temperature has been seen for T3, this is due to carbide release by turntable rotate.

3.2 Composition analysis during water gas production process

Mass carbonization yield is 20% under 1,200ºC combustion. The total produced gas amount is 110 m^3/h (input material amount is 200 kg/h), and the hydrogen and CO content of aqueous gas are about 66.27 vol% and 8.23 vol%, respectively (Figure 5). Other components except for N_2 carrier gas, are CO_2 23.41 vol%, CH_4 1.55 vol%, O_2 0.53 vol%, C_2H_4 0.01 vol% measured by gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan). Both endothermic reaction and aqueous gas shift reaction occurred under 700ºC.

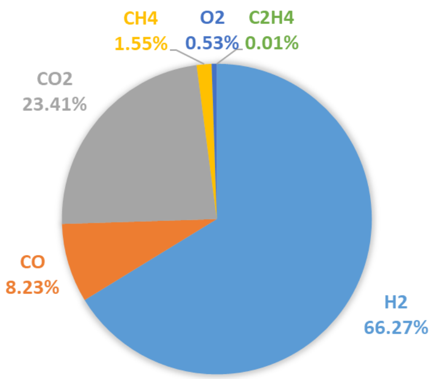


Figure 5. Detected gas components and ratio tested on January 19th, 2016 (except for N_2 as a carrier).

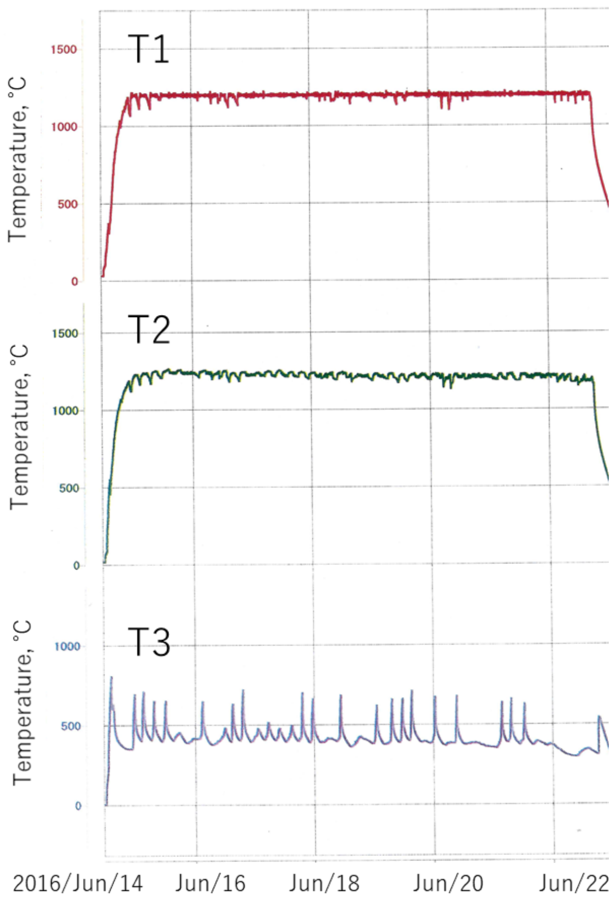
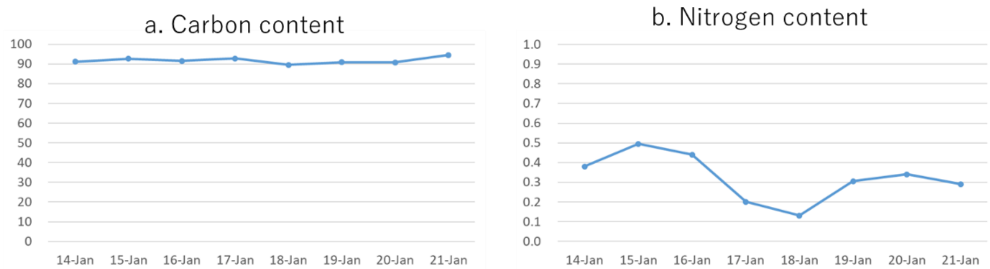
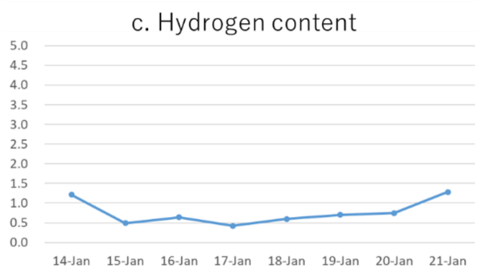


Figure 4. Time series of temperatures at tar combustion zone, T1, material combustion/ carbonization zone, T2, and smelting/extinction zone, T3 for 7days test.

The averages of 8 char samples of carbon, nitrogen, and hydrogen contents analysed by CHN coder (MT-3, Yanaco, Kyoto, Japan) produced from carbonizing furnace for 8 days continuous operation, from January 14th in 2018 to January 21st in 2018, were 91.5% (s.d.:1.5), 0.39% (s.d.:0.12), and 0.66% (s.d.:0.46), respectively (Figure 6(a-c)). From the sample piece provided within the equipment, to investigate the tar content in water gas and the exhaust gas, tar component as dissolved organic carbon was not detected by the total organic carbon analyzer (TOC-5000A, Shimazdu, Kyoto, Japan).





Figures 6. Results of element analyses by CHN coder for char from carbonizing furnace. (a) Carbon, (b) Nitrogen, (c) Hydrogen (Unit: %)

Stable supply of uniform gas composition and uniform water gas amount was demonstrated. For 8 days test, coal dust explosion did not occur, and there was no tar adhesion in the plant system.

3.2 Other characteristics

Released CO_2 gas can be used for activation of the charcoal, and the surface area of charcoal will be greatly increased. The present surface area is over 300 m^2/g. The comparison of other temperature treatment results is shown in Figure 7. It will be expected around 1,000 m^2/g, if it is activated. At this moment unreacted char is released as 2% weight of raw woody chip. This can be utilized as soil remediation equipment (e.g., Lehmann et al., 2003). As the other characteristics of the system, since the temperature of waste heat after the gasification reaction is over 600°C., the exhaust can be used for drying the raw materials which has 55% of the water content.

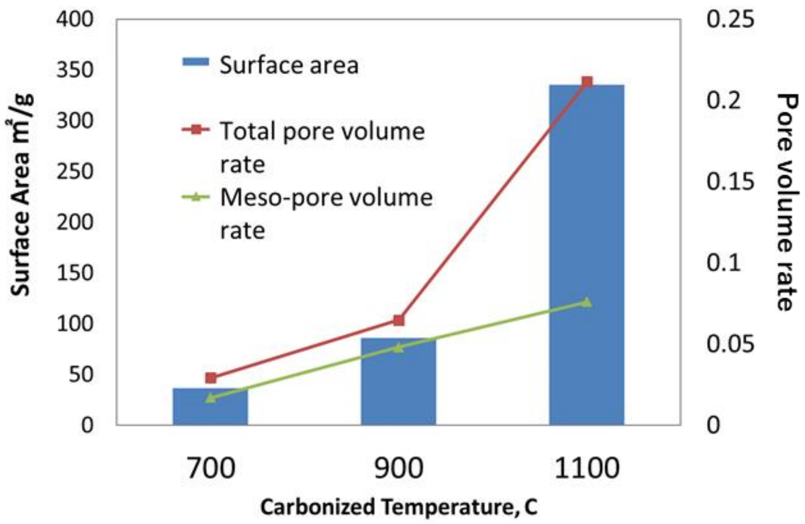


Figure 7. The surface area of unreacted char products from gasification react furnace.

4. Conclusions

The system that we introduced here, is not use of aqueous gas directly generated by combustion which contains tar, but gases obtained by pyrolysis gasification reaction of “purified char”. Purification of this carbide is 90% or more based on the "white coal" or “Bin-cho-tan” production method cultivated for a long time by craftsmen (1st step). This is the reason why smooth gas conversion into hydrogen, carbon monoxide by simple endothermic reaction and shift reaction with water vapor is satisfied (2nd step). The proportion of hydrogen in the water gas reaches the theoretical value of 60%, and impurities in the raw material are extremely small, so that high purity water gas.

Benefits of the presented system are summarized as below,

- Tar-free: Complete decomposition of tar component at 1,200°C in carbonizing furnace and the released exhausted gas is utilized for the gasification reaction and drying the raw materials, respectively.
- Carbon offset: No necessary auxiliary fuel other than for the ignition at start-up. Only the energy of wood chip combustion is released.
- High-quality Gas: No raw material remaining in gasification furnace after carbonization of chips due to char refining process, “Smelting/extinction”. The four nines of hydrogen content of water gas reached about 60 vol%, which is equal to theoretical value.
- Power generator or hydrogen production: The final stage of the facility, generator or hydrogen separation device, can easily be selected on depending on the use.

5. Acknowledgements

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Direct Contact Membrane Distillation for Decolorization of Reactive Dye Wastewater

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Abstract

It is addressed that the dye wastewater from textile industry causes significant impact to water environment. The treatment of textile wastewater is important because of its undesirable color and the breakdown process. Nowadays, the membrane distillation is getting interests for treatment of various wastewater as well as for water purification. In this work, the efficiency of the direct contact membrane distillation system for decolorization of synthetic Black-5 reactive dye wastewater (400 ADMI) has been investigated with the polyvinylidene fluoride hydrophobic membrane at three different feed temperatures of 40ºC, 50ºC and 60ºC as the feed temperature is one of the driving forces for this system by theoretically. The obtained permeate fluxes were 0.75 kg/(m^2·h), 2.12 kg/(m^2·h) and 3.29 kg/(m^2·h), respectively for each feed temperature. It was found that the feed temperature of the system could affect the obtained permeate fluxes. High quality of permeate water and high performance of decolorization efficiencies were achieved. The effect of temperature polarization coefficient, TPC value on permeate flux was also discussed.

Keywords: Dye wastewater/ Direct contact membrane distillation (DCMD)/ Feed temperature/ Flow rate

1. Introduction

The textile industry is becoming bloom among the industrial fields. This industry consumes the large amount of water especially in dyeing and finishing processes.

The compositions and the characteristics of wastewater are dependent on each process. The types of dyes and the auxiliary chemicals make the severe pollution to the environment. The primary source of wastewater in textile industry is from dyebath and the wash water. The water consumption is more than 100 m^3 per one ton of product from textile industry in Thailand (Tubtimhin, 2002).

Since 1963, membrane distillation has been introduced as an innovative technology. In recent years, it becomes as a popular technology among the researchers and the scientists. It has an attraction that it can give the 100 percent rejection of non-volatile components theoretically. Membrane distillation has been tested and researched in different fields e.g., desalination, food production, brine concentration and others. And other applications are the removal of ethanol and other volatile compounds. For the wastewater treatment, it can be considered to apply for various sources; textile and colored industry, olive mill, petroleum industry, mining industry, dairy industry, coal gasification and rubber plant industry (Thomas et al., 2017).

2. Theory

Membrane distillation method is the thermal-driven process using the hydrophobic material for the water treatment. There are four configurations of the membrane distillation as direct contact membrane distillation (DCMD), vacuum membrane distillation (VMD), sweeping gas membrane distillation (SGMD) and air gap membrane distillation (AGMD) (Belessiotis et al., 2016).

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DCMD process is mainly focused in this paper. The wastewater from the hot compartment can give the passage of the water vapor through the membrane sheet. The cooling water can induce the condensation of water vapor coming out from the membrane sheet. The permeate flux occurs and the volatile elements only can pass through the membrane sheet at the stable condition.

The driving force for DCMD is the partial vapor pressures through the membrane. The mass flux J (kg/(m^2·h)) is directly proportional to the vapor pressure difference through the membrane material and it also has the relationship with the membrane coefficient. The vapor pressure can be calculated by the Antoine equation with the temperature difference. So, the driving temperature can govern to the flux. (Lawson and Lloyd, 1996). The vapor pressure for pure water can be calculated by the Antoine equation as below.

$$P_{vapor} = \exp\left(23.238 - \frac{3{,}841}{T_m - 45}\right) \quad (1)$$

Where; P_{vapor} and T_m are the pressure and the membrane temperature difference (K).

Membrane distillation is the non-isothermal process. There is the heat loss during the separation process because of the heat transfer regions. The heat transfer region can be divided into three parts; the heat transfer from the feed bulk to the hot side boundary layer of membrane, the heat transfer through the membrane layer and the heat transfer from the cold side boundary layer of membrane to the permeate water in the cold side. The convection also takes place in this condition (Qtaishat et al., 2008).

2.1 Temperature polarization coefficient effect (TPC)

The temperature from the membrane surface differs from the temperature from the bulk water because of the loss of thermal driving force by the thermal boundary layer resistances. The temperature from the feed bulk (T_f) is higher than the temperature of the membrane surface of permeate side (T_{mf}) and the temperature of the membrane surface of permeate side (T_{mp}) is also higher than that of the permeate bulk (T_p). So, the temperature polarization is the ratio of the boundary layer temperature difference to the feed and permeate bulk temperature difference. The reduction of TPC makes to increase the mass and heat flux. To improve the TPC, the flow design, the membrane characteristics, the temperature difference and the spacers to promote can be controlled for the optimum flux (Manawi et al., 2014).

$$TPC = \frac{T_{mf} - T_{mp}}{T_f - T_p} \quad (2)$$

Where TPC is the temperature polarization coefficient and T_{mf}, T_{mp}, T_f and T_p are the temperatures at the feed membrane surface, the permeate membrane surface, the feed bulk and the permeate bulk respectively (K).

3. Methodology

The type of membrane is flat sheet type (hydrophobic polyvinylidene fluoride (PVDF) from Sterlitech Corporation) and 0.1 µm pore size, 45 µm thickness and the membrane area is 140 cm^2. The membrane cell is acrylic from Sterlitech Corporation. The feed solution was heated by the hot water bulk (WNB-7 from memmert) and the permeate water was cooled down by the chiller (Xi'an Heb Biotechnology Co., Ltd (CCA-420)) as a closed loop system. The membrane sheet was sandwiched between the hot and cold compartments.

The system layout is shown in Figure 1. The two peristaltic pumps were used to transport the water and the accumulated permeate water was measured by the electronic balance from A&D Company (GX-6100) within the time interval. The wastewater was prepared in the laboratory as the synthetic reactive dye wastewater using the Reactive Black dye (molecular formula - $C_{26}H_{21}N_5Na_4O_{19}S_6$, molecular weight - 991.82 g/mol, maximum wavelength -597 nm, color index number - 20505) from SIGMA-ALDRICH company. The color measurement (ADMI) was determined by Spectroquant® Prove Spectrometer 100 and prepared by the RO water to get the required concentrations.

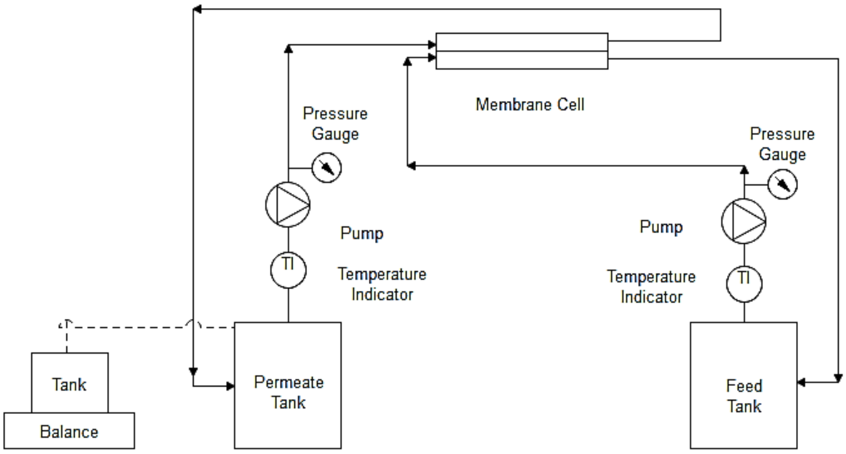

Figure 1. Schematic diagram of DCMD experimental setup

In this research, chloride (50 mg/L), sulfate (100 mg/L), phosphate (5 mg/L), nitrate (5 mg/L) and color (400 ADMI) were considered as the auxiliary components. Sodium chloride, magnesium sulfate, potassium dihydrogen orthophosphate and sodium nitrate were also added for the synthetic wastewater.

3.1 Permeate flux and removal efficiency

The flux can be calculated by the membrane as the below equation.

$$J = \frac{\Delta W}{t \times A} \tag{3}$$

Where; J is the flux (kg/(m^2·hr)), ΔW is the difference weight of permeate water in time interval (kg), t (h) is the time that is accumulated by the permeate water and A is the membrane area (m^2).

The color removal efficiencies can also be calculated from ADMI unit of the permeate water. Spectroquant® Prove Spectrometer 100 was used to measure ADMI unit for the color. It can give the direct ADMI value by measuring the absorbance. The removal efficiency can be calculated by the below equation.

$$\text{Rejection} = \left(1 - \frac{C_p}{C_f}\right) \times 100 \tag{4}$$

Where; C_f=initial concentration, C_p=permeate concentration after treatment.

4. Results and Discussions

4.1 Effect of feed temperatures

According to the theory, with the higher temperature difference, the higher flux is obtained. To study this test, the system was operated with 1 L/min flow for both sides and the permeate temperature was kept constant at 20°C. The resulted fluxes can be shown in Figure 1 for the different feed temperatures.

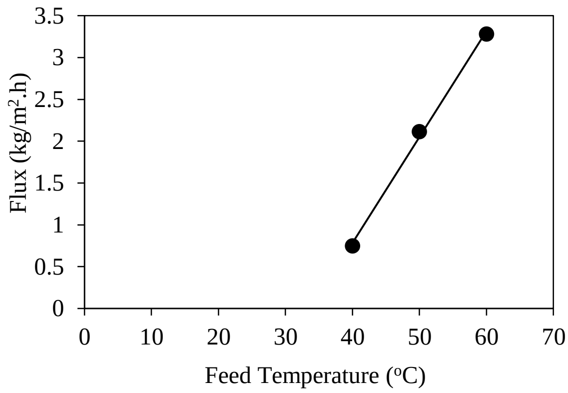

Figure 1. Flux with different feed temperatures

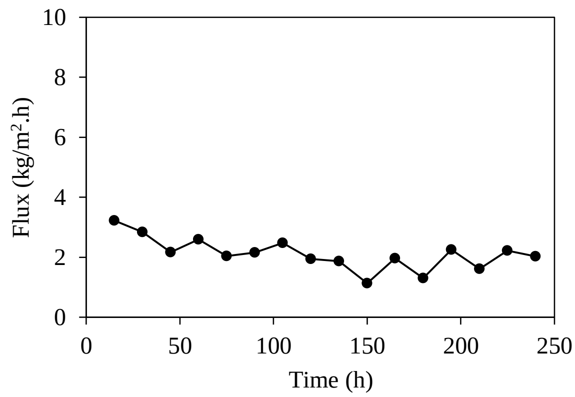

Figure 2. Flux (T_f=50°C, T_p=20°C and Q=1 L/min)

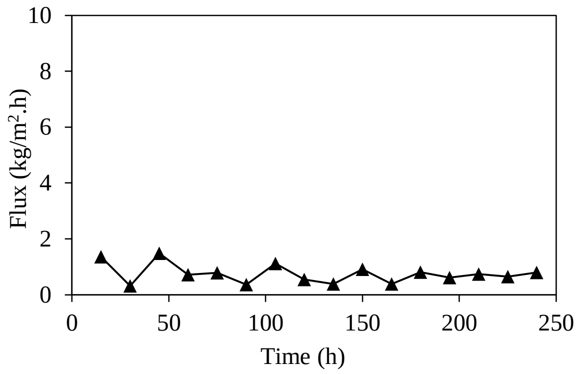

Figure 3. Flux (T_f=40°C, T_p=20°C and Q= 1 L/min)

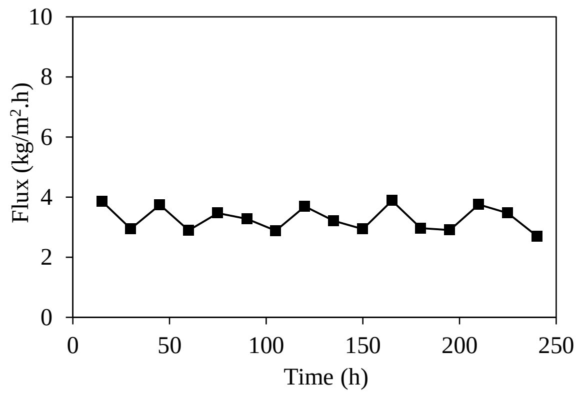

Figure 4. Flux (T_f=60°C, T_p=20 °C and Q= 1 L/min)

The permeate fluxes for 40°C, 50°C and 60°C were 0.75 kg/m^2·h, 2.12 kg/m^2·h and 3.29 kg/m^2·h, respectively. The higher feed temperature can produce more vapor and can increase the partial vapor pressure across the membrane surfaces that can create the more flux.

4.2 Flow rates with fluxes

The effect of flow rate on permeate flux is shown in Figure 7 with the feed temperature and permeate temperature is kept at 50°C and 20°C. The effect of flow rate is related the mass resistance of the system to get the permeate flux. It can reduce the temperature reduction on the membrane surface due to the higher flow rate. The flux 1.97 kg/m^2·h, 2.12 kg/m^2·h and 3.54 kg/m^2·h for the flow rate 0.5 L/min, 1 L/min and 1.5 L/min were obtained. The increased 7% of permeate flux was obtained at 1 L/min and 79% increased at 1.5 L/min more than the flux of 0.5 L/min.

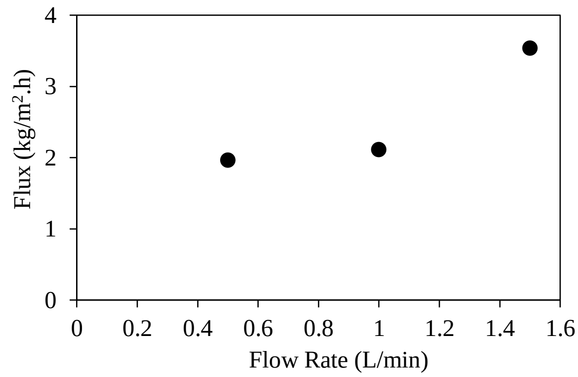

Figure 7. Effect of flow rate for the flux (T_f=50ºC and T_p=20ºC)

The flow rate also affects to the temperature polarization coefficient (TPC) value that can influence to the water permeate flux. The effect of higher flow rate is to increase the heat transfer coefficient and can reduce the effect of temperature polarization. This means that the higher the temperature polarization coefficient can give the greater transmembrane temperature difference (Martínez-Díez et al., 1999). The greater driving force enhances the flux. The resulted experimental values are shown in Figure 9. The TPC values are ranged from 0.5 to 0.63. The flow rate 0.5 L/min can reduce TPC value up to 0.5.

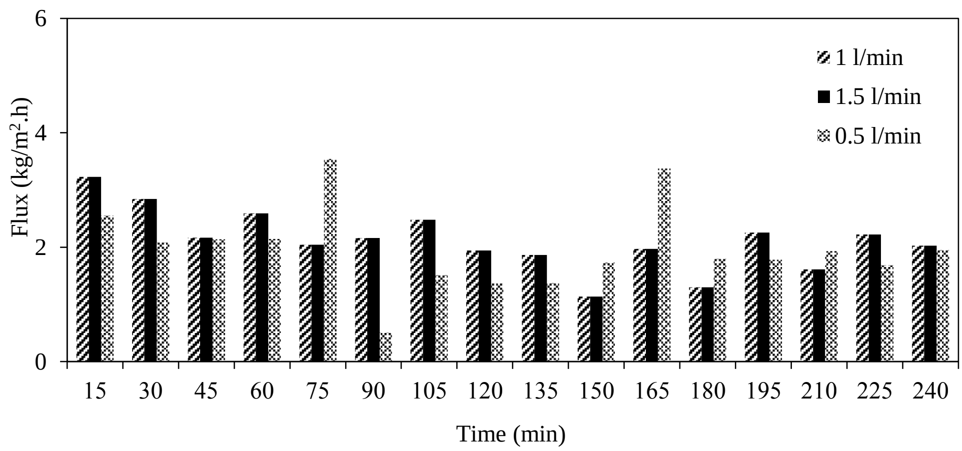

Figure 8. Flux Vs flow rate (T_f=50ºC and T_p=20ºC)

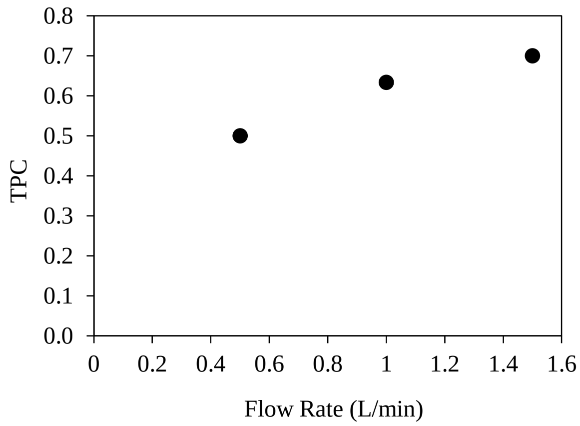

Figure 9. TPC (T_f=50°C and T_p=20°C)

4.3 Removal efficiency

The rejection efficiency for color is shown in Table 1. The flow rate is 1 L/min.

Table 1. Water parameters for different temperatures (T_p=20 °C)

	Reactive dye wastewater	Permeate water		
		T_f = 40°C	T_f = 50°C	T_f = 60°C
pH	7±0.3	7±0.3	7±0.3	7±0.3
Color (ADMI)	400	5.4	12.7	10.9

There are no significant changes for pH of every temperature condition. The efficiency of color removal is calculated by Equation Error! Reference source not found.. The color removal percentage is in the range from 96 to 98%. The color removal efficiency is highly satisfied for direct contact membrane distillation. The operation time is 4 hours, so for a long- term operation, the membrane fouling and the breakage of membrane might change the water quality and the permeate flux. For the real application of industry, the operating conditions and the performance of DCMD system should be stable during the long- term operation condition.

5. Conclusion

In conclusion, DCMD is the feasibility method for the treatment of textile wastewater. It can give the satisfied results for the treatment of reactive dye wastewater with the operating parameters (temperature and flow rate). By system the higher the temperature, the more flux can be given with low energy. The effect of flow rate and TPC for the colored wastewater were also described in this research. It could be suggested that the direct contact membrane distillation is feasible as the effective treatment system to recover and reuse treated water in the textile industry.

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The Application of Tannin Extract from Plants to Reduce the Concentration of Arsenic

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Abstract

This research prepared the tannins extract from agricultural wastes i.e., rice straw, tea leaves, longan leaves and banana leaves with fresh water for 2 months. The highest concentration of tannin was banana leaves (6,464.98 mg/L) followed by longan leaves (4,478.99 mg/L), rice straw (4,000.00 mg/L) and tea leaves (1,397.95 mg/L) respectively. Tannins extract were used for arsenic treatment in synthetic waste water. The result showed the efficiency of arsenic removal by 10, 20, 30, 40 and 50 mg/L of tannins concentration from banana leaves were 50.82%, 52.54%, 54.56%, 58.42% and 51.01% respectively, tea leaves were 69.74%, 73.42%, 60.29%, 63.60% and 70.54% respectively, longan leaves were 61.03%, 56.07%, 54.36%, 40.01% and 51.80% respectively and rice straw were 53.68%, 46.70%, 55.23%, 49.22% and 54.11% respectively which indicates that there are no significant differences to arsenic reducing. In addition, increasing of pH in solution from 2.5 to 5.5, 6.5, 7.5 and 8.5 led to decrease of arsenic removal efficiency.

Keywords: Tannin extract/ Banana leaves/ Longan leaves/ Tea leaves/ Rice straws/ Arsenic

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Life Cycle Assessment of Two Limestone Quarries in Thailand with Environmental Footprint Technique

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Abstract

Limestone is the natural resources that is commonly found in every part of Thailand through quarrying which is widely used in construction industry due to its mass volume and constant qualification. Limestone also has a major part in both economic growth in Thailand. This study accumulated the data of raw materials, resources and energy consumption based on the production process of 1 ton of 1 inch limestone from 2 limestone quarries in 2016. The data obtained is used to analyze and compare environmental impact in 3 aspects including global warming, fossil depletion and acidification through Life Cycle Assessment and Environmental footprint techniques. According to the study, the manufacturing process can be divided into 5 processes including, (1) preparing and drilling (2) blasting (3) excavating (4) transporting and (5) milling. These processes have consumed diesel fuel, electricity and ammonium nitrate which caused an environmental impact on human health at 0.2893 pt./ton for limestone quarry A and 0.1759 pt./ton for limestone quarry B. In addition, for ecosystem aspect, limestone quarry A caused 17.1437 pt./ton while limestone quarry B caused 6.6873 pt./ton. Lastly, acidification aspect, quarry A caused 0.0872 pt./ton while limestone quarry B caused 0.0819 pt./ton. In conclusion, ReCiPe endpoint calculation of quarry A can cause impact on human health at 39.20%, species loss per year at 60.99% and higher price of future generations available resources at 6.08% more than quarry B. It must be noted that the large difference of the impact is due to many factors in the manufacturing process, consumption rate, machine sizes and technology, purpose of production, quarry and organization size. These factors are relate to a different problem that each quarry has engaged, and also produce the environmental impact as shown in this study.

Keywords: Limestone quarry/ Environmental impact/ Life cycle assessment/ Environmental footprint

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Efficiency of Activated Carbon and White Charcoal from Textile Dyeing Industry in Synthetic Wastewater

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Abstract

This research prepared the tannins extract from agricultural wastes i.e., rice straw, tea leaves, longan leaves and banana leaves with fresh water for 2 months. The highest concentration of tannin was banana leaves (6,464.98 mg/L) followed by longan leaves (4,478.99 mg/L), rice straw (4,000.00 mg/L) and tea leaves (1,397.95 mg/L) respectively. Tannins extract were used for arsenic treatment in synthetic waste water. The result showed the efficiency of arsenic removal by 10, 20, 30, 40 and 50 mg/L of tannins concentration from banana leaves were 50.82%, 52.54%, 54.56%, 58.42% and 51.01% respectively, tea leaves were 69.74%, 73.42%, 60.29%, 63.60% and 70.54% respectively, longan leaves were 61.03%, 56.07%, 54.36%, 40.01% and 51.80% respectively and rice straw were 53.68%, 46.70%, 55.23%, 49.22% and 54.11% respectively which indicates that there are no significant differences to arsenic reducing. In addition, increasing of pH in solution from 2.5 to 5.5, 6.5, 7.5 and 8.5 led to decrease of arsenic removal efficiency.

Keywords: Tannin extract/ Banana leaves/ Longan leaves/ Tea leaves/ Rice straws/ Arsenic

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Tracking the Sedimentary Heavy Metals for Better Understanding the Anthropogenic Impact on Watershed Environment: A Study in Lower Chao Phraya River Watershed, Thailand

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Abstract

Developing countries in Asia such as Thailand, are facing a common target of sustainable development, particularly sustainable urbanization. Degradation of the environment, especially the aquatic environment, with urban expansion and intensive human activities is reported all over the world, reflecting an adverse impact due to unsustainable urbanization and industrialization. Heavy metals are a group of highly concerned environmental contaminants that release into the environment due to various human activities such as mining industries effluent, vehicle exhaust, fossil fuel combustion, and waste incineration. The persistence of heavy metals in the environment meanwhile provides a possible approach to explore the roles of anthropogenic activities on changing the environment. The Great Chao Phraya River, originating from four major tributaries of the north and flowing southward to the Gulf of Thailand, plays a dominant role in the social-economic development of the nation. It serves as the main source of water supply in the watershed for domestic, agricultural, and industrial uses. However, the river, especially in the downstream, is facing severe aquatic environmental risk with organic and pathogenic pollution, which are potentially related to anthropogenic inputs. It was reported that over 60% of wastewater from industries, urban, and rural inflows are directly discharged into the river without treatment. This study aimed to identify the temporal and spatial distribution of eight concerned heavy metals, namely lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn), in the lower Chao Phraya River via sampling and analyzing the sediment cores from the upstream and downstream of each tributary of the river. The contaminant distribution pattern was further linked with the land use-based and energy-based anthropogenic impact to understand the potential mechanisms on human impact on environment change. Dramatic increase in sediment heavy metal concentration was found at the downstream of the Chao Phraya River and Tha Chin River, suggested the significant impact on environment caused by urban expansion. On the other hand, stable Pb isotope analysis was adopt to tract the potential sources of Pb in sediment. A clear shift of the Pb emission source from natural base to gasoline emission was identified in Tha Chin River, indicating the energy-based anthropogenic activity is another major driver of contaminants distribution. However, the contaminant source identification faced a great challenge due to the little end member information in Thailand. Further researches are needed to address these limitations in terms of better understands on the impact mechanisms caused by anthropogenic activities in this region.

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Sampling and Isolation of *Ganoderma Lucidum* in Que Phong, Nghe An Province

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[b]*Student of Da Nang University of Science and Technology*

Abstract

A giant mushroom which was found in Que Phong, Nghe An province, was determined as *Ganoderma lucidum* by observing the morphological characteristics. The best medium for the first propagation was Rapper medium and the hyphae filled up erlenmeyer flask in 7 days. The best medium for the second propagation was brown rice and the hyphae filled up in 15 days. The medium which was appropriate for giving high yield of fruiting bodies was acacia logs and fruiting bodies could be harvested after 60 days with the biological efficiency of about 0.947%.

Keywords: *Ganoderma lucidum*/ Mushroom

1. Introduction

Ganoderma lucidum also known as Van Nien Nhung, Truong Tho mushroom are classified as a giant family. It is a noble herb that is classified as a super high-quality preservative (protection of the liver), detoxification, strength, brain (brain), sputum, diuretic, is a precious medicine used to make medicines for treatment (Tibuhwa, 2012). *Ganoderma lucidum* was found in some primeval forests in Laos and Vietnam. This mushroom only grows on dead green limon so picking is difficult.

Nghe An is a region with high biodiversity, especially in northwestern of Nghe An, including Que Phong. This is where there is a lot of primary forest-ideal conditions for *Ganoderma lucidum* growing and development.

Currently, Vietnam has not researched on *Ganoderma lucidum*, so the topic will *provide Ganoderma lucidum* for research and conservation of rare species of fungus.

2. Methodology

2.1 Materials

Sample source: *Ganoderma lucidum* collected in Que Phong district, Nghe An province.

The isolation of PDA: 200 g of potato, 20 g of dextrose, distilled water of 1000 mL (Nguyen Lan Dung, 2003).

Raper media: 2 g Pepton, 2 g yeast extract, 0.5 g $MgSO_4H_2O$, 1 g K_2HSO_4, 20 g Glucose, 1,000 mL distilled water (Nguyen Lan Dung, 2003).

PSA media: 100 g of potato, 200 g of glucose, distilled water of 1,000 mL (Nguyen Lan Dung, 2003).

Fruiting bodies media: Natural Acacia logs, rubber sawdust and rubber sawdust + other components.

2.2 Methods

2.2.1 Sample collection and identification

Ten samples of *Ganoderma lucidum* in Que Phong, Nghe An delta were investigated to identify, isolate. The samples were collected in sterile boxes and transferred to the laboratory. After pickled mushroom, washed surface with sterile distilled water, soaked in alcohol at 70^{o}C for 5 minutes, removed for natural drying, then immersed in 0.1% javelin solution for 1 minute, wipe dry, double mushrooms in sterile culture, use cuttings of inoculated fungal tissue into sterile agar media were prepared. The culture is incubated in the dark at room temperature (28^{o}C) and tested every day,

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ensuring no infection with other microorganisms. Then purified by continued culture of the cultures on PDA surfaces supplemented with chloramphenicol 2-3 times. The cultured were cultured on an SNA medium on the slanting method (Nguyen Lan Dung, 1983).

The study mushroom strain was identified based on the identification tag and morphological characteristics: Using the identification key of Mossebo et al. (2009) (Mossebo et al., 2009). This is a revised index and added to Heim (1977) and Pegler (1977) (Heim, 1977; Pegler, 1977). Based on Tibuhwa (2012), Karun and Sridhar (2013) describe the shape, color, size of mushroom capsule, mushroom stem, spore size (Tibuhwa, 2012; Karun, 2013).

2.2.2 Propagation of mushroom spawn

Pure mushroom culture were grown on PDA medium at 26-28^{o}C in darkness, multiplication of class I and class II according to Nguyen Lan Dung (2003) (Nguyen Lan Dung, 2003).

After the fiber spread, put in the house to open the neck neck temperature of 24-28^{o}C, the air humidity 85-90%.

2.2.3 Raise

Embryo transfer after incubation to 26-28^{o}C incubation, dark, airy.

2.2.4 Evaluation of biological performance

Picking mushrooms weighed dry weight, determination biological efficiency of mushroom through the first harvest after netting for 60 to 70 days.

2.2.5 Statistical analyses

The data is processed using Microsoft Excel 2010 and Minitab 17.0.

3. Results

3.1 Identification method

The natural *Ganoderma lucidum* is yellow or brownish-black. The top of the mushroom has many shiny spots, the bottom smooth. Mushroom stem 10-15 cm long. Stem mushroom and mushroom capsule, thin and smaller mushroom growing, difficult to break. Mushroom stalk with wood sticks of lima (Figure 1) (Trinh Tam Kiet, 2011). Based on the fungal morphological characteristics of the *Ganoderma lucidum*, this mushroom is similar in character to the mushroom named science *Ganoderma Lucidum* is described by the key of Mossebo et al. (2009), Heim (1977) and Pegler (1977) Tibuhwa (2012), Karun and Sridhar (2013), also known as Van Nien (Mossebo et al., 2009; Heim, 1977; Pegler, 1977; Tibuhwa, 2012; Karun and Sridhar, 2013).

3.2 Determination of *Ganoderma lucidum* propagation media of I grade

Ganoderma lucidum isolation on PDA receiving media. This mushroom growing is quite strong, uniform fiber system, dense silk (Figure 2). At grade I, the treatment PSA, PSA supplemented coconut water and PSA supplements green bean sprout, sporangular silk spread slowly filament yarn small pieces, thin spars and weak mycelium. Treat PGA and Raper on the surface of the orchid fast, uniform filament, dense silk. However comparison between treatments, the results showed that have only difference radial of sporangular silk spread in all 3 states after 4, 6 and 8 days with statistically significant 95% confidence, (Table 1).

Table 1. Results of the survey of primary propagation medium

Treatment	Media	Radius of sparse (cm)		
		Day 4	Day 6	Day 8
1	PGA	1.21±0.01	2.15±0.02	3.88±0.02
2	PSA	0.84±0.009	2.05±0.008	3.61±0.01
3	PSA + coconut water	0.95±0,01	1.97±0.03	3.72±0.009
4	PSA + Green peas water	0.92±0,008	1.87±0.009	3.69±0.02
5	Raper	1.42±0,03	2.47±0.03	4.48±0.04

Note: Tables 1, 2, 3, 4, 5, 6: The mean values in the same column followed by the same letters indicate no statistically significant difference at probability levels 95%.

Figure 1. *Ganoderma lucidum* collected in Que Phong, Nghe An

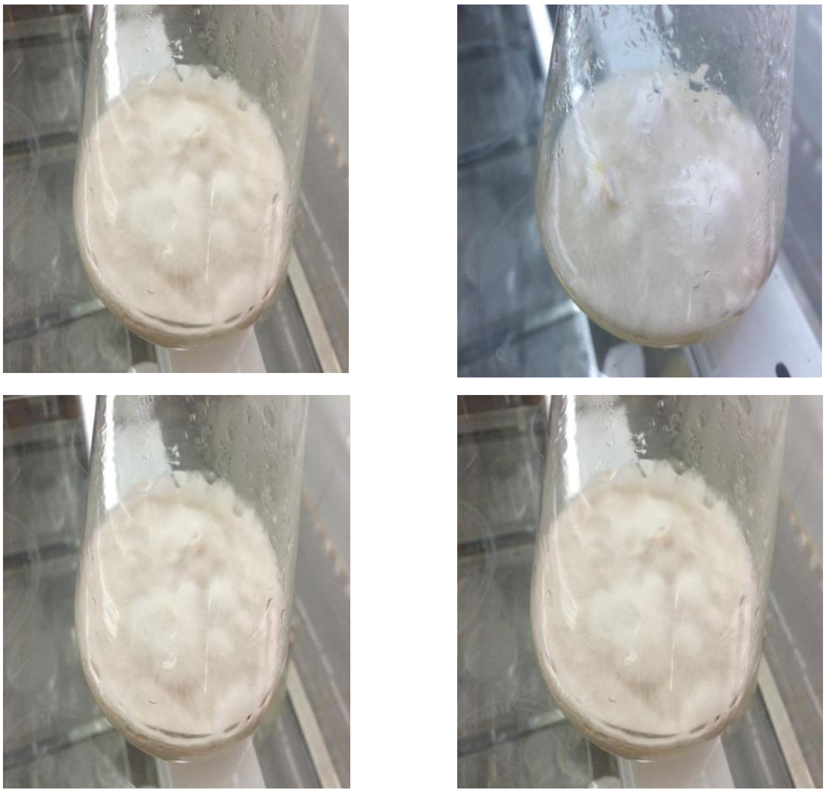

Figure 2. Mushroom hyphae on grade I media

3.3 Determination of secondary propagation media

In the secondary breeding medium, the spawning time in brown rice is very fast (11.4±0.01 days) than in rice (18.2±0.02 days) and corn (17.2±0.01 days). Brown rice contains many nutrients suitable for silk growing system. Result among the treatments showed, in the rice media the fastest spread and there are obvious differences 95% confidence level (Table 2, Figure 3).

Table 2. Results of the secondary propagation media

No	Medium	Spawning times (day)	
		50%	100%
1	Rice	7.7±0.01	18.2±0.02
2	Brown rice	5.6±0.01	11.4±0.01
3	Corn	7.3±0.03	17.2±0.01

Figure 3. Mushroom hyphae on secondary media

3.4 Determine the media of the fruit

Cultivation media was selected the natural acacia wood media with a full flowering time of 30 days. The medium which was appropriate for giving high yield of fruiting bodies was natural acacia and fruiting bodies could be harvested with the biological efficiency of about 0.947±0.04% (Table 3, Figure 4).

Table 3. Results of aquaculture medium survey

Treatment	Media	Quantity (gram)	Biological performance
1	Natural Acacia wood	11.29±0.10	0.947±0.04
2	100% rubber sawdust	10.09±0.09	0.801±0.03
3	90% rubber sawdust + 5% rice bran + 5% corn flour	11.21±0.08	0.941±0.02
4	95% rubber sawdust + 5% rice bran	10.65±0.06	0.897±0.03
5	95% rubber sawdust+ 5% corn flour	10.33±0.07	0.858±0.01

Figure 4. Hyphae after 30 days and cultured mushrooms

4. Discussion

The results of this study are similar to those of Le Dinh Hoai Vu (2008); Ho Thi Ba, Tran Nhan Dung, (2017). Continuing research to develop the optimal cultivation process for mushrooms to achieve the highest medicinal value.

5. Conclusions

Mushroom was discovered at Que Phong, Nghe An province are identified *Ganoderma lucidum*. Grade 1 of media is best rapper in 7 days silk full test, brown rice media is the media optimal breeding of the second grade in 15 days. Make the most suitable media is natural acacia logs for 30 days. Thu Corpses after 60 days of white silk and the biological efficiency of about 0.947%.

6. Acknowledgements

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Factors Affecting the Efficiency of Applying the Green Office Principles in Organization

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Abstract

This research investigated the factors that affect the efficiency of applying the green office principles in the organization. All of the participants in this study were from 30 organizations who joined the Green Office Project between 2016-2017 in Thailand. To use the green principles in the office would mean saving the energy consumption and reducing greenhouse gases released. In this study, an assessment to identify any factors that affect the efficiency of applying the Green Office principles from the project was done. A set of questionnaire was used in data collection and were analyzed using the descriptive statistical analysis and factor analysis. The results showed that there were 5 factors that affected the efficiency of applying the green office principles in the organization including Personal and Organization Action, Technology and Environmental of Organization, Organization Policy, Personal Status, Types and Size of Organization. These factors could be used for guiding the organization which joined the green office project and in planning to join another project to be better prepared and to be more efficient in managing future projects.

Keywords: Green office/ Green office principles/ Green operation/ Energy efficiency/ Worker participation/ Environment

1. Introduction

In recent years, the world economic growths are likely to be increasing as well as the numbers of the population. According to these, there have been increased energy using and resources using which grow apart in natural resources nowadays. The large amount of consuming may lead to lacking of natural resources. Most organization, both in government and public sectors whether it is public companies, university and collage etc., all of these organizations contribute the various social activities which benefit to the social potentiality. To get these activities well going need to use a lot of resources. These consuming could lead to the pollution such as water pollution, air pollution, and waste pollution and also released the Carbon dioxide to the atmospheres which is the main reason of Greenhouse effects as well. As we know, the Greenhouse effect could lead to the climate change and the Global warming (Ratanavaraha and Jomnonkwao, 2015).

In present, the global warming rate will still continue or accelerate. The greenhouse effect caused from several ways. Many of human activities can cause the global warming. As in Thailand, the economic sector played one of the major roles to running the country. The economic growth was related to the energy using as the real GDP is growing up in the same direction as the energy consuming.

The international organization foresee to the global warming issues, therefore most companies or the related organization push their sector forward to be the green office (Green office policy) and motivating their staffs and others who related in to concerned and joined into an environmental friendly way by their everyday activities and also improve the environmental awareness. In addition to the green office conception also bring cost saving for the organization and benefits to the environmental sectors as well. The global warming and also the climate change were occurring by one of the major caused by the human activities which created and released a lot of CO_2 and other greenhouse gases (Ratanavaraha and Jomnonkwao, 2015). The higher

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of greenhouse gases in the atmosphere will be the higher temperature. The majority of the world's emissions result from the electricity generation, transportation and other forms of energy production and use (USEPA, 2018).

Nowadays, many countries including Thailand are also concerned about the problem of greenhouse gases in the economic and society dimensions. Many related organizations starting to create the environmental helping policy which called the Low Carbon Economy.

2. Methodology

The number of people and organization for using to analysis in this study was the people in company that joining in the Green office project of Department of Environmental Quality Promotion (DEQP) Ministry of Natural Resources and Environment and also run by the Faculty of Environment and resources studies, Mahidol University. There were 39 corporations in this research which was the data in between 2016-2017.

This study was conducted to surveying and collecting data of the office or organization that joining in the Green Office Project to studies factor affecting the efficiency applying the green office principles in the organization. This research designed to use the questionnaire to collect the information data (Pei et al., 2014). For the questionnaires used for collected the data in this study was divided in to three parts as the biography of the participants, general information of the organization and questions about the factor affecting the efficiency of applying the green office principles in the organization (Kaliannan and Adjova, 2015).

First part consisted of the overall knowledge about the Green Office. For this part, the researcher has to use the scoring to set the gain by defined scoring by correct (1 point) and wrong (0 point) and measure by percentage.

Second part consisted of questions of factor affecting the efficiency of applying the green office principles in the organization. The questions in this part were closed ended included the areas of personal evaluation, organizational evaluation and environmental evaluation. In this part the researcher was used the Likert Scale and rating the data into 5 priorities which are Maximum, Most, Moderate, Few and Least (Keeves, 1988).

In explanation the analyzing data collection according to the characteristics of the query by types of organization which divided into 4 types (Government organization, Private sector, State enterprise and Educational institution) (Aroonsrimorakot and Phuynongpho, 2016). This set of questionnaires would query into Likert scale and analyzed by factors analyzing.

3. Results

According to the analysis of the survey, there were all 431 participants from 30 organization divided into 4 types of organization (the government organization, the private sectors, the state enterprise and the educational institution). Most participants were from the government organization which is 44%. And the educational institute was 29%, the state enterprise was 17% and private sector was 10% by respectively.

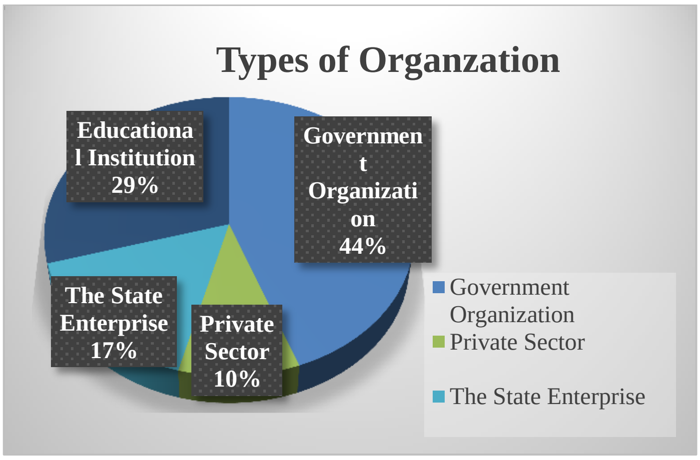

Figure1. Types of Organization Chart

The results of well-known about the Green Office

Every type of organization more than 80 percent considered that the participants and organization have all the activities to get less effect to the environment by efficient uses of resources. Also reduce the amount of waste by recycling would one way of the green operating. The efficient green operating would have the convincing system and also give the information to all workers to be follow. Furthermore, to having the green procurement policy to purchase only environmental-friendly products in the organization. The communication in the office was important such as public relations and news informing. The good management should have the communication within organization and also outside the organization. For the conclusion, most of participants were having a good well-known about the green office and the state enterprises having the highest scores in the well-known test.

Factors analysis of factors effecting the efficiency of applying the green principles in the organization

Kaiser-Meyer-Olkin Measure of Sampling Adequacy	0.964
Bartlett's Test of Sphericity Approx. Chi-Square	13,634.280
df	990
P-value	<0.0009

KMO = 0.964 means that the factors analysis was suitable for this data set.
Bartlett's Test = 13,640.280 concluded the 45 factors were related from P-value < 0.0009

Table 1. Factors effecting the efficiency of applying the green principles in the organization

Com po nent	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	19.441	43.202	43.202	19.441	43.202	43.202	10.350	22.999	22.999
2	3.520	7.823	51.024	3.520	7.823	51.024	7.516	16.702	39.701
3	2.051	4.557	55.581	2.051	4.557	55.581	6.000	13.334	53.035
4	1.253	2.784	58.365	1.253	2.784	58.365	2.260	5.023	58.058
5	1.140	2.533	60.898	1.140	2.533	60.898	1.278	2.840	60.898
6	0.972	2.161	63.059						
7	0.929	2.064	65.123						
8	0.883	1.962	67.085						
9	0.820	1.822	68.906						
10	0.776	1.726	70.632						
11	0.731	1.625	72.257						
12	0.682	1.516	73.773						
13	0.677	1.503	75.276						
14	0.630	1.399	76.675						
15	0.605	1.344	78.019						
16	0.561	1.247	79.266						
17	0.555	1.233	80.499						
18	0.521	1.157	81.656						
19	0.506	1.125	82.781						
20	0.489	1.086	83.867						
21	0.462	1.026	84.892						
22	0.454	1.008	85.901						
23	0.412	0.916	86.817						
24	0.400	0.888	87.705						
25	0.381	0.847	88.552						

Table 1. Factors effecting the efficiency of applying the green principles in the organization (cont.)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
26	0.361	0.803	89.356						
27	0.350	0.777	90.132						
28	0.345	0.766	90.898						
29	0.336	0.747	91.645						
30	0.324	0.721	92.366						
31	0.311	0.691	93.057						
32	0.294	0.653	93.710						
33	0.293	0.651	94.361						
34	0.274	0.610	94.971						
35	0.259	0.575	95.545						
36	0.254	0.565	96.111						
37	0.247	0.548	96.659						
38	0.230	0.511	97.170						
39	0.224	0.497	97.667						
40	0.217	0.483	98.150						
41	0.204	0.454	98.603						
42	0.185	0.411	99.014						
43	0.177	0.393	99.407						
44	0.154	0.343	99.750						
45	0.113	0.250	100.000						

From the table using the Principal Component Analysis from 45 factors can be extraction all factors into 5 factors which data variation was 60.898%.

For the factors analyzing in this study could be extracted into 5 factors which were following:

Factor 1: Personal and Organization Action

Revising and having addition study from green office operation handbook or related published, to setting clearly environmental policy and also the long-plan of operation from the executives also setting the objectives of the operation. Having the green meeting to brief about green office to the workers and also having the green operating report to the workers and board. Connecting and expressing with another organization be helpful to the operation processes and this consisted to the study by Pfeffer (1997) has discussed about the interdisciplinary of the organization efficiency that the organizations have an inducement for social actors to participate in them and in return there obtain contributions that become inducements for others. An organization is viable and survives by inducements-contributions balance because resources and energy must be expanded in order to keep the organization going.

Factors 2: Technology and Environmental of Organization

The organization that has the appropriate technology development and prepared the technology or devices to supported the green operation appropriately. Having the document about green operation in their office appropriately. To having the green areas in the organization which being comfortable atmosphere in the office that could encourage workers to cooperate and always has neatly and clean office would easy to be well manage and could develop the green operation. And this is consisted to Miao et al. (2017) that the green technological inefficiency is the one of the major factors influencing the utilization of resources

Factor 3: Policies in the Organization

The organization visions to the priority to the environment dimension and encourage workers to realize about the green operation. To set the person and working team that play responsible to the operation in organization and having an appropriate man-work in the green office operation in organization. Having the rules or regulations of reducing resources usage in the organization and this would consistence to Taylor (1911) that based on the concept of planning of work to achieve efficiency, standardization, specialization and simplification. Acknowledging that the approach to increased productivity was through mutual trust between management and workers.

Factor 4: The Personal Status

Position in organization, education level, the period of working was the important key that could affected to the efficiency of the green office operating also the opportunities to express the workers' opinions about the green operation. And the specific education about the green office with would consisted to Kaliannan and Adjova (2015) that the employee Engagement as an engine to be the management drive the effectiveness of various environmental factors from within and outside and organization.

Factor 5: Types and Sizes of Organization

The size of organization structure and types of organization has affected to the well green operation and the budget management for the green office operation could be affected to the efficiency of applying the green principles in the organization (Yimeng, 2017).

4. Conclusion

According to the analysis of the survey, there were all 431 participants from 30 organization divided into 4 types of organization (the government organization, the private sectors, the state enterprise and the educational institution). Most participants were from the government organization which is 44%. And the educational institute was 29%, the state enterprise was 17% and private sector was 10% by respectively. And the factors analyzing would be attracted into 5 factors which were Personal and Organization action, Technology and Environmental of Organization, Policies in the Organization, Personal Status and Types & Sizes of Organization.

5. Discussion

Factors affecting the efficiency of applying the green office principles in the organization in this research was consist of all 45 factors. In all 45 factors was having divided to 3 main categories which were human evaluation, organization evaluation and the environmental evaluation which this consistent to Pinprayong B. & Siengthai S. (2012) that organizational efficiency has to focus on the improvement of internal processes of the organization. There are the dimensions for the measurement of organizational efficiency were Organizational Strategy, Corporate Structure Design, Management System, Development of Corporate and Employee Styles, Motivation of Staff Commitment and Development of Employee's skills.

The factor analysis was about the factors that effecting the efficiency of applying the green office principles in the organization and these consistent to Sakalyte E. (2013) that the performance in the organization has been the major element of the discussions in term of theories conception in organization and one of the most common ways to get the performance of the entity was to measuring the effectiveness of the efficiency of the organization. To be the efficiency green office in the organization, the organization would be continuous follow the principles of green office with this consistence to Lawrence (2012) claimed that time is an important factor needed to enhance various organizational performances. From the factors analysis in this research could be analyzed into 5 factors which this could be the guidance to the company or organization that interested to register in the Green Office project in the future.

6. Acknowledgements

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Food Safety and Consumption Quality Potentials of Cassava Lines Grown in Three Rain-Fed Plantation Areas in Thailand

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Abstract

Cassava has been used as a major source of carbohydrate in many countries especially in Sub-Saharan Africa and South America. However, in Asia, particularly in Thailand, cassava is cultivated to be used domestically and exported for industrial purposes, but not for food. Even though Thailand remains in the first rank of the world cassava exporting countries, Thai farmers still suffer from the fluctuated prices upon global demand of fresh root over the years. Therefore, this research aimed to evaluate cassava lines from breeding program to use them as a source of carbohydrate, with a potential for substituting rice in drought-prone areas, for serving in household or for making value-added products which can be sold in higher prices. Three promising cassava lines namely 52-12, 52-97, and 55-689 from breeding program compared with four check varieties, Kasetsart50, Huay Bong80, Pirun2, and Hanatee, were evaluated in three rain-fed plantation areas in Nakhon Ratchasima province and Chachoengsao province in the North-Eastern and Eastern parts of Thailand. 52-12 line had high root starch content, but also high cyanogenic potential which was not qualified for safety food. 52-97 line had high root yield, but relatively low starch content; while, 55-689 had yellow flesh root with high starch content. Both lines had moderate cyanogenic potential in the same level as that in Pirun2. For these reasons, 52-97 and 55-689 lines were suggested for cooking with the similar potential as Pirun2; whereas, Hanatee which is the most safety variety for cooking had the lowest root yield in most rain-fed areas.

Keywords: Cassava/ Food safety/ High root yield/ Low hydrocyanic acid/ Good cooking quality/ Rain-fed cultivation

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial plant which can be grown in various environmental conditions including sub-tropical arid zones due to C3-C4 intermediate photosynthetic characteristics. Therefore, cassava also has higher efficiency for carbon dioxide exchange than C3 plants such as rice and wheat (El-Sharkawy and Cock, 1987). Moreover, cassava has been promoted as food crop for under-nourished population in Sub-Saharan Africa, Asia, and the Pacific through crop biofortification program. For these reasons, cassava has a potential for food security which positively response to the global elevating carbon dioxide level (Rosenthal and Ort, 2012). Sundaresan *et al*. (1987) classified cassava by taste into two groups which were sweet type tending to have low cyanogenic potential (lower than 100 mg kg^{-1}) and bitter type which tended to have high cyanogenic potential (upon 100 mg kg^{-1}). Cyanide or hydrocyanic acid, produced from cyanogenic glucoside namely Linamarin and Lotaustralin in cassava, is toxic for animal by blocking electron transport chain from generating energy to support cell activity (Bokanga, 1994). Cassava root considered to be safe for consumption should have hydrocyanic acid lower than 50 ppm (mg kg^{-1}). Hydrocyanic acid content

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between 50 to 100 ppm was considered to be moderate toxic for consumption and hydrocyanic acid over 100 ppm was highly toxic for consumption (Balagopalan *et al*., 1998) because consuming cyanide 50 to 100 mg per day can be acutely intoxicate for adult (Montagnac *et al*., 2009). For food safety, World Health Organization allowed hydrocyanic acid content in food to lower than 10 ppm (FAO/WHO, 1991). Cassava has been grown mainly for food and feed in Africa and South America. It can be processed into many kinds of food such as sticky dough namely "Fufu", toasted fermented flour called "Gari", fermented sun dried called "Lafun", and roasted flour namely "Farina de mandioca" (Parmar *et al*., 2017). In Thailand, there is small application of cassava for food. Hanatee is the popular variety for cooking due to low hydrocyanic acid and mealy taste. However, Hanatee is mostly grown in the irrigated areas because it gives a low root yield in rain-fed areas. Therefore, Hanatee plantation in Thailand was around 148 hectares (Department of Agriculture Extension, 2017) which was relatively small compared to 1.48 million hectares of all cassava plantations in Thailand which were mostly in rain-fed conditions (Office of Agricultural Economics, 2017). In addition, cyanogenic glucoside content in cassava root was found to be increased in drought condition (Cardoso *et al*., 2005) which can cause cultivating cassava for consumption in rain-fed areas to become a challenge.

Most of cassava breeding objectives in breeding programs in Thailand in the past focused on high root yield, high root dry matter, and high starch content. Before releasing variety for farmer, regional yield trials are required for evaluating stability of cassava through various environment conditions for root yield and starch content (Sipunya *et al.*, 2007). However, recently, cassava production in Thailand becomes over supplied in some seasons because the domestic industrial uses and cassava product export has reach the ceiling. Thus, breeding cassava for food safety and good consumption quality such as low hydrogen cyanide content, mealy texture, non-bitter taste as Hanatee, but also maintain high root yield in rain-fed areas is a challenging objective to give farmer a new market for cassava production as well as to prepare food security option in Thailand as we move towards turbulent global climate change. The objective of this research was to evaluate a potential of three cassava lines namely 52-12. 52-97, and 55-689 as promising lines for food safety and consumption qualities as well as yield potentials in three rain-fed plantation areas in Nakhon Ratchasima province and Chachoengsao province in the North-Eastern and Eastern parts of Thailand.

2. Methodology

2.1 Experimental design

Three promising cassava lines coded as 52-12 (from a cross between Rayong2 and Hanatee), 52-97 (from a cross between Huay Bong 60 and Hanatee), and 55-689 (from a cross between Huay Bong 80 and Kolog) from cassava breeding program for low cyanogenic potential and high root yield were grown in regional yield trials together with four check varieties namely Kasetsart 50 (KU50) as check for high root yield, Huay Bong 80 (HB80) as check for high starch content, Pirun 2 (PR2) as check for moderate cyanide content, and Hanatee (HNT) as check for low cyanide content and good texture. Seven lines/varieties were grown in plot size of 5x 10 plants per plot in randomized complete block design with four replications with plant density of 1x1 m^2. Experiments were conducted in three rain-fed yield trials in Dan Khun Thot district and Sikhio district in Nakhon Ratchasima province and Phanom Sarakham district in Chachoengsao province. In Dan Khun Thot district, the soil texture in the experimental site was loamy sand with 85% sand, 6% silt, and 9% clay with 0.33% organic matter. Soil contained 0.04% total Nitrogen, 6 mg kg^{-1} Phosphorus, 98 mg kg^{-1} Potassium, 505 mg kg^{-1} Calcium, 29 mg kg^{-1} Magnesium with 0.49 dS m^{-1} under pH 6.7. Plants were fertilized with 46.9 kg ha^{-1} of total N, 21.9 kg ha^{-1} of P_2O_5, and 56.3 kg ha^{-1} of K_2O applied at 30 days after planting. In Sikhio district, The soil texture in the experimental site was loam with 45% sand, 30% silt, and 25% clay with 1.40% organic matter. Soil contained 0.07% total Nitrogen, 15 mg kg^{-1} Phosphorus, 248 mg kg^{-1} Potassium, 2,975 mg kg^{-1} Calcium, 116 mg kg^{-1} Magnesium with 0.47 dS m^{-1} under pH 7.1. Plants were fertilized with 46.9 kg ha^{-1} of total N, 21.9 kg ha^{-1} of P_2O_5, and 56.3 kg ha^{-1} of K_2O applied at 30 days after planting. In Phanom Sarakham district, The soil texture in the experimental site was loamy sand with 81% sand, 10% silt, and 9% clay with 0.73% organic matter. Soil contained 0.05% total

Nitrogen, 7 mg kg^{-1} Phosphorus, 67 mg kg^{-1} Potassium, 267 mg kg^{-1} Calcium, 27 mg kg^{-1} Magnesium with 0.26 dS m^{-1} under pH 5.4. Plants were fertilized with 46.9 kg ha^{-1} of total N, 21.9 kg ha^{-1} of P_2O_5, and 56.3 kg ha^{-1} of K_2O applied at 30 days after planting.

Cassava stakes were planted in June 2017 and were harvested in April 2018. The monthly weather data was provided in Table5. Hand weeding was taken twice in rainy season.

2.2 Data collection and analysis

At harvesting time, fresh weight of root, leaf, stem, stomp were measured from 3x8 plants inside of each plot. Harvest index (HI) was calculated from the ratio of fresh root yield to total biomass. Cyanide content (HCN) in fresh root was measured from 100 mg fresh root tissue, taken from root parenchyma without cortex part, using picrate paper from method of Bradbury *et al*. (1999). Cassava texture and bitterness was measured, after cassava was peeled and boiled in water for 20 minutes, by sensory test from five trained people. Texture were scored as 1 for mealy texture, 2 for mealy firm texture, and 3 for firm texture. Bitterness scores were ranged from 0 to 3 toward more bitter taste. Statistical analysis was performed using ANOVA by STAR software (IRRI) and GGE biplots were analysis using R program.

3. Results

3.1 Yield, starch content, cyanide content, and consumption quality of cassava grown in three rain-fed areas

Grown in early rainy season and harvested at 10 months after planting, cassava yield in these trials could be represented a regular yield gained from farmer practice. In Phanom Sarakham district, Chachoengsao province (Table1), 52-12, 52-97, and 55-689 had root yield in the same level of HB80 and PR2. HNT had the lowest root yield in this trial, but still was not significantly different from KU50. 52-97, HNT, and PR2 had the highest stem weight in this trial which was correlated to plant height, but all three lines had harvest index in the same level of HB80, KU50, and PR2. Only HNT had the lowest harvest index in this trial. 52-12 and 55-689 had high starch content above 20% which was in the same level of HB80 and KU50. 52-97 had starch content lower than 20% which was in the same level of HNT and PR2. For cyanide content, only KU50 had the highest HCN content. Varieties which had HCN above 100 ppm were KU50 and HB80. Those having HCN content in root lower than 50 ppm were 55-689 and HNT and others had HCN between 50 and 100 ppm. According to HCN level of toxicity in Balagopalan et al. (1998), only 55-689 line had food safety potential in this trial. However, for texture sensory test, no lines or varieties had the same mealy texture as HNT. This data indicated that farmer who prefer boiled cooking might not like to consume 55-689 compared to HNT. Therefore, suggestion for cooking of 55-689 is to make flour or fly it. The other advantage of 55-689 is the yellow flesh color (as shown in Figure 5) which contain high carotenoid (Kongsil *et al*., 2016). For bitterness, all three lines had lower bitterness than KU50. Among check varieties, only PR2 had the same level of bitterness as KU50. Thus, bitterness of cassava flesh in this experiment related to HCN content which was quite similar to the report of Sundaresan et al. (1987).

For trial in Dan Khun Thot district (Table2), Nakhon Ratchasima province, all three lines had similar root yield as other check varieties. HNT also had low root yield, but in this site, it's root yield was significant different from only 52-97. Stem weight and plant height pattern in this trial was different from those in Chachoengsao province. Harvest index of 52-12 and 52-97 were as low as those in HNT and PR2. HI information indicated the ability to distribute biomass to root. Thus, 55-689 had higher ability for carbohydrate partitioning than other two lines in this trial. Starch content of 52-12 was as high as HB80 which was recommended for high starch content. For HCN content, no varieties or lines in this trial had HCN lower than 50 ppm which mean grown cassava in this area under rain-fed condition can cause problem for some risky level of intoxication which is not recommended for food safety practice. PR2 in this trial had similar mealy texture as HNT. Even though 52-12 and 52-97 had firmer texture than HNT, but still not significantly different from PR2. There was no significant difference of bitterness among varieties and lines because of high %CV value reported here which might be due to sensory practice.

Table 1. Yield, starch content, Cyanide content, and consumption quality of cassava grown in Phanom Sarakham district, Chachoengsao province

Var	RW	LW	SW	PH	HI	ST	HCN	TX	BT
52-12	27.6 ab	3.3	4.5 b	164.50c	0.7074b	24.88 a	92.39 bc	3.00 a	0.47 bc
52-97	31.4 ab	3.1	7.1 a	216.25a	0.6926b	18.58 b	88.33 bcd	3.00 a	0.53 bc
55-689	27.0 abc	2.4	4.0 b	172.00c	0.7431ab	24.68 a	48.39 cd	3.00 a	0.56 bc
HB80	32.4 a	2.4	4.5 b	188.00bc	0.7688a	25.73 a	122.10b	3.00 a	0.50 bc
HNT	20.5 c	3.3	7.9 a	226.50a	0.5814c	19.98 b	22.38 d	1.25 b	0.06 c
KU50	24.4 bc	2.1	4.3 b	186.50bc	0.7251ab	23.77 a	195.63a	3.00 a	1.25 a
PR2	31.2 ab	3.0	7.3 a	204.25ab	0.6918b	19.12 b	70.48 bcd	2.75 a	0.78 ab
F-test	**	ns	**	**	**	**	**	**	**
CV (%)	11.02	22.63	13.54	5.74	3.28	6.78	31.24	9.57	43.5

Note: *Different alphabets below values indicate significant difference among values at 95% confidence.*
Var = Varieties/Lines, RW = Fresh root weight (Tons/hectare), LW = Fresh leaf weight (Tons/hectare), SW = Fresh stem weight (Tons/hectare), PH = Plant height (cm), HI = Harvest index, ST = Starch content (%), HCN = cyanide content (ppm), TX = Texture score, BT = Bitterness score

Table 2. Yield, starch content, cyanide content, and consumption quality of cassava grown in Dan khun thot district, Nakhon Ratchasima province

Var	RW	LW	SW	PH	HI	ST	HCN	TX	BT
52-12	42.3 ab	5.2 b	11.6 ab	194 b	0.6483 c	29.85 a	113.17 bcd	2.50 ab	0.00
52-97	47.3 a	8.7 a	13.9 a	231 a	0.6258 c	19.18 e	70.85 cd	2.50 ab	0.00
55-689	42.1 ab	4.7 b	7.3 b	185 b	0.7216 a	24.55 c	153.02 bc	3.00 a	0.56
HB80	40.7 ab	4.8 b	7.7 b	219 ab	0.7086 ab	27.80 ab	185.37 b	2.75 ab	0.25
HNT	32.4 b	6.9 ab	8.4 b	216 ab	0.6241 c	20.12 e	54.55 d	1.00 c	0.00
KU50	42.5 ab	6.2 ab	8.0 b	202 ab	0.7016 ab	26.02 bc	290.79 a	3.00 a	0.75
PR2	42.3 ab	6.5 ab	10.2 ab	214 ab	0.6590 bc	22.43 d	59.25 d	1.75 bc	0.25
F-test	*	**	**	**	**	**	**	**	ns
CV (%)	12.3	19.8	21.6	6.9	3.3	3.7	28.0	19.8	170.8

Note: *Different alphabets below values indicate significant difference among values at 95% confidence.*
Var = Varieties/Lines, RW = Fresh root weight (Tons/hectare), LW = Fresh leaf weight (Tons/hectare), SW = Fresh stem weight (Tons/hectare), PH = Plant height (cm), HI = Harvest index, ST = Starch content (%), HCN = cyanide content (ppm), TX = Texture score, BT = Bitterness score

At Sikhio district (Table3), HNT was the only variety that had the lowest root yield. Due to HNT harvest index, it was not significantly different from those in 52-12, 52-97, and PR2. Therefore, the reason of low root yield in HNT could be from low total biomass. Starch content of 52-12 here was still high in the same level of HB80 and KU50. Interestingly, HCN content of some lines and varieties in this trial were lower than those in Dan Khun Thot district. In the harvesting month in April 2018 (Table5), rainfall in Sikhio district was lower than that in Dan Khun Thot district. According to Cardoso et al. (2005) suggestion, drought might cause increase in HCN content in cassava in both root and leaf. However, from the result in these trials indicated that there should be more factors involving with HCN content in root other than soil moisture. For consumption quality, even though 52-12, 52-97, and 55-689 had low bitterness in the same level HNT, but texture of those were still firmer than that in HNT. Therefore, 55-689 is as safe as HNT to be grown for food in this area, but not be suggested for boiling for consumption. Flour making or flying are recommended.

For combine analysis (Table 4) due to homogeneity of information of three locations, all three lines had the same level of root yield as other commercial varieties which were HB80, KU50, and PR2 except for HNT which had the lowest yield. Starch content of 52-12 and 55-689 were in the same level as HB80. HCN content of 52-97 and 55-689 were in the same level of HNT and PR2. However, only HNT had HCN level that lower than 50 ppm. Therefore, food safety for these three lines and PR2 is still a concerned issue.

Table 3. Yield, starch content, Cyanide content, and consumption quality of cassava grown in Sikhio district, Nakhon Ratchasima province

Var	RW	LW	SW	PH	HI	ST	HCN	TX	BT
52-12	57.3 a	18.7 ab	15.3 a	251.75ab	0.5801bc	28.50 a	144.4 abc	2.75 a	0.75 cd
52-97	58.5 a	23.3 a	18.0 a	272.00 a	0.5455bc	20.70 b	81.9 cd	3.00 a	0.65 d
55-689	51.0 a	13.7 ab	13.2 ab	252.25ab	0.6103b	27.25 a	40.2 d	3.00 a	0.15 d
HB80	50.6 a	7.7 b	8.6 bc	236.75b	0.7108a	26.65 a	207.7 ab	3.00 a	1.40 bc
HNT	18.9 b	8.9 b	5.5 c	241.75ab	0.5021c	20.27 b	40.1 d	1.00 b	0.40 d
KU50	49.8 a	11.2 b	11.9 ab	250.00 ab	0.6423ab	25.80 a	236.6 a	3.00 a	2.52 a
PR2	53.3 a	17.2 ab	11.9 ab	238.25b	0.5976bc	19.30 b	131.3bcd	2.75 a	1.83 b
F-test	**	**	**	*	**	**	**	**	**
CV (%)	13.19	32.87	22.26	5.32	7.07	7.57	33.32	10.39	26.31

Note: *Different alphabets below values indicate significant difference among values at 95% confidence.*
Var = Varieties/Lines, RW = Fresh root weight (Tons/hectare), LW = Fresh leaf weight (Tons/hectare), SW = Fresh stem weight (Tons/hectare), PH = Plant height (cm), HI = Harvest index, ST = Starch content (%), HCN = cyanide content (ppm), TX = Texture score, BT = Bitterness score

Table 4. Combine analysis of yield, starch content, cyanide content, and consumption quality of cassava grown in three rain-fed areas

Clones	Fresh root yield (Tons/hectare)	Starch (%)	Harvest index (%)	HCN (ppm)
52-12	42.4 a	27.74 a	0.6453	116.68 bc
52-97	45.7 a	19.48 c	0.6213	80.36 cd
55-689	40.0 a	25.49 ab	0.6917	80.55 cd
HB80	41.2 a	26.73 ab	0.7294	171.73 b

Table 4. Combine analysis of yield, starch content, cyanide content, and consumption quality of cassava grown in three rain-fed areas (Cont.)

Clones	Fresh root yield (Tons/hectare)	Starch (%)	Harvest index (%)	HCN (ppm)
HNT	23.9 b	20.12 c	0.5692	39.02 d
KU50	38.9 a	25.20 b	0.6897	241.02 a
PR2	42.3 a	20.28 c	0.6494	87.03 cd
F-test	**	**	ns	**
CV (%)	28.91	8.19	8.95	40

Note: Different alphabets after values indicate significant difference among values at 95% confidence

3.2 The best performance of root yield, starch content, harvest index, and cyanide content among three yield trials

Due to rainfall data (Table5), Phanom Sarakham district, Chachoengsao had the highest total rainfall, while; Dan Khun Thot district and Sikhio had relatively similar level of rainfall. There were pattern in rainfall reduction in December 2017 until March 2018 of three locations regarding as dry season. However, in Phanom Sarakham district, rainfall was still available even in dry season. The ratio of rainfall and pan evaporation (RF/PE) which were tremendously lower than 1 together with low relative humidity also confirmed that dry season occurred from December 2017 through March 2018. However, in January 2018 at Phanom Sarakham district, RF/PE was higher than 1 which mean relatively high amount of rainfall occurred in dry season. According to RF/PE values, Sikhio district tended to have longer and more severe dry season than others, but yields of cassava biomass were higher than those in Phanom Sarakham district. This might be due to the high acidic soil properties in Phanom Sarakham district which reduced macronutrients solubility making slow growth and loss of yield production according to yields and plant height data. However, more rainfall in Phanom Sarakham district could cause reduction in HCN content in root compared to those in other two locations, but did not affect starch content. Starch content tended to vary upon genotype, but not upon environment. For temperature, there was no difference of this parameter in three locations and averaged temperatures of all three locations were in the range of optimum temperature for cassava growth and development (Alves, 2002).

From GGE biplot analysis, 52-97 had the best root yield performance (Figure1) in Sikhio district and Dan Khun Thot district. 55-12 had the best starch content in all three locations. 55-689 had the best harvest index in Dan Khun Thot district. KU50 and HB80 had the highest HCN content in all three location and HNT had the lowest one.

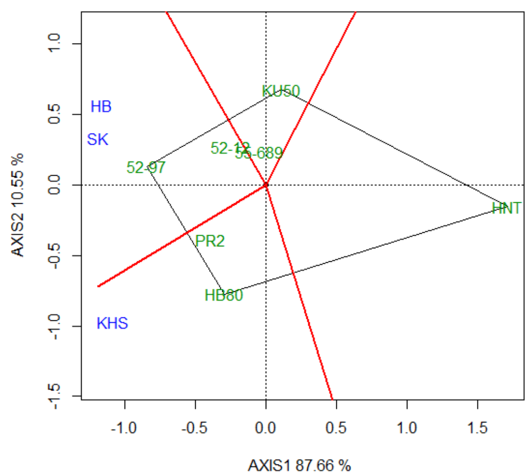

Figure 1. GGE plot of fresh root yield

Table 5. Monthly rainfall (mm) data from three districts in the experiments

Factor	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18
Dan Khun Thot district, Nakhon Ratchasima province											
RF	132	75	185	102	104	157	15	68	6	14	170
PE	153	164	154	129	121	133	172	139	148	192	162
RF/PE	0.86	0.46	1.20	0.79	0.86	1.18	0.09	0.49	0.04	0.07	0.80
RH%	78	80	80	83	83	74	66	70	69	70	76
Temp	27.0	26.2	26.8	26.4	25.5	24.5	22.9	24.1	24.0	26.4	26.4
Sikhio district, Nakhon Ratchasima province											
RF	65	112	240	124	171	66	10	0	63	9	154
PE	211	217	200	143	133	142	139	115	130	161	144
RF/PE	0.30	0.51	1.20	0.86	1.28	0.46	0.07	0	0.48	0.05	1.06
RH%	69	68	74	83	81	78	72	72	65	67	74
Temp	28.3	28.1	27.1	26.1	25.6	25.7	24.5	25.6	25.6	28.6	28.7
Phanom Sarakham district, Chachoengsao province											
RF	274	258	151	203	228	59	9	196	19	78	239
PE	128	132	140	125	124	114.	125	112	121	148	137
RF/PE	2.12	1.94	1.07	1.61	1.83	0.51	0.07	1.74	0.15	0.52	1.73
RH%	84	85	84	86	85	81	73	78	77	80	82
Temp	28.1	27.3	27.9	27.6	27.0	25.9	24.5	25.4	25.3	27.0	27.3

Note: RF = monthly rainfall (mm), PE = monthly pan evaporation (mm), RF/PE = ratio of rainfall to pan evaporation, RH% = averaged percent relative humidity, Temp = air temperature (°c)

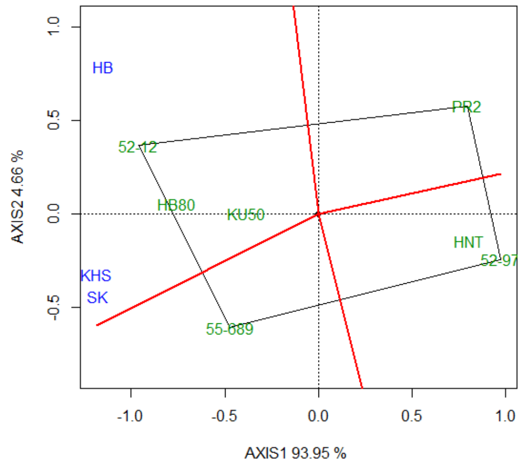

Figure 2. GGE plot of starch content

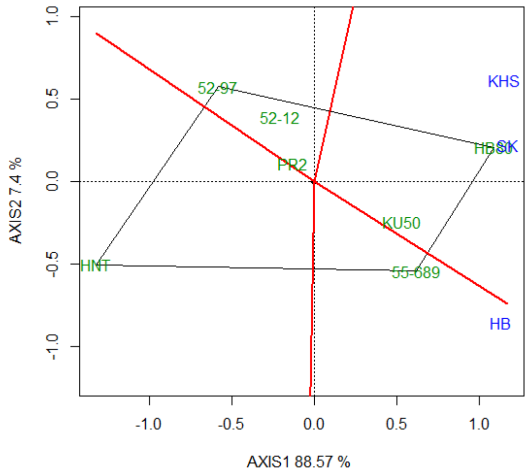

Figure 3. GGE plot of harvest index

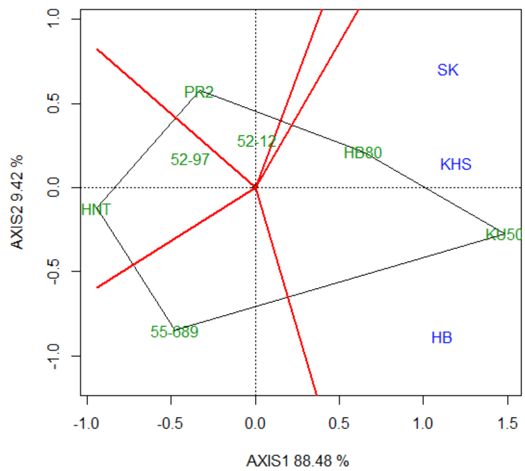

Figure 4. GGE plot of cyanide content in fresh root

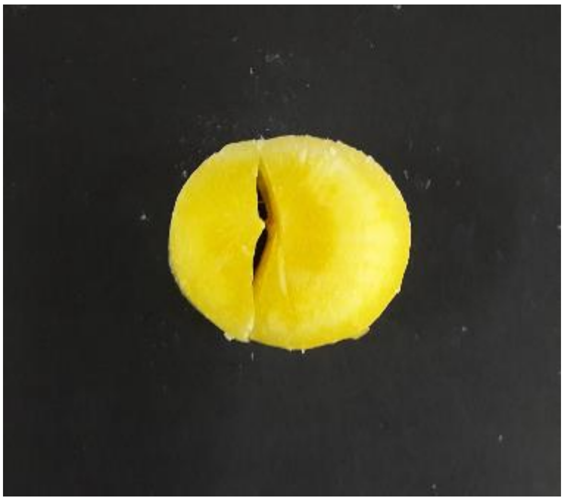

Figure 5. Yellow color of boiled cassava flesh of 55-689

4. Conclusions

From regional yield trials in three rain-fed locations, 55-689 seems to have high potential to be promoted for food security in these areas due to high root yield, yellow flesh root and moderate HCN content. However, with concern about fluctuating HCN content that sometimes higher than standard, Flour making is the most safety practice for cooking of cassava. The other concern about 55-689 is the yellow flesh color that suitable for household consumption, not for industrial needed. Therefore, it should not be grown with other commercial varieties which have white flesh color preferred by starch industry. 52-97 was high root yield line with moderate HCN content as well, but it may not good for making flour due to low starch content.

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Manage the Environment for Security Development with the Legal Method at Vietnam

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Abstract

In the current period, the Party and State are actively promoting the industrialization and modernization, step by step integrating deeply and comprehensively with the international. Harmonizing the relationship between sustainable development and environmental protection has become one of the key requirements in the socio-economic development strategy. To do this, environmental protection is very important and one of the basic and effective management tools is the law. The paper focuses on analyzing the role of law in environmental protection management and current situation analysis and suggests some solutions to improve the effectiveness of using legal instruments in environmental protection management in Vietnam. Male.

Keywords: Environmental management/ Environmental protection/ Sustainable development/ Management tools/ Law

1. Introduction

It can be seen that rapid urbanization and industrialization in Vietnam is changing the environmental conditions in both urban and rural areas. "The environment and development have a very close relationship: the environment is the area and the object of development is also the cause of environmental change" (Mai Hoang Thinh, 2017, 101). Therefore, the demand for environmental protection of sustainable human development has become one of the important objectives in the strategic orientation of socio-economic development in Vietnam. The Constitution of 2013 states: "Everyone has the right to live in a clean environment and to be obliged to protect the environment" (Article 43).

In the light of the above challenges, the system of legal normative documents on environmental protection has been continuously improved. The Law on Environmental Protection was first promulgated in 1993 and amended twice in 2005 and 2014 (Law No. 52/2005 / QH11 and Law No. 55/2014 / QH13). The law provides a legal basis for environmental protection. Among the important legal tools contributing significantly to the protection of the environment directly and effectively include the Law on Handling of Administrative Violations and the documents detailing the implementation of the Law on Handling Administrative Violations.

With the above-mentioned legal documents, the environmental protection management has a firm legal basis. However, in practice, the use of legal instruments in environmental protection management or in other words the application of environmental legislation is still inadequate to be overcome.

2. Methodology

2.1 Goal and Scope of the Study

In this article, we have used some analyzes and conclusions of Vietnamese researchers on the environment and environmental management by law in Vietnam as a reference. The results of the study are based on the methodology of systematization, analysis - synthesis and comparison of theories, views and methods. In addition, we synthesize and analyze data provided by the Ministry of Natural Resources and Environment on reports on the handling of violations in the field of environment.

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3. Results

3.1 Some basic theoretical issues on environmental protection management for sustainable development through legal instruments in Vietnam

3.1.1 Environmental management and sustainable development principles

The environment is a very broad concept and is used in many different fields. In life, people use many environmental concepts such as pedagogical environment, social environment, educational environment ... The environment used in the field of legal science is understood as interrelationship. interpersonal and natural systems in which the environment is understood as the elements, circumstances and natural conditions surrounding human beings. Clause 1 of Article 3 of the Law on Environmental Protection adopted by the 18th National Assembly of the Socialist Republic of Vietnam at its Seventh Session on June 23, 2014, defines as follows: natural and man-made elements that have an impact on the survival and development of humans and organisms. " Thus, according to the definition of the Law on Environmental Protection, man becomes the center in relation to nature.

The environment is made up of innumerable physical factors. Among them, natural elements such as earth, water, air, light, sound, flora and fauna are of special and important significance. These elements are considered to be the basic components of the environment. They form and develop according to the natural laws inherent and beyond the ability of human decisions. Humans can only affect them in a certain way. In addition to the physical elements, the environment also includes artificial elements. These factors are created by humans to influence the natural elements to cater to their own needs.

The current environment is changing for people, especially natural factors such as water, soil, air, flora and fauna. Bad environmental change is taking place globally as well as within each country. The environmental status of Vietnam also has the common features of the world environment: "In large cities, pollution is also at a dangerous level. These are wastewater, household waste, medical waste, air, noise, etc. "(Pham Ngoc Lang, 2016).

The process of industrialization and modernization in Vietnam is in the process of vigorous development. Not only in the city but also in the countryside. This process has brought great achievements for the country. But the problem of natural environment is heavily polluted, causing negative impact on production and life. Industrial parks, industrial clusters, and modern new urban areas have sprung up, and commodity production and processing of agricultural products and foodstuffs in rural areas have become increasingly active. Therefore, solving environmental problems through economic development policies in parallel with environmental protection is an urgent issue that is set by the State at present. On June 9, 2014, the Prime Minister signed Decision No. 879 / QD-TTg approving Vietnam's industrial development strategy up to 2025 with a vision to 2035. In relation to the environment, The development of industry on the basis of green growth, sustainable development of environmental protection.

In any country, industrial development often involves the risk of environmental pollution. If you do not care about prevention from the beginning, the danger will be unfathomable. In fact, there are already so many fast-growing industrialized nations that have paid dearly for this. Industrialization is often accompanied by rapid urbanization, high levels of emissions, the rate at which natural resources are exploited, and environmental degradation. Exhaustion affects public health and threatens the entire ecosystem.

After more than 30 years of innovation, Vietnam's industry has an average growth rate of more than 10% per year, contributing positively to the socio-economic development of the country. But industry is also one of the industries that produce the most waste, negatively affecting the ecological environment. High polluting industries such as coal and mineral mining; electricity production; chemical industry; metallurgical industry; food industry, textiles, leather and footwear ..., the government has to solve the problems with high efficiency. The policy of protecting the environment in the process of industrialization is really an urgent matter and requires that these policies must both ensure the goal of industrialization and modernization succeeds. ensuring the ecological environment to reach the safety threshold.

This situation requires that the environment is managed strictly and scientifically. "Environmental management is a combination of appropriate economic, technical and social measures, laws and policies to protect the quality of the living environment and sustainable development of the national socio-economic" (Pham Ngoc Mausoleum, 2016). The main objectives of the state management of the environment include:

- To overcome and prevent degradation and environmental pollution arising in human life.
- Sustainable development of the national economy and society according to nine principles of a sustainable society proposed by Rio-92. The aspects of sustainable development includethe Sustainable development of the economy, protection of natural resources, zero pollution and degradation of habitat quality, enhancement of commune civilization and justice. Assembly.
- Develop tools that are effective in managing the national and territorial environment. The above tools should be appropriate for each sector, locality and community.

With the main objectives of the state management of the environment, we see that environmental management is not separate from the principle of sustainability. Sustainable development is a category formed by the need for environmental protection. The essence of sustainable development is the combination of development with environmental sustainability or, in other words, the fundamental element of sustainable development, the right to development and the need for environmental care. Although there is no comprehensive and consistent definition of sustainable development, it is an inescapable link between development and protection of the environment.

The concept of "sustainable development" has emerged in the environmental movement since the early 70s of the 20th century. In 1987, the World Council on the "Common Future" Environment and Development (WCED) of the United Nations, "Sustainable Development" is defined as "development that meets the requirements of the present but does not hinder the fulfillment of the needs of the future generations".

In Vietnam, the viewpoint of sustainable development has been affirmed in the Party's policies and policies (such as the Political Bureau's Directive No. 36-CT / TW of June 25, 1998, on intensifying the protection of the environment. During the national industrialization and modernization, Resolution No. 41-NQ / TW dated November 15, 2004, of the Politburo on environmental protection in the period of accelerating the industrialization and modernization of the country. and the legal documents of the State (Strategic Orientation for Sustainable Development in Vietnam (Agenda 21 of Vietnam) issued together with Decision No. 153/2004 / QD-TTg dated 17 / 8/2004 of the Prime Minister, the National Strategy for Environmental Protection up to 2010 and orientation to 2020 was approved by the Decision No. 256/2003 / QD-TTg dated December 2, 2003, of the Prime Minister). The Vietnamese government has a common vision of sustainable development: "Sustainable development is a development that meets the needs of the present without compromising the ability to meet that need of the generation. "(Article 4, Article 3 of the Law on Environmental Protection, 2014).

To ensure the principle of sustainability in environmental management requires the State to use a variety of measures because "environmental management is a combination of measures, laws, economic and technical policy, commune "(Pham Ngoc Lang, 2016), therefore, the law is one of the measures to manage the environment or in other words the law is a particularly important tool in the management and protection environment.

In short, the threat of environmental damage to its serious consequences has forced states to pay more attention to effective measures to protect the environment. Many economic, social and organizational measures have been implemented to effectively protect the environment. The environment can be protected under many different levels and measures, including Organizational and Political Measures, Economic Measures, Scientific and Technological Measures, Educational Measures, Legal Measures physical. It can be said that it is difficult to list all the measures that countries have taken to effectively protect the environment. However, when it comes to environmental protection, we cannot ignore legal measures.

3.1.2 Law is a particularly important tool in environmental management for sustainable development

Environmental management tools are the means of action to implement the environmental management of the state, scientific organizations and production. Each tool has a certain function and scope of influence, links and supports each other.

Environmental management tools can be classified by nature into the following basic categories:

"Legal or policy instruments, also known as legal instruments, include documents on international law, national law, other legal documents (ordinances, decrees, regulations, schools, environmental permits, etc.), national environmental plans, strategies and policies, economic sectors and localities "(Minh Phuc, 2018).

Economic instruments include taxes, fees charged on cash income from production and business activities. These tools only apply effectively in the market economy.

Technical management tools play the role of controlling and supervising the state of the quality and composition of the environment, on the formation and distribution of pollutants in the environment.

As such, the law belongs to the group of instruments of policy law in environmental management. The role of the law in environmental management is particularly important. The environment is mostly ruined by human destruction. It is the people who exploit the elements of the environment that lose ecological balance, pollute. Therefore, the need to protect the environment must first affect people by means of state management in which the law must be mentioned. Law as a system of rules regulating human behavior will have a great effect in protecting the environment. The significance of the law in environmental management is reflected in the following aspects:

First, the law provides for the rules of conduct that humans must take when exploiting and using elements of the environment. Laws have guided human behavior in an environmentally beneficial way, ensuring that human behavior does not harm the environment, limit harm, prevent degradation and environmental pollution.

Second, the law provides for criminal, economic and administrative penalties to force individuals and organizations to fully comply with legal requirements for the exploitation and use of environmental factors. In reality, subjects who participate in socio-economic activities tend to pay attention to their own interests but ignore the common interests of the environment and the community, ignore the obligation to do with the environment and not Be aware of the responsibility of environmental protection. For example, when carrying out environmental impact assessments, project owners often do not foresee their interests, so they always seek to shirk their legal obligations to the environment. At the same time, the sanctions prescribed by the law play an important role in protecting the interests of the organization itself, the individual and the long-term common interests of the society. These sanctions are not only punitive measures that violate the environmental law, prevent, educate and rehabilitate violators but also detain other subjects so that they can voluntarily follow the legal norms of protection. the environment, thereby preventing and limiting the negative impact of man on the environment. Therefore, the law provides for administrative and civil sanctions. to enforce the law on the exploitation and use of environmental factors.

Third, the law defines the functions, tasks and powers of environmental protection organizations. As we all know, all aspects of social life require state management and the environment is no exception. Moreover, environmental protection is a complex task, because the environment is a large and complex structure, so it is necessary to have a suitable and effective organizational management system. Legislation has played an important role in creating a working mechanism for organizations and agencies to protect the environment. The promulgation of legal documents creates the legal basis for these agencies to perform their functions, tasks and powers, ensuring the good performance of the State management over the environment. The legislation also divides tasks among agencies, avoids overlapping management, and creates a harmonious and synchronous coordination among agencies and enhances the effectiveness of state management of the environment.

Fourth, the law promulgated environmental standards. Environmental standards are the permissible limits of environmental quality parameters and the concentration of pollutants in wastes designated by competent state agencies as the basis for management and protection. environmental protection. Adopt legislation that environmental standards will be strictly observed by organizations and individuals when exploiting and using elements of the environment. At the same time, environmental standards also serve as a legal basis for identifying environmental law violations and prosecuting those behaviors.

Fifth, the law stipulates the reward and support for environmental protection In Paragraph 2, Article 63 of the Constitution, the Constitution states: "The State encourages all activities of environmental protection, development and use of new energy and renewable energy." Accordingly, the law stipulates organizations and individuals that record achievements in environmental protection activities, detect and promptly report signs of environmental incidents, overcome environmental incidents and environmental degradation. , to prevent acts of destroying the environment, shall be commended. Participants in environmental protection, remedying environmental incidents, environmental pollution, environmental degradation and combating acts of violating the legislation on environmental protection but suffering damage to property and health or their lives shall be compensated in accordance with law.

Sixth, the law deals with disputes related to the protection of the environment. The environment plays a particularly important role in the human life, the organism and the development of the nation as well as the whole of humanity. Consequently, environmental protection is the duty of every individual, organization as well as every nation and all of humanity.

Therefore, effective management of the environment to ensure sustainable development can not be lacking in legal instruments. Since then, sustainable development has become one of the fundamental principles of environmental law. In Vietnam, the process of industrialization and modernization of the country has brought many great achievements but it poses no small challenge to the environment and sustainable development. Especially now, when the environment of our country is degraded rapidly, environmental protection becomes a task, a constitutional obligation. In environmental protection, the great role of law is undeniable.

3.2 The status of environmental protection and management for sustainable development through legal instruments in Vietnam

3.2.1 Evaluate the legal basis for environmental management in Vietnam

It can be said that in terms of the institution, the work of building and perfecting the legal system on environmental protection has been paid much attention by the Party and the State. The number of dozens of Laws, Ordinances, hundreds of Decrees, Decisions, Directives of the Prime Minister, documents of the Ministries and agencies concerned quite comprehensive measures and fields of environmental protection This is a clear proof of that.

Environmental legislation has a high legal force, which is strong enough to regulate relations that arise in the field of environmental protection. In the system of legal documents on environmental protection, in addition to the central law - Law on Environmental Protection 2014, there are many documents at the level of Law, Ordinance on the protection of each environmental element. Among them, the Law on Forest Protection and Development 2004, the Law on Water Resources 2012, the Law on Minerals of 2010, the Law of Fisheries 2003, the Law of the Sea, the Land Law 2013 ... These are the legal bases. It is important to mobilize the participation of all sectors, levels and the entire population to participate in the cause of environmental protection.

The legal system for environmental protection in Vietnam is relatively comprehensive. In particular, the content and mode of state management of environmental activities have been identified. Including regulations on rights and obligations, organization and operation of state management agencies in environmental protection. Legal documents regulating these relationships constitute a system of documents regulating the establishment, organization and operation of the system of environmental protection institutions. The system of environmental protection agencies is established at both central and local levels and is integrated into many relevant agencies. In addition, the law also regulates the reward and handling of violations of environmental protection law.

Environmental law has ensured openness and transparency. Environmental protection activities will be difficult to achieve the desired effect if there is no mechanism to mobilize broad participation of people in environmental protection activities. One of the measures to create the premise for people to participate in environmental protection activities is the publicization of information on environmental protection activities for people, news agencies and press conditions. To supervise the observance of environmental law by state agencies, organizations and individuals.

In general, environmental legislation is still inadequate and limited to the requirements of sustainable development: lack of consistency and consistency, perspectives on sustainability. However, the law on environmental protection in Vietnam is built on a consistent and solid political basis, with links between specialized legal documents that provide the legal basis for management agencies. the state, all individuals and organizations, in general, carry out the activities of environmental protection.

3.2.2 Practical application of the law in environmental protection management in Vietnam today

In the past time, with the socio-economic development, the issue of sustainable development and environmental protection has always been the Party and State's priority to direct and organize the implementation. Along with the efforts of all levels, sectors and people in implementing the Party's guidelines, the State's policies on environmental protection and environmental protection in our country in recent years There have been many positive changes contributing significantly to improving the quality of life and the socio-economic development of the country.

However, in general, environmental protection is not commensurate with the increasing level of environmental pollution and the negative impacts on the environment of the development process. Many natural resources are exhausted and used inefficiently. The natural environment in many places is seriously damaged, polluted and degraded to an alarming level. It can be said that, along with the rapid development of the economy, our country is indeed facing many challenges of sustainable development, in which the most fundamental challenge is how to harmonize Socio-economic development with environmental protection.

Firstly, activities to handle law violations and environmental crimes

When it comes to dealing with environmental law violations we first mention the activities of sanctioning administrative violations. Many acts of violating the administrative law in the field of management and protection of natural resources and environment have been detected, promptly prevented and severely dealt with, the results of violation handling expressed through some data as follows:

2013: issued sanctioning decisions for 1,875 individuals and organizations violating the amount of 41 billion 632 million. Of which, the units under the Ministry of Natural Resources and Environment will sanction 178 organizations with a total fine of VND11.645 billion (Ministry of Natural Resources and Environment, 2015).

2014: issuing sanctioning decisions for 1,751 individuals and organizations violating the amount of 140 billion 990 million. In which units under the Ministry of Natural Resources and Environment sanctioned 247 organizations with a total fine of VND42 billion and 201 million (Ministry of Natural Resources and Environment, 2015).

In the first six months of 2015: Units under the Ministry of Natural Resources and Environment (Ministry Inspector, General Department of Land Administration, Vietnam Environment Administration, General Department of Geology and Minerals of Vietnam) sanction 229 organizations with a total fine of 23 billion 987 million (Ministry of Natural Resources and Environment, 2015).

In addition, the Party's and State's guidelines on strengthening the work of preventing and combating environmental crimes and violations, the people's police force at all levels, the environmental crime prevention and fighting police have carried out synchronously the professional measures and intensified propaganda to raise the sense of observance of the environmental law. Environmental crime prevention and control have detected, investigated and discovered over 7,200 cases of environmental law violations, transferred 200 investigating bodies to prosecute, sanctioned administrative violations and collected arrears. The environmental fee is over 200 billion VND.

In addition to the advantages gained, the difficulties in preventing and fighting against environmental crimes and violations of the People's Police force have also been encountered:

The detection and handling of law violations and environmental crimes are not yet exhaustive. Cause of the method, the tricks of this kind of crime is more sophisticated, there is the deal with the authorities. Another difficulty in the investigation and handling of violations is that there are many offenses involving foreign factors, in some cases when dealing with the factors of diplomacy.

The handling of violations of environmental law has not been uniform, uniform and not really serious, caused by the viewpoint of handling between localities, some ministries and branches are not uniform. In many places due to economic development priority should call for investment spread and licensed business massively, regardless of the assessment and assessment of the impact of the project on the environment, especially the project in key areas or when dealing with state economic groups and enterprises.

The system of legal documents, though amended and supplemented, is still lacking and incomprehensible, unclear, sanctions are not strong enough to deter, there are still many holes for the object " " The newly created Environmental Police Force, despite many efforts, has limited experience.

Second, environmental dispute resolution

Environmental disputes are a form of social conflict involving management, use of resources and environmental protection. Any environmental conflicts arise from the issue of rights, the appearance of opposing parties, and the ability to resolve them. The nature of environmental disputes in Vietnam is widespread, causing great damage to many. However, the authorities have not resolved to thoroughly solve the environmental problem but stop at the settlement of security and order.

At present, environmental disputes in localities throughout the country are mainly resolved by administrative orders. When environmental disputes occur, the people's committee of the commune or ward is the first to receive the complaint. However, with their capacity and authority, the CPC is often unable to handle the higher level, usually the Provincial People's Committee, even the Government. Therefore, the treatment process lasts, sometimes takes 2-3 years is not resolved thoroughly. As the case goes on, it slows down the people's urge to increase and the risk of conflictharms the property and health of people as well as businesses. The main cause of this situation is that the cases are dealt with bureaucratic, hierarchical and time-consuming administrative procedures without a negotiable, conciliatory procedure (out of court)., with the full participation of stakeholders. In addition, there is a lack of technical tools in determining damages and compensation levels such as standards and technical regulations on pollution; Guiding the determination of damage caused by environmental pollution.

Thirdly, on the inspection, examination and coordination of the inspection of the enforcement of the law on environmental protection. Over the past years, the inspection and examination activities have been identified by the Ministry of Natural Resources and Environment as one of the key tasks of state management along with the improvement of the system of legal documents on finance. raw materials and environment. In the area of environmental protection, the annual inspection and examination are of great concern to the leaders of the Ministry and the leaders of the General Department of Environment for a deep and comprehensive implementation and good results. Thereby, raising the awareness of the business community and people in environmental protection. However, the inspection and examination activities are overlapped between the environmental police force and the environmental management agencies at all levels, leading to troubles for enterprises; Inspection activities are lack of initiative, flexibility ...

Thus, the process of development and integration has helped the country's economy and society to develop and people's life improved. In addition, the process of development and integration also poses new challenges for environmental pollution for the sustainable development of the economy of the country. In order to achieve sustainable development goals in the future, we need to improve the state management activities first to have more solutions to use the legal instruments in the management more and more effective.

3.3 Some solutions to improve environmental protection for sustainable development through legal instruments in Vietnam

In order to effectively use legal tools in managing the environment in Vietnam, we need to implement the following measures:

To concentrate on renewing, supplementing and perfecting policies and law on environmental protection and intensifying the inspection and supervision of the implementation.

To carry out a comprehensive review of the system of policies and laws in the socio-economic fields, to supplement and perfect them in the direction of formulating a coordinated and synchronous policy environment to promote socio- The society is harmonious with nature, friendly with the environment, green growth and sustainable development.

To carry out a comprehensive review of the system of policies and laws on environmental protection, supplement and complete in a synchronous manner in line with the policy on economic restructuring, transformation of the growth model, synchronous infrastructure, institutional improvement and human resource development.

To review, amend and supplement the Law on Environmental Protection and relevant laws in the direction of clearly defining principles and policies of the State, contents, tools, mechanisms, technical regulations and criteria. assessment, responsibility for environmental protection in accordance with the context of climate change, policy of economic restructuring, transformation of growth model. Study and develop the environmental law in the direction of unifying the protection of environmental components and responding to climate change. To study the development of the Law on Minimization, Recycling and Reuse of Waste; Clean Air Law; Law on Restoration and Improvement of Environmental Quality, Natural Ecosystems; Developing the legal system, processes, standards and guidelines for the zoning of degraded functions.

To step up the inspection, examination and handling of law violations, the fight against environmental crimes and the enforcement of policies and law on environmental protection.

To intensify the close and effective cooperation with the environmental police forces in the fight against crimes and violations of the environmental protection law. To coordinate with the Ministry of Justice, the Supreme People's Court and the Supreme People's Procuracy in guiding the implementation of the regulations on environmental crimes in the Penal Code in order to quickly put these regulations into practice. .

Promote the socialization of environmental protection activities; To adopt policies to promote the participation and promotion of the role of socio-political and professional organizations.Continue to improve the environmental law in the direction of sustainable development. Towards sustainable development, the issue is not only to
harmonize environmental legislation but also economic, cultural and social legislation. This system requires co-ordination. in line with the common goal of ensuring better and better human rights, including the right to live in a healthy environment. In addition to improving the law, special attention should be paid to the ability to implement this issue in practice.

Assure the right to live in a healthy environment. The rule of law is that the state protects human rights by law, including the right to live in a healthy environment. But now we do not have a clear, complete and consistent understanding of the right to live in a healthy environment.

Strengthening the implementation of environmental law. This is perhaps the most important issue that needs to be addressed in improving the current environmental law. Practical implementation of the law on environmental protection shows that there are too many inadequacies, effective protection of environmental resources is low.

Improve law in the context of regional and international integration today. Improving the environmental law should pay attention to international cooperation related to this issue. Because international cooperation helps Vietnam to have many advantages to protect and develop the environment, such as; In addition, in the context of Vietnam's integration into ASEAN, this year has formed the ASEAN Common Community, with three pillars economic, political and security. In that sense, the improvement of the environmental law in Vietnam will have to be in line with international and regional treaties. Besides, it is necessary to strictly handle violations of environmental laws by organizations and individuals. In the context of building and perfecting the rule-of-law state in Viet Nam, improving the environmental law also needs to pay attention to addressing the responsibilities of state management entities.

4. Discussion

To perfect the legal system on the environment and enhance the effectiveness of applying the environmental law in state management to ensure sustainable development in Vietnam.

5. Conclusions

The environment is an important factor in ensuring the sustainable development of a country; Environmental degradation has had a negative impact on the community's quality of life and human health. In Vietnam, with the development of economy and society, more and more acts of violation of laws, conflicts and disputes on the environment require appropriate legal mechanisms to deal with protection of rights., legitimate interests of individuals, agencies and organizations concerned. Environmental management by effective legal instruments will address these shortcomings and contribute to sustainable socio-economic development.

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Solutions for the Sustainable Development of Marine Resources in Vietnam in the Current Period

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Abstract

Vietnam is located on the coast of the East Sea, with a coastline of 3,260 km (27th in coastline of 157 coastal countries in the world). The sea level in Vietnam is six times higher than the global average. Vietnam has more than 3,000 islands, including Hoang Sa, Truong Sa archipelago; The continental shelf is under the sovereignty, sovereignty and territorial jurisdiction of over 1 million square kilometers (3 times the land area). There are 28 provinces and cities in coastal areas, accounting for 42% of land area and 45% of the national population. With these characteristics, Vietnam is considered a country with great advantages for marine resources. The sea is truly the sacred territory of the motherland of Vietnam, the natural heritage of the nation, the spiritual and material support for the Vietnamese people today and tomorrow. The East Sea has rich and diverse marine resources. However, protecting the marine environment in Vietnam is challenging. Many marine resources are exploited exhausted, many marine environment is polluted. The level of pollution has caused serious damage, hindering the socio-economic development in Vietnam. The purpose of this paper is to understand the current situation of marine resources and marine environment in Vietnam and to present some specific solutions towards the sustainable management of biological resources. The results of the article include: Surveying statistics on marine resources in Vietnam. Identify causes that lead to depleted marine resources. From there, solutions will be developed to protect marine living resources in a sustainable way.

Keywords: Marine resources/ Vietnam/ Marine environment/ The East Sea

1. Introduction

In addition to Vietnam, The East Sea is bordered by eight other countries: China, the Philippines, Indonesia, Brunei, Malaysia, Singapore, Thailand and Cambodia. The potential of this sea is a rich source of life in the countries of the region and in the world. Therefore, The East Sea is seen as an endless source of life. But the South China Sea will no longer be an endless source of life, as the exploitation of the sea, often enriched by the sea, is accompanied by unsustainable modes of exploitation. Harvesting activities are focused only on meeting economic development objectives to achieve immediate expectations, without taking into account the consequence of environmental degradation such as lack of planning. Along with the lack of strict management mechanism, the impacts of climate change leading to the decline of marine biological resources, increasing pollution of the marine environment and negative repercussions for economic development. Along with the depletion and depletion of many marine resources due to exploitation, unreasonable use and lack of sustainability, coastal areas in Vietnam are facing the challenges and pressures of population growth. The development of the coastal economy, especially the development of industry, tourism services, and agriculture directly discharged into estuaries and coastal areas. Vietnam is pursuing the basic objective of the Vietnam Sea Strategy until 2020 "to develop Vietnam to become a strong country on the sea, to get rich from the sea, to integrate closely the economic development-social security and defense, international cooperation and environmental protection" (Resolution No. 09-NQ/ TW dated February 9, 2007 of the Fourth Plenum of the Party Central Committee on the Vietnam Sea Strategy to 2020).

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2. Methodology

In this article, we have used some analyzes and conclusions of Vietnamese researchers on marine resources and marine environment in Vietnam as a reference. The results of the study are based on the methodology of systematization, analysis-synthesis and comparison of theories and views. In addition, we synthesize and analyze data provided by local researchers on marine biological resources in Vietnam. In addition, we synthesize and analyze data provided by the Ministry of Natural Resources and Environment of Vietnam.

3. Results

3.1 Marine resources in Vietnam

Location, geography and climate have created a high biodiversity in the East Sea compared to other countries in the world, both in terms of species composition, ecosystems and genetic resources. Differences in natural conditions from north to south such as changes in temperature along the latitude, the degree of exchange of environment with the surrounding areas, the shape of the continental shelf has created the characteristics of ecosystems between the waters of Vietnam.

According to the Seafood Research Institute under the Ministry of Agriculture and Rural Development of Vietnam on marine resources. Vietnam is on the list of 10 marine biodiversity centers and lists 20 of the world's most economically viable marine fisheries. Up to now, there have been about 12,000 species of marine life in Vietnam with over 2,000 species of fish, of which about 130 species have economic value; Marine fish stocks of the whole region are about 4.2 million tons; The yield is about 1.7 million tonnes/ year, including 850,000 tonnes of bottom fish, 700,000 tonnes of floating fish, 120,000 tonnes of floating fish. In addition, the sea of Vietnam has many other marine resources with about 1,600 species of crustaceans, yields that allow exploitation of 50-60 thousand tons per year, of which high value seafood is shrimp, lobster, shrimps, crabs, crabs; About 2,500 species of molluscs, of which the highest economic value are octopus and octopus (60-70 thousand tons per year). Sea birds in Vietnam are extremely rich, including: gulls, pelicans, birds, swift. In addition to animals, the sea also provides humans with a variety of valuable seaweed. This is a source of nutritious food and a rich source of medicinal herbs. Vietnam Sea has about 638 species of seaweed.

Significant marine resources have given advantages to the livelihoods and economic development of Vietnam. The fishery catch is about 3-3.5 million tons, the seafood structure is very rich, has high economic value can be exploited every year. Coral reefs, seagrass beds, seagrasses, mangroves, zooplankton, zooplankton, sea bass, seabirds, marine mammals and reptiles with high economic value, have been exploited, serving people and socio-economic development in coastal areas and on the islands.

However, according to the Vietnam Administration of Sea and Islands, deterioration in the quality of the marine environment has caused the habitat of marine creatures to be destroyed or lost biodiversity. Many species of marine life are declining in numbers, some species is extinct locally. There are 236 species of rare and endangered marine species, of which more than 70 species of marine life are listed in the Red Book of Vietnam. Many of these species are still exploited in various forms, including chemical and explosive extermination. The decline in biodiversity has led to a decline in the number of economically valuable species. As the fisheries resources are being exploited in an unsustainable manner, it is increasingly exhausted in quantity and decreasing in quality. Fish stocks declined from 4 million tons in 1990 to 3 million tons today. The average size of fish and species diversity is also significantly reduced.

The value of coral cover and species diversity have also been declining in recent years, in some areas more than 30%. Some important fish species living on reefs are severely degraded, such as Doctor shrimp, lobster, Sea ginseng, Butterfly fish, Angel fish, Dauai fish ... Density of big size fish group has commercial value High is severely reduced. Similar to coral reefs, seagrass beds are also being gradually diminished to encroach upon sea for the construction of aquaculture ponds, coastal structures and pollution. The hot spots for seagrass decline are Ha Long Bay, Tam Giang-Cau Hai Lagoon, Phu Quoc Island. At the same time, the area of mangroves was reduced dramatically, from 408,500 ha in 1943 to only 155,290 ha in 2000.

The above data show that, although the sea of Vietnam brings great value and resources, it is also facing alarming risks. It is necessary to find out what factors influence marine resources in Vietnam and find solutions.

3.2 Factors affecting marine resources in Vietnam.

According to the assessment of the Ministry of Natural Resources and Environment of Vietnam, the main cause of marine pollution is the development of industry and tourism rampant; unreasonable aquaculture; increasing population and poverty; simple lifestyle and low education; Institutions, policies are inadequate. In this article, we focus on the following underlying causes:

3.2.1 Unsustainable mining methods.

At present, the rate of population growth is increasing rapidly, the demand for economic development is increasing in the context of increasingly depleted land resources as well as promoting the tendency to go to sea, exploit the sea, get rich from the sea, but often accompanied by unsustainable modes of operation. Harvesting activities mainly focus on economic development objectives to achieve maximum aspirations, while minimizing environmental protection. The issue of exploiting marine resources and protecting the marine environment in Vietnam is facing many challenges, many resources are exploited exhausted, the marine environment in many places are so polluted. The main reason is due to the increase in fishery exports, which results in annual catches in excess of available reserves. On the other hand, illegal and destructive fishing methods, such as the use of explosives, cyanides, electric pulses and small meshes, are not strictly controlled, not only reducing marine resources, but also Harms the habitat of marine species. Exploitation by using mines, using toxic chemicals to rapidly deplete aquatic resources and causing serious consequences for marine ecological zones. Due to low awareness of fishermen, fishermen in the habitat of destruction are quite popular. That is not to mention the effects of pesticide residues, pesticides accumulated in the water that flow through the nutrient levels of the food chain, which are considered as one of the last chains.

3.2.2 Factories, factories, industrial zones and residential areas that discharged waste water, untreated solid waste into rivers in the coastal plains or directly discharged into the sea.

Between 70% and 80% of marine waste comes from inland waters when factories, factories, industrial parks and residential areas discharged waste water, untreated solid waste into the rivers coastal plain or discharge to the sea. For example, in the aquaculture process, there is a significant increase in the amount of solid waste directly to the sea. The main sources of waste are fertilizers and artificial feeds used in aquaculture. On average, one hectare of shrimp ponds will emit about 5 tons of solid waste and tens of thousands of m3 of waste water in one crop. With a total shrimp farming area of more than 600,000 hectares, it will emit nearly 3 million tons of solid waste each year. Specifically: In the provinces from Quang Ninh to Quang Binh, over 37,000 hectares have been exploited and used for aquaculture (accounting for 30-35% of the area of salt water). A large number of establishments have come into aquaculture on the industrial scale leading to the habitat of living creatures, spawning grounds, breeding grounds, disease outbreaks. A newly published study shows that Vietnam is the fourth country in the world to dispose of plastic waste to the sea, but there are no policies and regulations governing waste management.

Along with the most alarming cases of marine pollution, the most serious cases of Ha Tinh formosa discharge, causing serious consequences to the marine environment and socio-economic situation in four provinces Central Vietnam in early 2016. According to Dictionary Encyclopedia open Wikipedia: Mass mortality in Vietnam 2016, also known as Formosa Incident, refers to mass mortality in Vung Ang waters (Ha Tinh). From the coast of Quang Binh, Vung Chua has hundreds of individuals of grouper species from 40 to 50 kg drifted to the shore and died (Thanh Long, 2016)). On April 25, Ha Tinh province has 10 tons, Quang Tri 30 tons, to April 29 Quang Binh more than 100 tons of sea fishing suddenly. This disaster has a great impact on the production and life of fishermen, the coastal aquaculture households, affecting the marine tourism and life of the Central residents. There are 18 communes specializing in marine fishing with more than 14,000 households and 24,000 marine workers. VNExpress cited information from the National Tourism Authority in November saying that pollution from the Formosan Company along the Central Coast in April had almost completely destroyed the region's tourism sector as revenues from Tourism reduced to 90%. On 30 June 2016, the Government of Vietnam held a press conference, announcing the cause of dead fish is due to polluting wastes from Formosa Hung Nghiep Co., Ltd. exceeded the permitted concentration. Large wastes from the factory complex of Formosa Company Ha Tinh contain toxic to form a complex, moving to the South for seafood in the Dead Sea floor, which causes the disaster of marine pollution. The Vietnamese government thinks that the waste that the Formosa plant in Ha Tinh acknowledges discharges into the sea affects the lives of more than 200,000 people, including 41,000 fishermen. The marine environment incident caused by Formosa Ha Tinh in April 2016 was ranked by the Ministry of Natural Resources and Environment in the list of outstanding environmental pollutants in 2016. According to the Ministry of Natural Resources and Environment and environment in Vietnam, environmental problems caused by Formosa caused serious economic, social and environmental damage: The most affected are the fisheries sector, followed by the business, service, tourism and the life of the fishermen. Formosa has accepted responsibility, apologized to the Government, people and made a $ 500 million compensation. In my opinion, the amount of compensation is never compensated. The losses caused by the failure of Formosa to the Vietnamese people are the consequences of the Vietnamese people's long-term consequences.

3.3 Solutions for the sustainable development of marine resources in Vietnam in the current period.

In order to achieve the objective of sustainable development, measures must be taken to manage and protect the environment and marine resources. Within the scope of this article we focus on the following solutions:

3.3.1 Strengthen control and prevention of marine pollution sources.

Strengthening the control and prevention of marine pollution sources should be carried out through the following specific tasks:

Promote the propaganda and raise the awareness of the people about the importance of the sea, the need to protect the marine environment, protect the environment is to protect our lives.

+ Strengthening environmental inspection, inspection and supervision. Prepare monthly reports on marine environment, coordinate with units to receive timely information on marine pollution incidents.

+ Building a standard waste treatment system in factories and industrial zones.

+ Have sanctions to sanction the violations. To formulate regulations on sanctions for each case of polluting such offshore activities, acts of deliberately polluting the marine environment from outside the borders or acts of submerging without permission.

+ Continue to improve and implement effective policies and regulations on sustainable fisheries development, combat illegal and destructive fishing.

+ To review the planning on development of seaports, special economic zones, marine economic zones, open economic zones and marine technologies so as to ensure efficient investment in line with the country's resources.

3.3.2 Ensure sustainable livelihoods for coastal communities.

It has been shown that most of the coastal population is poor and dependent entirely on marine resources. In order to minimize the pressure on resources and protect the marine environment, the emphasis should be placed on the application of market-based solutions to resource management while addressing career transition solutions. Sustainable livelihoods for coastal communities are also of great concern. So far, in many countries, especially in countries with large numbers of fishermen such as China, Indonesia has many activities, programs for sustainable livelihood diversity for coastal residents are deployed as training handicrafts; support sustainable aquaculture, build ecotourism programs in association with marine protected areas, train tourist guides for local communities, and have obtained encouraging results. For example, in China, statistics show a sharp decrease in the number of fishers involved in fishing (a decrease of 13% from 2001-2004), while the number of fishers changing their livelihoods through aquaculture Fishery has increased in recent years. In the Philippines, the establishment of Protected Areas in the Apo Islands has created more employment opportunities in the coastal tourism sector, according to estimates by more than half of Apo's households involved in the work. Or in California, some fishermen have been involved in support to monitoring and researching protected areas. Ensuring sustainable livelihoods for coastal communities.

3.3.3 Community-based management/ co-management model:

Community-based marine resource management has been adopted in many countries, especially in developing countries, and is recognized as a cost-effective and efficient way to maintain and manage resources. Fisheries, biodiversity protection, and meet other conservation objectives as well as the needs of human livelihoods. In the region, the Philippines, Indonesia ... are the first countries to soon implement the model of community-based management and achieved certain success. Through this model, local coastal communities are given specific and controlled rights to manage coastal resources. This has strengthened the initiative, promoting greater community participation in shared responsibility with the state in the effective management and conservation of marine resources.

3.3.4 Establishment of marine protected areas

Marine protected areas are designed to protect and preserve biodiversity, natural resources and cultural values. Vietnam's waters are recognized as one of the high biodiversity centers in the world and include the major marine geographic subdivisions: the Tonkin Gulf, the coastal waters of Central Vietnam and coastal waters. The South West, the coastal areas of the Southwest and the Hoang Sa-Truong Sa archipelago. Based on the survey data collected by scientists from the research institutes, the Ministry of Fisheries proposed a marine protected area system of Vietnam comprising 15 marine areas representing the maritime zones of the whole for submission to the government for approval.

No. Location Depth Recommended type Province / city Remarks

I. The Gulf of Tonkin

1. Tran island 10 m Not yet identified Quang Ninh Island forwards, lack of surveying
2. Coto Island 20 m Marine Sanctuary of Quang Ninh An island district, not yet zoned
3. Cat Ba Island National Park On the island was decided by the state to establish a national park (1986) with about 540 ha of sea (the area of Southeast Sea Cat Ba Island)
4. Bach Long Vi Island 30 m Hai Phong Marine Reserve and Natural Resources The island of pepper island, the highest coral cover
5. Hon Me Sea Conservation Area The broad coral distribution, but with high threat, there are 11 new species for Vietnam.
6. Con Co island, Quang Tri marine protected area, intact coral reef, high diversity, located in the baseline system
7. Hai Van-Hon Son Tra 30 m Thua Thien Hue Natural Reserve and marine resources Including Son Tra, North Hai Van and Lang Co Lagoon, the highest habitat diversity

II. Coastal waters of Central Vietnam

8. Cu Lao Cham 30 m Quang Nam Marine Reserve The whole of the coral reef is intact, with unique structures, diverse coral reefs and precious germplasm.
9. Ly Son Island Anonymous Quang Ngai Marine National Park Reefs develop on a volcanic background with many rare species.
10. Hon Mun - Bich Dam 30 m Khanh Hoa Marine National Park The most diverse reef fish communities, the richest coral reef fish fauna, many rare species.
11. Hon Cau - Vinh Hao 27 m conservation area/ habitat Binh Thuan Reefs intact, high biodiversity, rare species, spawning grounds of fish and sea turtles
12. Phu Quy Island Not identified Binh Thuan the most diversified and diversified fishery resources in Vietnam, fishery logistics and ecotourism.

III. The coastal waters of the Southeast

13. Con Dao National Park (50m) Ba Ria-Vung Tau Sea National Park High biodiversity, typical coral reefs, 60 rare species in the Red Book, a national park (1993) with a sea area of 9000 ha. Internationally accredited region

IV. Coastal waters of the Southwest

14. Phu Quoc National Park 10 m Kien Giang National Park Including An Thoi island group, high biodiversity, turtles and dugong, many predatory islands

V. The Truong Sa-Hoang Sa sea area

15. Truong Sa 3000 Khanh Hoa Marine Nature Reserve International Center for Biodiversity, Coral Reefs, Also Disputed Sovereignty (Nguyen Quang Hung, 2009).

4. Discussion

The author's article is formed on the basis of a comprehensive analysis of the research results in Vietnam on marine biological resources and the protection of marine resources in a sustainable manner. The paper presented in logical order. Starting from the survey of reliable statistics on marine resources in Vietnam. From there, find out the causes leading to the depletion of marine resources. Finally, draw up solutions to protect marine living resources in a sustainable way. In particular, the improvement of the system of measures to protect marine living resources in Vietnam in the current period in order to ensure the sustainable development of marine resources in Vietnam is the most important issue that the author has done.

5. Conclusion

After more than 30 years of policy reform, the economy of Vietnam in general and in the coastal area in particular has developed strongly. Economic development moving towards industrialization and urbanization tends to go to sea. In recent years, the marine economy has been estimated to contribute about 48% of GDP, of which marine economic sectors have contributed significantly to oil and gas 64%, fisheries and seafood processing 14%, shipping and water seaports account for 11% and marine tourism about 9%. But the growing pressure on the environment and natural resources as well as other coastal and marine values is always a problem exposure now.

In the past few years, in order to solve the problems related to the sea in Vietnam, aiming at rational exploitation of natural resources and environmental protection for sustainable development of the sea, Continuously develop and implement many policies, measures, programs and plans to exploit and rationally use natural resources and protect the marine environment and sustainable development of the country remarkable progress and success.

Vietnam is rapidly establishing a coastal protection corridor. According to the Law on Natural Resources and Environment of the Sea and Islands, by February 2020, the People's Committee of the province or city directly under the Central Government has the responsibility to set up a coastal protection corridor under its management. However, to date, no local authority has implemented this task. Therefore, in the coming time, the establishment of coastal protection corridors in localities should be speeded up.

Finally, special attention should be paid to marine planning. There are regulations on the development and promulgation of a number of master plans in the sea such as master plan for resource use and environmental protection of the sea and islands; Planning on the use of the sea in Vietnam; Master plan for sustainable exploitation and use of coastal resources or recent national marine space planning. These plannings, though different in name, scope ..., are still interdisciplinary, overall and contribute to reducing overlapping conflicts between sectors and areas of marine use, while protecting the systems marine ecology. However, none of these plans has been issued yet. Therefore, it is necessary to urgently carry out this task to ensure harmony in the exploitation and use of natural resources and the protection of the marine environment while ensuring the right of people to approach the sea.

Some criteria for assessing sustainability in the exploitation and use of marine living resources should be proposed in the context of Vietnam. By the end of 2020, people of all walks of life will be aware of the value of marine life resources and the basic steps that they can take to preserve and use it sustainable way. The value of biological resources and marine biodiversity must be integrated, integrated into national criteria and indicators for sustainable development, poverty reduction strategies and the planning process plan; national accounting system and reporting system.

By 2020, to limit and eliminate all unreasonable incentives for the exploitation of marine biological resources. Abolish or renew (new) policies with specific policies to minimize, mitigate, and promote policies that encourage the conservation and sustainable use of biological and biological resources learn. These positive policies are developed and applied in accordance with national regulations and in harmony with the Convention and other relevant international obligations, taking into account the country's socio-economic conditions. Governments, businesses and stakeholders at all levels have completed the development of a roadmap, concrete steps to achieve or have implemented strategies, planning and plans for exploitation, production and Sustainable consumption of marine biological resources. There is a specific program of activities that contribute to mitigating negative impacts, making use of biological resources within ecological safety limits.

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Type of Story Related to “Human-Fairy Marriage” in Vetnam and Other Southeast Asian Islands Countries Mount with Environment

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Abstract

This study attempts an analysis of the similarities and differences of an intrinsic structure, the patterns of intrinsic structure, and their influence on the fairy tales. The research samples consisted of 60 fairy tales. The study finds that the plots consist of: a male character, who wandering in a jungle, accidentally comes across a lake where fairies are bathing. He steals a fairy’s shawl and forces her to marry him. The fairy findsher shawl that is stolen and she finally goes back to heaven. The similarity among some fairy tales seems to have occurredbecause of the mutual correlation among them and the context of dissemination.

Keywords: Fairy tale/ Intrinsic structure/ Similarities/ Differences/ A male character/ Fairies

1. Introduction

According to Wellek and Warren (111), the history of comparative literature study begins fromstudies on oral literature, folklore and its migration, that is, how and when it comes into the more artistic literary writing. In this terminology, comparative literature is encompassing the study of relationship between two or more literature. Furthermore, Darma (2007) says that comparative literature is drawn by the awareness that literature is not singular but pluralistic in nature.According to Block (Saman 95), the study of effect is the important study within comparativeliterature. Block says that the effect can be elaborated into several parts, such as (1) part of the art or creative art, use of past as an inspiration, (2) the authors’ relationship and relevancies factor, (3) an accidental element, (4) as the source of the creation process, and (5) an aesthetic interaction that is not easily seen by the eyes. Based on the background mentioned above, this article will discuss the similarities and differences found in some fairy tales in VietNam and other Southeast Asian Islands Countries. Those similarities and differences are reviewed from the intrinsic point of view, thus repeated similarities will eventually form the pattern of the fairy tales.

2. Methodology

In this research we have applied the following method:

Sorting statistics: We researched 60 folk tales in Viet Nam and other Southeast Asian Islands Countries folklore collections and folk culture pages in different Indonesian islands with the same content about the marriage between man and fairy.

Methods of classification: We have selected 60 copies and then compare them basing on criteria such as Findings and Discussion, Character (Male Character, Fairy Character), The Fairies and Their Children, Setting.

Using comparative method is to compare two phenomena with similar signs in order to highlight the characteristics and attributes of the phenomenon through the other phenomena. Comparative features highlight the type of story and the culture hidden deep in the story.

Review of related literature

Intrinsic structure of fairy tales

In order to comprehend a story, an interpretation toward its elements is needed. Bacon (244) asserts that in the attempt to understand a novel (in this case, a folklore), which illustrates complete action from a unique world, the elements of the story needs to be described in terms of its plot, setting, and character. Plot according to

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Stanton is a pattern that contains a sequence of events; but each event is only connected in cause and effect, an event which is caused or cause other events (Nurgiyantoro 113). The occurrences in a plot are usually cause and effect. The first occurrence causes the second one, and so on. On the other hand, the time-orderly occurrence cannot be defined as plot. Setting defines the general circumstance, giving a concrete and concise illustration to a story. Setting provides readers with realistic pictures, so that they are able to operate their imagination in the manner that the author wants to. Nurgiyantoro (185) stated that setting can be divided into three main elements, namely the place, time and social. Setting of place shows the location where the events are recounted in a fiction. The place element used may be some places with a specific name, particular initial, may be a specific location without clear information. Setting of time relates to the time when an event occurs in literature. A matter of time usually associated with a factual, time related to historical events. Knowledge of reader about the incident is used by the reader to understand the story. Social setting related to the behavior of social life in a place which is told in fiction. Characterization is often equated with the character and disposition, and it shows the placement of certain characters with certain characteristic in a story. Characterization present a clear picture of someone featured in a story. This view seems to put characterization as an important part in constructing a story. The characters in the story do not only serve to play the story, but also serve to convey ideas, motives, plots, and themes (Jones 33). The characters are the people appeared in a work of narrative or drama. That is why the characters occupy a strategic position as a carrier and transmitter of messages, mandate, moral, or something deliberately to convey to the reader (Abram 34).

Method

The VietNam fairy tales which will be analyzed in this study are the following:

A Human Married a Fairies (Kinh), Morning Star and Evening Star (Kinh), The Legend of Tiên Sơn Cave (Kinh), The Legend Of The Milky Way (Kinh); The Hunter (Muong); Ò Pjạ (Tay), The Legend Hang Nga (Tay), Tung Mariried a Fairies (Tay), Pó Tiên (Tay), Giraffe Gods (Tay); Nai's rice (Thai), Lục Pịa (Thai), The Maiden Married The Orphan (Cao Lan), The Story of Thi Thon (Lao), Ku and Ki (PuPeo), The Seventh fairy (Dao), The Deer Horns and The Demon (Dao), The Legend of Mien Stream (H'Mong), The Human-Fairy Marriage (Ha Nhi), Gió Lòng (Ha Nhi), The Seven Fairies (Lô Lô), The Magic Knife (Cham), Why don't Muslims eat pork? (Cham), The lengend O Loan Stream (Cham), The Lazy Men (Cham), The Maiden with Rum Du (E De), Fairytale Lồ Ô Stream (Raglay), Tiều Ca Lang (Van Kieu), BocRo's Son (Bana), Nineth fairy (H're), Duom Be (Ca tu), Taking Care of His Field (CaTu), The Human-Fairy Marriage (Cor), Orphan K'ong (Ma), A-Set A-Tieng (Xo dang), BocRo's Son (Bana), The Poor Boy Married a Fairies (Hoa).

The fairy tales other Southeast Asian Countries which will be analyzed in this study are the following:

Arya Menak (Indonesia), Jaka Tarub (Indonesia), Oheo (indonesia), Lahilote (Indonesia), Telaga Bidadari (Indonesia), Datu Pulut (Indonesia), Mahligai Keloyang (Indonesia), Mambang Linau Princess (Indonesia), BulaloLo Limutu (Indonesia), Raja Omas dan Bidadari (Indonesia), Tumatenden(Indonesia), Raja Pala(Indonesia), Lengend Horn Bills (Indonesia) , The Legend of Pattuddu Dance(Indonesia), Polopadang and Deatanna (Indonesia); The Handsome Young Man (Malaysia), Mayang Sari (Malaysia), The Story of Bayagong (Malaysia), Malim Deman (Malaysia); Kimod and The Swan Maiden Story (Philippin), The Maiden Star (Philippin), Taraw Siblaw (Philippn), Si Butatal (Brunei), Nang Nora (Singapo).

3. Results and discussion

Description

Swans are the largest of the aquatic birds, closely related to the Goose. They are known for their grace and beauty and have long been considered 'ornamental birds' which float on ponds in zoos, parks, and botanical gardens. Swans are long necked and web-footed. The most common swan, the Mute Swan, is a large, all white bird with a pink bill that ends in a black knob. The bill of a swan is so sensitive that it serves as an underwater feeler.

Swans have the longest neck of any bird, with 23-25 neck vertebrae. Swans have as many as

25,000 feathers. They are long-lived birds, and can live up to twenty years in the wild, even fifty years in captivity!

Habitat

Swans prefer wetlands and land surrounded by water, where they build their nests on mounds. The Tundra swan builds its nest in the tundra wetlands, where they maintain a territory of one square mile and defend it from other swans.

Mating

Swans will both display before mating, then mate for life. They are devoted to each other, and remain together throughout the year. They keep their young with them until they nest again, some staying through a second clutch. If one of a pair of swans dies, the survivor usually takes a new mate, and they form a dedicated pair. Breeding Swans usually mature in two to three years, and breed at around 3-4 years of age. The northern birds do not breed until their fifth or sixth year. Birds kept in captivity take much longer to establish a breeding pair.In their bulky nests, females do most of the egg incubation. The average clutch is about five eggs, but may be as many as ten. Incubation lasts about 30 days. Cygnets first learn to float in the water, then start to fly in about 60-75 days. Swans molt in July and August, when their cygnets are too young to fly.

Both the whooper Swan and Mute Swan are found throughout Europe and Asia, known for mating in the far north and returning south in the winter. For inhabitants of northerly regions, swans, and perhaps geese as well, would have been symbols of summer, whereas for those living further south, from the British Isles to Japan, they symbolized the coming winter. In Western Europe, swans arrived in the fall and left in the spring. When whooper swans and greylag geese migrated northwards from Scotland in the spring, it was said they were carrying the souls of the dead 'north beyond the north wind'. In the British Isles, the swans would arrive in late autumn, usually around the period known to the Celts as Samhain. Interestingly, these swans often travel at night, and they may have contributed to beliefs about the Wild Hunt and other processions of the dead found throughout Europe. The whooper swan has a musical call that would pierce through the quiet dark, while the mute swan's wings in flight produce a haunting musical sound. It is no wonder these unearthly sounds may have, for some people, heralded the return of the spirits of the dead.

Speciality of nature birds swan has been wishfully for the folk creation of the light type of story related to "Human – Fairy marriage (swan)

3.1 Plot

- **Similaritie**

Related to the fairy tales, the plot begins with the introduction of a male character that wanders into the wood. Some of the characters in different stories simply take a walk; others are doing something related to their respective job, such as catching birds, looking for firewood, gardening, and looking for a break in their journey. The male figure then finds a lake where fairies take a bath. The beauty of the fairy figure leads him to steal a shawl or wing of the fairy. This makes the fairy that loses her shawl unable to return to heaven. With this condition, she is forced to accept the marriage proposal of the man who stole her shawl. Before her marriage, she offers some requirements that must be made by the male character. The climax stage of the plot is that those requirements are violated by the male; so it becomes the cause of the fairy's finding her shawl. Finally, the fairy goes back to heaven, leaving her husband and her children.

- **Differences**

The differences of storyline are found in certain parts of the plot. Generally it is on the climax part when the fairy finds the shawl.

At Indonesia, in the story Arya Menak (Indonesia), Arya Menak violated fairy's agreement that prohibits him to open the lid of the pot so that the fairy could not cook with a grain of rice. Formerly she was cooking rice with just one grain, but when her power was gone, the supply of rice in the barn was empty. Arya Menak hid the fairy's shawl under a heap of rice and consequently the fairy finds her shawl back. This story has a similar climax with Jaka Tarub fairy tale. In Lahilote fairy tale, the fairy found her shawl when she took some rice at the bran; therefore, she was back to heaven with her children. The climax stage of Raja Omas dan Bidadari reaches when the fairy tries to find her clothes. Raja Omas did not predict that his wife still had an intention of returning to heaven. The fairy tried to take her children from him, but she often failed.

In The Story of Bayagong (Malaysia), when a child was playing with marbles behind the house, one marble rolled beneath the paddy storage bin. The child stretched out his arm and left around. Suddenly he touched some – thing soft. He pulled it out and ran with it to his mother. The mother told her two children that it was the garment she had lost some time ago. She explained who she really was and why she should returned to Fairyland. Sadly, she bade them farewell and putting on the robe, flew up into sky.

At Philippin, in the story Kimod and The Swan Maiden, The maiden found her shawl in the sumpitan

The Legend of The Milky Way, A-Set A-Tieng, The Magic Knife (VietNam) is when the fairy found her shawl in the granary. However in A-Set A-Tieng, The Magic Knife, she discovered it accidently when she pour out the rice to sun dried.

Thus, in conclusion, there are differences in terms of the plot as follows:

a, The story in Indonesia often tell about the fairy returns to heaven and separates like the plots in Arya Menak and Nawangwulan, Jaka Tarub, Putri Mambang Linau, and Mahligai Keloyang, Rajapala. But the story Raja Omas dan Bidadari tell about the fairy was back to heaven, but her family was not willing to accept her. So she was transformed into saringgon.

b, The fairy returned to heaven and her husband followed her and this occurs in The Legend of The Milky Way (VietNam), A Human Married a Maiden, The Youngest Maiden (VietNam)…,

c, The husband died on the road when finding his wife as : Orphan (Ý Pơ-ja, Luc Pia..), The Hunter .. Giraffe Gods VietNam

3.2 Character

There are three main characters in the fairy tales, namely, a young unmarried man, a beautiful, young woman coming from the heaven, and a child born from the marriage of the man and the woman. However, not all tales mention the latter character.

- **Similarities**
  - **Male character**

The main characters of fairy tales consist of a male character who stole fairy's shawl and fairy character as the targeted theft.

  - **Fairy character**

The fairy character is illustrated as a young and beautiful heavenly woman having wings (or shawl). The beautiful lady loses her shawl as it is stolen by a man when she is taking a bath with her sisters in a lake.

- **Differences**
  - **Male character**

Male characters consist of two groups of society, namely lower social class and upper social class. Lower social class characters can be found in the TieuCaLang, Orphan, Orphan K'ong, Ku and Ki, The idle servants, Mayang Sari, Jaka Tarub, Arya Menak, Oheo, Lahilote, PutriMambang Linau, Datu Pulut and Rajapala, … Si Butatal is about an ugly young man with skin disease who isostracized by society…

The upper social class can be found in tales other southest asia islands countries as story The Handsome Young Man, Telaga Bidadari, Raja Omas dan Bidadari, Tumatenden, and Mahligai Keloyang fairy tale. In Telaga Bidadari fairy tale, Awang Sukma who titled datu was a district ruler. Raja Omas in the story of Raja Omas dan Bidadari came from the royal family. However, his stepmother who hated Raja Omas washed him away to a river then he was found by a poor old woman.

  - **Fairy character**

The fairy character is generally the youngest daughter. The fairy bathing in the lake was commonly not alone. Most of the fairy tales other Southeast Asian Islands countries stated that the fairy was bathing with her 7 sisters (According to 24 kinds of fairy tales being analyzed, there are 20 fairy tales mentioned that information (see table). But, tales in VietNam often talk about the fairies harvesting rice as: The legend Hang Nga, Giraffe gods, Luc Pia, The Hunter, Nai's rice, the maiden married The orphan, The seven fairies.

Table 1. The table of man character

The fairy tales other Southeast Asian Islands Countries					The fairy tales VietNam			
No	Title	Upper class / Rich	Lower class / Poor	Occupation	Title	Upper class / Rich	Lower class / Poor	Occupation
1	Tumatenden	√		The rich man	A human married a fairies		√	The Hunter
2	Raja Pala		√	Bird catcher	Morning star and evening star		√	Firewood seeker
3	Lengend Horn Bills		√	Bird catcher	The Legend Of The Milky Way		√	The shepherd
4	The Legend of Pattuddu Dance	√		Raja	The legend of Tien Son cave		√	Fisherman
5	Polopadang and Deatanna		√	Farmer	The Hunter		√	Hunter
6	Arya Menak dance		√	Fond of wandering	Ò Pjạ		√	Finding Snails
7	Jaka Tarub		√	Dove catcher	The legend Hang Nga		√	The shepherd
8	Oheo		√	Open farmland cane	Tung Married a Fairies		√	Firewood seeker
9	Lahilote		√	Polahi has black Magic	Pó Tiên		√	No explanation
10	Telaga Bidadari	√		A Leader (Datu)	Giraffe Gods		√	Hunter
11	Datu Pulut		√	Bird catcher	Nai's rice		√	No explanation
12	Mahligai Keloyang		√ (Datu Skati)	A Leader	Lục Pịa		√	No explanation
13	Mambang Linau		√ (Bujang)	Firewood seeker	The Maiden Married The Orphan		√	Firewood seeker
14	Bulalo lo Limutu	X	X	Come from heaven	The Story of Thi Thon		√	Hunter
15	Raja Omas dan Bidadari	√		Son of the king (Tapper cane)	Ku and Ki	No explanation	No explanation	The Hunter

Table 1. The table of man character (cont.)

The fairy tales other Southeast Asian Islands Countries					The fairy tales VietNam			
No	Title	Upper class / Rich	Lower class / Poor	Occupation	Title	Upper class / Rich	Lower class / Poor	Occupation
16	The Handsome Young Man	√		Prince	The Seventh fairy		√	Farmer
17	Mayang Sari		√	Fish catcher	The Deer Horns and The Demon	No explanation	No explanation	No explanation
18	The Story of Bayagong		√	The Hunter	The Legend of Mien Stream		√	No explanation
19	Kimod and The Swan Maiden story		√	The hunter	The Human-Fairy Marriage		√	Farmer
20	The Maiden Star	√		The sugar cane farm owner	Gió Lòng		√	Firewood seeker
21	Taraw Siblaw		√	The hunter	The Seven Fairies		√	Farmer
22	Si Butatal		√	Fish catcher	The Magic Knife		√	No explanation
23	Nang Manorah	√		Prince	Why don't Muslims eat pork?	No explanation	No explanation	No explanation
24					The legend O Loan Stream		√	farmer
25					The Lazy Men		√	had done nothing
26					The Maiden with Rum Du		√	Fish catcher
27					Fairytale Lồ Ô Stream		√	fisherman
28					Tiều Ca Lang		√	fisherman
29					BocRo's Son		√	No explanation
30					Nineth fairy		√	No explanation
31					Duom Be		√	Corn, sweet potatoes thieves
32					Taking Care of His Field		√	Farmer

Table 1. The table of man character (cont.)

The fairy tales other Southeast Asian Islands Countries					The fairy tales VietNam			
No	Title	Upper class / Rich	Lower class / Poor	Occupation	Title	Upper class / Rich	Lower class / Poor	Occupation
33					The Human-Fairy Marriage	No explanation	No explanation	Farmer
34					Orphan K'ong		√	Firewood seeker
35					A-Set A-Tieng		√	Growing Watermelons
36					BocRo's Son		√	No explanation
37					The Poor Boy Married a Fairies		√	No explanation

Table 2. The Fairy Character Profile

The fairy tales other Southeast Asian Countries				The fairy tales VietNam		
No	Title	The numbers of fairy who are bathing in the lake / Fairies' trip to earth	The status of fairy	Title	The numbers of fairywho are bathing in the lake / Fairies' trip to earth	The status of fairy
1	Tumatenden	9 fairies	The youngest	A human married a fairies	6 fairies	The youngest
2	Raja Pala	7 fairies	No explanation	Morning star and evening star	No explanation	No explanation
3	Lengend Horn Bills	1 fairies	No explanation	The Legend Of The Milky Way	3 fairies	No explanation
4	The Legend of Pattuddu Dance	7 fairies	The youngest	The legend of Tien Son cave	7 fairies	have swords
5	Polopadang and Deatanna	1 fairies	No explanation	The Hunter	5 fairies	No explanation
6	Arya Menak	7 fairies	The youngest	Ò Pịạ	A numbers of	The youngest
7	Jaka Tarub	7 fairies	The youngest	The Legend Hang Nga	A numbers of	No explanation
8	Oheo	7 fairies	The youngest	Tung Married a Fairies	1 fairies	No explanation
9	Lahilote	7 fairies	The youngest	Pó Tiên	A numbers of	seventh Fairy
10	Telaga Bidadari	7 fairies	The youngest	Giraffe Gods	A numbers of	No explanation
11	Datu Pulut	7 fairies	Having orange shawl	Nai's rice	2 fairies	No explanation
12	Mahligai Keloyang	A numbers of	No explanation	Lục Pịạ	3 fairies	No explanation

Table 2. The Fairy Character Profile (cont.)

The fairy tales other Southeast Asian Countries				The fairy tales VietNam		
No	Title	The numbers of fairy who are bathing in the lake / Fairies' trip to earth	The status of fairy	Title	The numbers of fairywho are bathing in the lake / Fairies' trip to earth	The status of fairy
13	Mambang Linau	7 fairies	Having orange shawl	The Maiden Married The Orphan	10 fairies	The youngest
14	Bulalolo Limutu	7 fairies	The eldest	The Story of Thi Thon	12 fairies	The youngest
15	RajaOmas dan Bidadari	7 fairies	The youngest	Ku and Ki	A numbers of	No explanation
16	The Handsome Young Man	7 fairies	The youngest	The Seventh fairy	7 fairies	The youngest
17	Mayang Sari	7 fairies	The youngest	The Deer Horns and The Demon	A numbers of	No explanation
18	The Story of Bayagong	A numbers of	The youngest	The Legend of Mien Stream	No explanation	No explanation
19	Malim Deman	7 fairies	The youngest	The Human-Fairy Marriage	A numbers of	The youngest
20	Kimod and The Swan Maiden story	7 fairies	The youngest	Gió Lòng	7 fairies	The youngest
21	The Maiden Star	A number of	Having white shawl	The Seven Fairies	7 fairies	The youngest
22	Taraw Siblaw	10 fairies	The youngest	The Magic Knife	3 fairies	The youngest
23	Si Butatal	1 fairies	The youngest	Why don't Muslims eat pork?	1 fairies	No explanation
24	Nang Norah	7 kinnaree	The youngest	The legend O Loan Stream	1 fairies	No explanation
25				The Lazy Men	1 fairies	No explanation
26				The Maiden with Rum Du	9 fairies	No explanation
27				Fairytale Lồ Ô Stream	1 fairies	No explanation
28				Tiều Ca Lang	A numbers of	eighth fairy
29				BocRo's Son	A numbers of	No explanation
29				Nineth fairy	9 fairies	The youngest
30				Duom Be	2 fairies	2 fairies
31				Taking Care of His Field	A numbers of	No explanation
32				The Human-Fairy Marriage	A numbers of	The youngest
33				Orphan K'ong	2 fairies	No explanation
34				A-Set A-Tieng	A numbers of	The youngest
35				BocRo's Son	A numbers of	No explanation
36				The Poor Boy Married a Fairies	7 fairies	The youngest

3.3 The fairies and their children

Similarities

Most of the fairy tales stated that the marriage between human and fairy produced a child. Children born by a fairy were living on earth when the fairy was back to heaven as in tales Indonesia: Jaka Tarub, Oheo, Telaga Bidadari, Datu Pulut, Mahligai Keloyang, Raja Omas dan Bidadari, and Rajapala; however, it is different with Bulalo La Limbutu fairy tale. In fairy tale the maiden star (Philippin), the children back to the heavens to flicker their last ray before the approach of dawn.

But Vietnamese fairy tales stated that the husband and his child who met an fairy in heaven had to return to earth with the fairy since man could not live in fairyland as: The Legend Of The Milky Way, A Human married a fairies, Gio Long, The Seven Fairies, The Youngest Fairy, Nineth fairy, The magic knife, The legend Hang Nga. On the contrary in Giraffe gods, Luc Pia, The Hunter, Ò Pjạ, Ku and Ki children met an fairy in heaven had to return to earth with the fairy since man could not live in fairyland.

However, there are some differences in characteristics not talking about children as: The Nang Norah (Singgapore),

3.4 Setting

- **Similarities**

The place where the male character and the fairy meet is set in a lake where the fairy and her sisters are taking a bath.

- **Differences**

- **Tales in other southeast Asian Islands countries**, the difference in setting occurs sometimes with the bathing in the lake other southeast Asian countries: Arya Menak, Lahilote and Mahligai Keloyang fairy tale implied that the fairies were bathing in the lake when there was a full moon. However, most fairy tales specified the fairies were bathing at day time.

- **Tales in Viet Nam**, the place where the male character and the fairy meet is set in field where the fairy and her sisters are harvesting rice as : The legend Hang Nga, Giraffe gods, Luc Pia, The Hunter, Nai's rice, The maiden married The orphan, The seven fairies

The fairies harvesting rice as : The legend Hang Nga, Giraffe gods, Luc Pia, The Hunter, Nai's rice, The maiden married The orphan, The seven fairies.

4. Conclusions

The Human – Fairy marriage tale is one of the best known and most popular tale types in folktale tradition around the world. The theme of this tale type in Europe, Vietnam and other Southeast Asian nations is similar and thus reflects the internationality of folklore. The swan maiden is a totem of beauty and grace. As in the story of the Ugly Duckling, it connotes inner beauty as well. If Swan is your totem animal, you are emotionally sensitive, and empathic towards the feelings of others, and you draw people to you. The pure white swan is a solar symbol, whereas the Australian Black Swan is a nocturnal symbol. The swan, with its long neck, acts as a bridge between the worlds, making it an oracular bird. Being a cool weather bird, its direction is North. Swans are excellent totems for children, those connected to the Fairy Realm, poets, bards, mystics, and dreamers.

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Female Bleds and Religious Beliefs of Type of Story Related to "Human-Swan (Kinnari) Maiden Marriage" in Folk Stories Southeast Asia

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Abstract

This article is devoted to the study of blend formation in Southeast Asia folktales. It aims at the study of heroines' origin and the revelation of other ethenic people traditions that gave rise to folktale plots. The research is built on the conceptual integration theory by G. Fauconnier and M. Turner. Images of folktale heroines are analyzed as blends yielded by separate input spaces; these input spaces in their turn are united by the generic space that is presented by cultural peculiarities and customs. Depending on types of input spaces all folktale blends were divided into three groups: anthropomorphic, zoomorphic and artefactual. The result of the research shows that Southeast Asia folktales appeared due to interaction of imagination and everyday experience by means of exaggeration and rethinking. The decodation of the tale context with the help of the blending theory reveals the origin of heroines' images is swan (Kinnari) maiden and helps to understand the mindset of ethenic people living in Southeast Asia.

Keywords: Blend/ Input space/ Southeast Asia/ Folktales/ Swan/ Maiden

1. Introduction

Folktales go back to the archaic epoch and reflect figurative comprehension of reality that surrounded our ancestors. It is traditionally believed that myths, in a close connection with rituals, ceremonies and rites of initiation, served as the originof folktales.

Folktales as well as myths, in this or that way, illustrate a primitive way of thinking. There are three main aspects that reflect the peculiarities of how ancient people interpreted the world. They are animism (animation of nature), anthropomorphism (imposition of human features onto nature, animals, and things), and totemism (belief in the idea that people originated from animals). Their features have been preserved in tales and now serve as evidences of a highly metaphorical content of this genre. Metaphorization due to its ability to explain one thing via another was an important tool of getting knowledge or comprehending the external world by means of something known. The metaphoric way our ancestors used to interpret the world is reflected in myths and folktales. Although many centuries later the real essence of this metaphoric context was forgotten and got to be perceived as something fantastic or unreal, it is still possible to reveal what's been hidden for centuries. The theory of blending by G. Fauconnier and M. Turner (2006) enables to decipher metaphoric context by addressing input spaces that serve as a basis for projecting one thing onto another.

2. Methodology

2.1 In this research we have applied the following method:

Sorting Statistics: We researched 69 folk tales in Indonesian folklore collections and folk culture pages in different Indonesian islands with the same content about the marriage between man and fairy.

We used the interdisciplinary approach, applying the research results of some related sciences such as foklore, culture, and biology to explain the problem. Methods of comparison, collation, synthetic analysis.

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2.2 Research Materials

The theory of blending is based on input mental spaces that, with the help of the cross-space mapping, yield another mental space, the blend. The blend partially inherits features of the input spaces and possesses an emergent structure of its own. One more essential component of this process is the so-called generic space. It reflects some common structure projected from the inputs (Turner, 2014). Blending works in the same way as metaphor does. G. Fauconnier points out various kinds of 'mappings between cognitive domains that are set up when we think and when we talk': pragmatic function mappings are based on metonymy and synecdoche, schema mappings refer to grammatical constructions, and projection mappings deals with metaphors (Fauconnier, 1999). In order to understand how folktale metaphors, or blends, were formed, it is important to analyze their input spaces and the peculiarity of mappings between them.

This research is based on studying southest asia female folktale images through the theory of blending. Female images are very metaphoric in their core because they carry traces of ancient rituals and beliefs. The collection of tales southest asia by Multi authors served as a material for analysis. We have chosen tales with women as main heroines and divided the received images into groups. Groups are determined by the types of input spaces used to form the blend. They are anthropomorphic (referring to a man), zoomorphic (referring to animals) and religious beliefs. Input spaces were analyzed from the point oftheir meaning in Southest culture.

The VietNam and Southeast Asia fairy tales which will be analyzed in this study are the table following:

Table 1. The fairy tales other Southeast Asian Countries. The fairy tales Vietnam

No	Title	Title
1	Sithon Manora	A human married a fairies
2	Lao Phen	Morning star and evening star
3	Princess Nan	The Legend Of The Milky Way
4	Preah Sothun Neang Monorea	The legend of Tien Son cave
5	The Tale of Phra Suthon – Manorah	The Hunter
6	Legendary peacock dance	Ò Pjạ
7	The Lazy Men	The legend Hang Nga
8	The Silver mountain	Tung Mariried a Fairies
9	Pindaya caves	Pó Tiên
10	The Story Dway Mal Naw	Giraffe Gods
11	Tumatenden	Nai's rice
12	Raja Pala	Lục Pịa
13	Lengend Horn Bills	The Maiden Married The Orphan
14	The Legend of Pattuddu Dance	The Story of Thi Thon
15	Polopadang and Deatanna	Ku and Ki
16	Arya Menak	The Seventh fairy
17	Jaka Tarub	The Deer Horns and The Demon
18	Oheo	The Legend of Mien Stream
19	Lahilote	The Human-Fairy Marriage
20	Telaga Bidadari	Gió Lòng
21	Datu Pulut	The Seven Fairies
22	Mahligai Keloyang	The Magic Knife
23	Mambang Linau	Why don't Muslims eat pork?
24	Bulalo lo Limutu	The lengend O Loan Stream
25	Raja Omas dan Bidadari	The Lazy Men
26	The Handsome Young Man	The Maiden with Rum Du
27	Mayang Sari	Fairytale Lồ Ô Stream
28	The Story of Bayagong	Tiều Ca Lang
29	Kimod and The Swan Maiden story	BocRo's Son
30	The Maiden Star	Nineth fairy

Table 1. The fairy tales other Southeast Asian Countries. The fairy tales Vietnam (cont.)

No	Title	Title
31	Taraw Siblaw	Duom Be
32	Si Butatal	Taking Care of His Field
33		The Human-Fairy Marriage
34		Orphan K’ong
35		A-Set A-Tieng
36		BocRo’s Son
37		The Poor Boy Married a Fairies

3. Results

Sotuthest asia folktale heroines are often associated with animals, things or other images. If to look back to the old of the Southeast Asian people culture, it comes as no surprise how these images appeared. Folktales as well as myths are the most ancient types of getting knowledge and sharing it with other generations. These genres appeared at the time when the man didn’t separate themselves from the natural, animal world, or the world of things, but conceptualized the existing reality via their own perception. This is the reason why anthropo-morphism in folktales is mostly expressed by carrying human traits over nature, animals and things.

3.1 Anthropomorphic blends

Projection mappings create female folktale blends with the help of the so called ‘mirror reflection’. On the one hand, nature is assigned with female anthropomorphic features, such as character traits, the manner of behaviour, and lifestyle.

On the other hand, natural images are projected on the woman, enduing her with features of natural phenomena, animals and plants. Female folktale images appeared from old of the Southeast Asian culture traditions and beliefs but were subjected to metaphorical transformations which can be revealed through blending. The most popular and recognizable blend that refers to the group of anthropomorphic blends is The story “Prince Suthon and Princess Manora”

The story *Preah Sothun Neang Monorea (Cambodia); Sithon Manora (Lao), Lao Phen (Lao), Princess Nan (Lao),), Legendary Peacock Dance (Thailand), The Tale of Phra Suthon – Manorah (Thailand):* shares several themes and elements that are found in other tales of celestial maidens. These themes and elements shape the characters and the plot of the story and could have emanated from religion and societal influences. Nature of the Divine. First and foremost is the presence of celestial beings. In "Prince Suthon and Princess Manora", first appeared Chomphuchit, the Dragon King, who lived in the pond. He acts as the provider of the people of North Panchan. He sustained the fertility of their land and ensured them of abundant harvest. Upon seeing this, the envious king of Panchan wanted to kill him. Fortunately, a hunter named Buntharik saved his life. Chomphuchit repaid the hunter by welcoming him to the place of the dragons, entertaining, and giving him jewels and the dragon rope/noose he requested for. The actions of the dragon king reflect the characteristics of a divine being that rewards those who give him favor. Beauty of Kinnaris. The main character of the story is Manora. She and her sisters were the seven kinnaris, half female- half bird beings, who bathed and played in the pond. They embodied beauty as they possessed attractive body figures. This was the primary reason why mortals such as Buntharik the hunter and Prince Suthon were lured by them.

Challenges to the Husband. In Prince Suthon's search for Manora, his wife, he encounters the King of Sawannakhon, Manora’s father. Prince Suthon is faced with challenges that are impossible to be achieved by an ordinary mortal such as shooting the arrow of his longbow through a series of articles, lifting up an incredibly heavy stone and picking his wife out of all her identical kinnari sisters. But since it had been established in the beginning that Prince Suthon is the reincarnation of Bodhisattva (a devout who aspired to be like Buddha), and a god in heaven aided him, he was able to surpass these, winning Manora back.

Impurities of Mortals. Looking now into the mortal characters, they are conceived as impure beings (as opposed to the divines). Though many humans are good inherently, some have been swayed by evil motives.

We may see the balance of good and evil in the character of the hermit. Initially, he connived with Buntharik by informing him that there were celestial maidens who came to the pond to bathe at full moon. He even advised Buntharik to ask for the dragon rope from Chomphuchit. In doing this, he partly was responsible for making the hunter selfish. Buntharik at that time just wanted to own one Kinnari, unmindful of what these maidens would feel if they lost one of their sisters. But on the other side, when Manora flees North Panchan, this same hermit served as a messenger to Manora and a guide to Prince Suthon in his quest for his wife.

The major antagonists were King Nantharat and the Wicked Soothsayer. King Nantharat envied North Panchan's wealth. Because of this, he intended to kill the dragon king with the help of the Brahman monks. The story then might imply a sort of religious bias on Hinduism since much of this story is based on Buddhist beliefs. In making the Brahmans appear to be criminals, the story might be stressing that these two religions have some areas of conflict or contradicting beliefs. While the Hindi implements the caste system, the Buddhists promoted equality among people. Buddhists also believe in the concept of Bodhisattva while Hindis do not.

While the whole kingdom of Panchan delighted in the presence of Manora, the Wicked Soothsayer was the only one who disliked her. He took advantage of the king's nightmare and interpreted it in a twisted manner. He tricked and purposively advised the King of North Panchan to sacrifice Manora. But then, after the king had discovered about his evil plan, he suffered banishment. This kind of situation is very prominent in society. Ironically, traitors emerge from the people who are expected to be loyal to the authorities.

Water and Dance. Elements that are also emphasized in the story would be water and dance. Water was represented by the crystal clear pond where the Kinnaris bathed and the water in the jars with which Manora was to be cleansed of human contact. In Buddhism, water symbolizes purity, cleanliness, clarity, and calmness. On the other hand, dance was reflected in the story when Manora performed before she is sacrficed. Dance's role is not only about entertainment but more about the art and grace that the Kinnaris possessed.

3.2 Zoomorphic blends

The motif of human transformation is also reflected in bird images, for example swan princess. The importance of this image has got its occurrence in Russian metaphoric expressions: swanneck, floating like a swan, white swan. The blend swan princessis often associated with beauty and purity, but why? In Southeast Asia mythology swans were treated as sacred birds. It was forbidden to kill them and eat their meat. The beauty and graciousness of these birds gave rise to legends about swan maidens. Swan maidens were supposed to be elegant, mystical creatures that rule the roost of nature. In Southeast Asia culture there was even a tradition among girls to put on white dresses with long sleeves and dance altogether to music (Gura, 1997). They imitated swan dance thus highlighting their chasteness. The reverence of these birds is reflected in folktales. The blending of the female and the bird images is brightly portrayed in the appearance of the main heroine. In the tales *A Human Married a Fairies (Kinh), The Legend of Tiên Sơn Cave(Kinh), The Legend Of The Milky Way (Kinh); The Hunter (Muong); Ò Pịạ (Tay), The Legend Hang Nga (Tay), Nai's rice (Thai), Lục Pịa (Thai), The Maiden Married The Orphan (Cao Lan), The Story of Thi Thon (Lao), Ku and Ki (PuPeo), The* <u>*Seventh fairy*</u> *(Dao), Arya Menak (Indonesia), Jaka Tarub (Indonesia), Lengend Horn Bills(Indonesia), The Legend of Pattuddu Dance(Indonesia), The Handsome Young Man (Malaysia), Mayang Sari (Malaysia),* The Story of Bayagong (Malaysia), *Malim Deman (Malaysia); Kimod and The Swan Maiden Story (Philippin), Si Butatal (Brunei), Nang Nora (Singapo).* For the modern reader the woman who has feathers and looks like a bird seems to be more than strange, but according to fairytale canons it is an ideal of perfection. Such an effect is achieved with standard folktale metaphors, lik swan-bird, beautiful maiden, from bone to bone the marrow flows, words like pearls intersperse, and frequent repetition of clipped forms of adjectives young girl, coloured dress. In this description the ritual of unmarried girls to get dressed as swans is discernible. Once again the generic space of two inputs is stipulated by cultural background.

Therefore, the belief of the people in the power of animals and birds as well as the ritual to disguise themselves for different purposes gave rise to folktale images. Of course male and female images are different because of different roles they played at those times, but these images are not spontaneous either. It is necessary to point out that not only animals were used as totems and symbols, man-made things were also conferred with powers.

3.3 Religious Beliefs

Birds have become a symbol of religious belief in Southeast Asian culture such as Kinnari.

Kinnara/Kinnari are often distinguished from other celestial creatures in the HinduBuddhist pantheon by their human heads, torsos, and arms, gracefully synthesized with avian legs, upswept tail feathers, and auspiciously articulated wings. As composite creatures, their very name in Sanskrit revolves around a riddle: "What kind (kim) of human being (nara)?" Inhabiting the mythical Himavanta forest of Buddhist legend, these paradigmatic couples are found throughout Asia, more commonly as half-horse, half-human (kimpurusa) in India, where they are thought to have originated. However, it is their bird-like qualities that come to the fore throughout Southeast Asia; each feather ascribed with protective powers, each proffered jewel of wisdom, transformative. Associated with music, poetry, and dance, these enigmatic beings are endowed with apotropaic possibilities to engender fear and to inspire the imagination. Whether found face to face on suspension brackets for sacred bells in Burma, or lending their weight to support post-Angkorian columns in Cambodia, their resonant presence resides at the interstices of all things, where moments of divine intervention are marked by the spontaneous giving of gifts. In Java, Indonesia, images of paired kinnara/kinnari can be found on stone temples (candi) like Borobudur, Mendut, Pawon, and Prambanan, where they are most often poised at the base of wish-granting trees (kalpataru), resplendent with garlands of jewels.

In Thailand, the most renowned kinnari is Princess Manora (derived from Mano-hara), whose love story with Sudhana, the Crown Prince of North Pancala is taken from a previous life of Buddha, the Pannasa Jataka. This story inspired a dance called "Manorah Buchayam," hailed as one of the more esoteric in the Thai classical dance repertoire, as well as the "Norah" dance of Southern Thailand. As one of the Divyavadana or "Heavenly Avadanas," the story appears as well in a detailed series of stone reliefs on the first gallery of the 9th century candi, Borobudur, in Central Java. When poised as couples at the base of wish-granting trees, kinnara/kinnari maintain their halfhuman, half-bird forms. In the case of Manohara and her celestial sisters on Borobudur, however, they are depicted in human form, the tale of Manora (Manohara) told in Southeast Asia has become conflated with the story of the Cowherd and the Celestial Weaver Girl, popular in China, Korea, and Japan, Indian, The Mentawei Islands, Celebes, The Moluccas, New Guinea, Micronesia, Polynesia, Australia and New Zealand.

It will thus be seen that all these lines of migration radiate from India, which fact seems clearly to point to India as the home of the *motif*. But if we look more closely at these routes which we have followed we will see that, to a large extent, they tell us the history of India itself. They tell us of the gradual expansion of Hinduism and Buddhism in the East and South-east, while in the North they exhibit the results of the invasion of Islam. That the great highways, both of land and sea, would be followed in any migration is natural enough, and we need not lay much importance on this side of the question as far as story-migration is concerned. It is the actual history of a country, both religious and political, that will tell us if it is likely to be a centre from which tales would radiate in all directions, or whether, on the other hand, it lies on one of the main routes from such a centre.

There but remains to discuss the interpretation of the *motif* to put the swan-maiden on the operating-table of criticism, to strip her of her feathers and any other ornaments she may have acquired in course of time, to dissect her, and by so doing hope to discover what she really is.

This is the cruel treatment she may expect from the scientific folklorist, who will not be happy till he has done it. He will then begin guessing, and perhaps give his opinion that the swan-maiden is nothing but a beautiful white cloud which is chased and captured by the spirit of the storm.[14] .Or he may look upon her as a being who has strayed from the Isles of the Blessed, where she rightly belongs.[29] He may, on the other hand, regard her as a founder of clans, taking into account only the totemistic aspect.[16] There is but one other theory he is likely to advance that which would attach most importance to the principle of taboo.[19]

Modern scholarship will at once discredit the two former opinions, and will hesitate on which of the two remaining theories to bestow its blessing. It will in all probability make a compromise and stretch out both hands at once, dividing the honours equally between totemism and taboo. I often feel that in seeking a scientific “explanation” for every *motif* we are very liable to forget what delicate and elusive material we have to deal with. Surely a story may be the result of a beautiful thought that by the merest chance flitted through the brain of some unknown person whose poetic imagination alone prompted its creation. The subsequent shaping of the tale may perhaps be governed by the creator’s subconscious obedience to the manners and customs of his own environment.

It is none the less a spontaneous and unpremeditated invention. In the case of the swan-maiden we have one of the most beautiful themes in the whole world of fiction. Her personal charm and elegance, the setting in which she appears, the manner in which she is captured, and the mystery surrounding her origin and abode, all add to her fascination, and make us love her.

The simile implied in the very term “swan-maiden” is beautiful in itself. The pure whiteness of the swan, the soft down of its breast, the grace of its movement, the poise of its head how could it escape being likened to a lovely woman? No wonder the swan-maiden was not easy to capture, and, being captured, was still harder to keep. It would require little less than a superman to make such a being from another world happy and contented in her new mortal home. And so the story grew.

Look upon her as you will, ascribe to her what origin you like, she still remains aloof and untouched a lovely thing whom we should be grateful to have met at all.

4. Conclusion

As any other folklore genre, folktales have passed from generation to generation in an oral form. This led to changes in plots and interpretations. Only several centuries ago folktales acquired a written form and started to be used only for esthetic function, thus having lost ritual and sacral meanings. Nowadays folktales are being studied in linguistics, philology, historical sciences, folkloristics and ethnology, but they still preserve many mysteries. The history of any nation has always been connected with its tale heritage. Folktales were initially created as an attempt to explain reality by means of imposing peculiarities of the natural world on the life of the society. The blending of ideas in tales carries a deep pragmatic sense. Folktales present a mixture of different ideas that at the first gaze seem to be quite ridiculous and fantastic, though these ideas were not blended spontaneously but are the result of long lasting traditions. As a lot of rituals were carried out by women, the later play an important part in folklore heritage. The notion of woman is the basic concept that is characterized by strongly marked national and cultural characteristics. Its interpretation is based upon stereotypes which have appeared due to biological and social functions carried by women. It is an input space that serves for yielding various blends both in everyday speech and in tales. Feature the symbol for the swan to the current text value, a signal, but sorry instead of a numbers of the angel but being aborting destroy must be protect for environment.

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Socioeconomic and Environment Challenges of Artisanal and Small-Scale Tin Mining Sectors in Bangka Barat Regency, Bangka Belitung, Indonesia

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Abstract

This paper attempts to investigate and elaborate the dynamism of Artisanal and Small Scale Mining (ASM) in Bangka Barat Regency, Bangka Belitung Province, Indonesia–most notably, the socioeconomic and environment challenges under Indonesia's Reform Era. Some questions embedded to ASM, whether this sector is short-term income gains at the local level can translate into medium and long-term poverty reduction or whether ASM gains outweigh its often-severe socioeconomic and environmental drawbacks. Qualitative method was used as research method with interviews, observations and document analysis were used in data collection stage. Some findings are confirmed important facts, firstly, the most significant environment degradation to ASM activities are deforestation and land degradation, erosion, biodiversity loss and water pollution from dumped tailings, alluvial river damage, acid rock drainage and river siltation. Secondly, Artisanal and Small Scale Miners' knowledge in terms of environment impacts due to their activities are very low. They got the information only from local newspapers, friends, and NGOs. It is believed that environment not become their mainly concern. Thirdly, environment assignments are not the priority of mining activities. Not only miners itself, local government also paid minimal attention to the need for maintaining environment degradation in ASM sectors. It is worsen by most of the reclamation attempts by government and large companies have failed due to weak of law enforcement. To conclude, different levels of government are trying to meet the ASM interests and environment assignments, but a coordinated response has not been formulated.

Keywords: Artisanal and small-scale mining (ASM)/ Tin mining/ Socioeconomic factors/ Human livelihoods/ Environment degradation/ Environment policy

1. Introduction

Since tin ore mined in 18th century by Chinese immigrant using traditional tools, Bangka Belitung Island had been exploited and cannot separated with tin for hundred years. When 1997 economic crisis hits Indonesia, followed by Reform Era, the national political system had been changing dramatically that begin the implementation of Regional Autonomy in early January 2001. Consequently, this changes triggered political constellations in local level.

In regard to Bangka Belitung Island, especially Bangka Barat Regency, it means crucial political economy and environment policy change, relate to tin management. One of the robust change to the previous mining regulatory is licensing structures–Contract of Work (CoW/CcoW) replaced by IUP (*Ijin Usaha Pertambangan*–Indonesian), either for foreign investment or local investment as described the table 1 below.

Artisanal and Small-Scale Mining (ASM)[1] stated as *people's mining* remains challenges until now. It is seen that tin mining accounts for about 70 percent of Bangka Barat's economy (BPS, 2015). Data showed an estimated 10,000 artisanal and small-scale mining operated across the island in 2010 with about more than 50,000 people depending on such mining for their livelihood (ITRI, 2012). This number must be increase in last several years.

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[1] In this paper, the abbreviation ASM will be used to refer to Artisanal and Small-scale Mining.

Table 1. Type of Indonesia's License in Mining Regulatory Framework

No	Type of License	Role
1.	Mining business license or *Ijin Usaha Pertambangan* (IUP)	A general license to conduct mining activities in a commercial mining area that is reserved for large-scale mining.
2.	Special mining business license or *Ijin Usaha Pertambangan Khusus* (IUPK)	A specific license to conduct mining activities in specific state reserve areas for a national strategic interest.
3.	People's mining license or *Ijin Pertambangan Rakyat* (IPR)	A license for mining in an area of limited potential that is served for small-scale mining. This category is available only to domestic investors.

Without a set of law and regulations, artisanal and small-scale miners have been massively expanded from 2001 in Bangka Barat Regency. Although they must expose themselves to harsh working conditions for minimal income in a high risk context, and endangering their health as well as the surrounding environment, the miners must digging deeper and deeper for living.

This paper trying to elaborate the dynamism of ASM in Bangka Barat Regency–most notably, the socioeconomic and environmental challenges and whether this sector is short-term income gains at the local level can translate into medium and long-term poverty reduction or whether ASM gains outweigh its often-severe socioeconomic and environmental drawbacks.

2. Methodology

In-depth and semi-open structured interviews were used in this paper. In-depth interviews were used to analyze the relationship between ASM and environmental challenges at local level that affect the sustainability development of miners' community. In-depth interviews were conducted with academician, NGO's which focus on environment policy, local mass media, local government officers, and tin mining experts. They were asked to examine the nature of specific research question. Besides, semi-open structured interview with miners also were conducted in six districts and twelve villages as fieldwork sites. Those villages were decided due to the intensiveness of artisanal and small-scale mining has been operated.

Key informants from miners were recruited from 12 (twelve) villages across Bangka Barat Regency, mostly in remote areas, where mining activities were carried out. These villages namely, *Air Belo* (*Muntok* District), *Berang* (*Simpang Teritip* Distict), *Puput* (*Parittiga* District), *Cupat* (*Jebus* District), *Mancung* (*Kelapa* District) and *Tempilang* (*Tempilang* District). The initial key informants consisted of 18 (eighteen) artisanal and small scale miners and in-depth interviews were conducted with 4 (four) informants who have the capacity on their own about tin mining in Bangka Belitung Province, such as academician, NGO, local mass media, and tin mining expert. It is important to mention here, the data collection, therefore, focused on depth rather than breadth. In doing so, the total number of informants were 23 (twenty-three) for research instrument.

3. Results

On a local scale, ASM activities remain the biggest socioeconomic and environment challenges. Due to no legal rights and permits over mineral, and often lack of the capacity to meet legal requirements of environmental assessment artisanal miners seen as an activity that's not only destroying the environment but also many rural Indonesians' livelihoods (Devi, 2013). But, in fact is, mining regulations often tailored to the medium or large-scale mining industries, instead of integrating it into broader governance discussions as often environmental degradation caused by ASM occurs within a vacuum of government regulation and presence (Spiegel, 2014).

Before going further, it is necessary to clarify what are the characteristics of Artisanal and Small Scale Mining (ASM) in the context of tin mining of Bangka Barat Regency. Table 2 describe type ASM miners of research locus. Basically, ASM characterized by the absence or low degree of mechanization, small covered area. It is also influx of migrant workers, low paid, low productivity, generally known as illegal activity, low safety standards, and poor environmental management systems.

Table 2. Thype of Artisanal and Small-scale Tin Mining in Bangka Barat Regency

No	Miner type	Mechanization in (%)			Area covered (ha) in %		
		H-m*	S-m**	U-n***	< 1	3 - 5	Other****
1.	Artisanal miner (lowest class)	-	-	100	33.3		66.7
2.	Small scale mining worker (middle-class)	-	100		83.3	16.7	
3.	Small scale mining owner (upper class)	-	100		100		

Source: primary data from fieldwork (researcher)
Notes:
*Highly-mechanized
** Semi-mechanized
***Un-Mechanized
**** In this category, cannot measured the area covered, where tin mining activities in the downstream rivers or riverbeds.

The table 2 above shows the main characteristics of ASM as follows, the miners had been mined 100% by low degree of mechanization (semi-mechanized, which is heavily used manual labours or workers), indeed, the artisanal miner absolutely not use mechanized tool for mining. The mine area covered which means the foremost of natural capital (mineral resources), based on the table presents is quantified low; 83.3% states working in *less* than 1 hectare of the total mine area. Only 16.7% estimates of miner working on area range from 3 to 5 hectares. Interestingly, there are differences in the ratios of mine area for artisanal miner, 66.7% of them works along downstream rivers, and can move easily from one river to river depend on the intensity of the streams. On the other hand, some miners said, if they worked on *less* than 1 hectare mine area (33.3%), actually the area was already abandoned by previous small scale miner, due to the unproductive area.

Figure 1. Artisanal miners who works in downstream rivers, heavily used manual labours. Primary data, fieldwork, taken April 9, 2016 (researcher)

Since the location, or access to mine location is priority within natural capital of ASM, therefore, the location of mining has been the subject of intense debate among many actors. Ranging from policy makers to academicians, mainly because of its diverse environmental degradation effects. Based on the interviews, just over half of those informants who answer the question about where are they doing mining activities, they were said is in ex-mine locations. Notwitstanding its economic importance, ex-mined locations mostly under the reclamation area by governments and large company, such as PT. Timah, Tbk. Due to conservation objectives, those reclamation areas are protected and prohibited to mine again. To put simply, those mining locations including reclamation areas are highly contested arena among miners, citizens, companies, and governments.

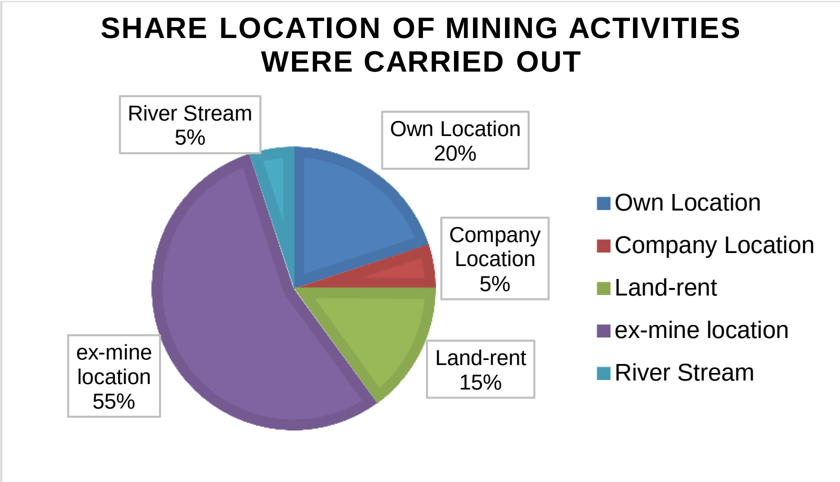

Figure 2. Share Location (%) of Mining Activities were Carried Out

The correlation between Bangka Barat government and ASM communities is interesting because as a policy making institution, basically, local government is aware of the great potential of artisanal and small scale mining for local people, however, putting the ASM sector as one of sustainable livelihoods options and brings the ASM sector to poverty reduction framework was far away from discussion. Although Indonesian mining policies and regulatory frameworks are exists, but still at infancy phase. Moreover, policy makers have little attention to make a radical change in terms of giving ASM communities opportunities to sustainable in mining sectors. Doing raid actions intensively do not make ASM's problem solved. Regarding this issue an informant reflected:

"Environmental problems in Bangka Barat cannot be blamed due to ASM activities only. If looked carefully, ASM miners doing mining operation in ex-mined locations that has been operated by PT. Timah or previous miners in the beginning of 2000s. I am not looking those miners doing mining practices in new locations. So, as far as I am concerned, the volumes or areas of environmental destructions have been not changed. Meanwhile, to date, large scale offshore mining operations that causing sea damage often be ignored. Whereas, there are many suction ships operates across shoreline. According to the laws, those operations are prohibited. In 2012, some of environmental activists in Muntok ever recorded the suction ship owned by Mario, which had operated under 10 mil. But, when we asked to the local officers about these operations, the local officers have not seen these operation as violation. I think this is the grim portraits how our legal system had been implemented unfair in West Bangka." (Interview, October 31, 2016)

Inappropriate government policies were found to cause the impediment ASM's sustainability. This inconsistency may be due to lack of understanding and efforts from government to over looked how ASM sector actually can be beneficial for local people in terms of socioeconomic improvement if implement '*good mining practices*', as one of the academician points it,

"So far, governments neither national, province nor local do not have formulate yet the policy in term of accommodating the ASM interests to extract tin minerals in Bangka Belitung in general, and Bangka Barat in particular. However, for sea-mining have been accommodated by local government, which is through partnership schemes with regional-owned enterprises (BUMD). The ASM contribution in providing informal job opportunities cannot be ignored. Although, their present is recognized yet, therefore, no adequate comprehensive policies in local level in term of tin mining." (Interview, Oktober 31, 2016)

A brief fieldwork was conducted from August to September of 2016, for the purpose of determining the miners' general perception of environmental deconstruction of mining activity within the ASM areas identified. The informants were included artisanal miners, small scale mining workers, and operator owners engage in basic mining practices. In short, this method particularly sought to elicit local people's perception of environmental challenges.

Table 3. Environment Degradation Perception from ASM Miners

Have you got the information of environmental effects due to tin mining activities?				Have you noticed the significant negatives effect during your mining activities?			
Yes	No	No response	Total Responses	Yes	No	No response	Total responses
11	6	1	18	15	3	-	18

Are you felt involved in maintaining the environments around mining sites?				According to you, what is the most priority should be taken in terms of small scale tin practices?
Yes	No	No response	Total responses	Responses
5	13		18	- Legal permit issues - Access to the markets - Technology enhancement

Source: primary data, fieldworks (researcher)

The majority of miners noted having got the information of environmental effects due to tin mining activities, some had also mentioned having the information from local newspaper, friends, and local government units. A substantial number of miners at one site in particular mentioned that their operations were significantly give negative effects during their operations. Then, some miners noted that they don't have any ideas in maintaining the environments around mining sites. However, only few of the miners said that the most priority should be taken in terms of small scale tin practices were environmental issue. Specifically, most key informants mentioned what is the most priority should be taken were legal permit issues, technology enhancement, and access to the markets.

4. Discussion

The Indonesian law No. 4 year of 2009 stipulated that local governments have power to giving the mining and smelting licenses to local entrepreneurs. According to Mining and Energy Department of local provincial government cited in (Fagotto, 2014) , 30 to 40 per cent of Bangka's population is active in mining, some working in private, licensed concession, but the vast majority operating in unlicensed areas. This is because increasing output by global demand toward tin. Consequently, not long after years since decentralization era, the island became overrun with thousands of "informal miners" or "artisanal miners".

In fact, there is no single definition of Artisanal and Small Scale Mining (ASM). In World Bank's assessment toolkit (2012), it defines ASM is part of informal sector, traditional practice, with skills passed from generation to generation. It may refer to small or microenterprises employing one or a few people or it can involve highly organized labor chains with complex and well-established organizational structures. Furthermore, it typically involves rudimentary tools, but in some cases, it can include basic equipment such as water pumps or jackhammers or even heavy machinery (Eftimie, et al., 2012).

In the same vien, (Spiegel, 2009) gives the terms "artisanal" mining and "small-scale" mining generally describe the use of rudimentary technologies of mineral extraction, undertaken by persons or groups who operate with limited capital investment. The activities can be illegal, legal or semi-legal. In addition, he demonstrates the using of this terms interchangeably, "artisanal" is generally regarded as a less technological sophisticated form of mining, often by panning or using no mechanization to crush rocks.

Accompanying pattern of socioeconomic benefit, however, it can be argued that artisanal and small scale miners tend to become vulnerable people, because they are characterized firstly as those with limited power and financial resource. In addition, ASM occurs in some of the most remote areas and involves some of the poorest people and trap to the "vicious cycle". Therefore, they are associated with places that suffer from high poverty and weaker long-term economic growth.

Turn to environmental challenges, which are most face by mining industries–reclamation. Admittedly, most of the reclamation attempts by governments and large companies in Bangka Barat Regency at post-mining have failed. As one key informant argued that despite reclamation has been failed, no efforts have been made by the government. Giving sanctions on the companies is one of the effort could be taken by governments. Reclamation system in which companies have to deposit money for reclamation in the post-mining stage has scarcely changed. Therefore, the funds and law enforcement has been not working properly. This was a significant positive correlation between environmental degradation and reclamation system has been failed over the years.

This paper also found several environment challenges as follows; the most significant environment degradation to be blame due to ASM activities are deforestation and land degradation, erosion, biodiversity loss and water pollution from dumped tailings, alluvial river damage, acid rock drainage and river siltation. Post-mining areas (ex-excavation) are become dangerous locations since they are not reclaimed and become a source of disease, such as dengue fever and malaria. Miners' lack of knowledge in terms of environment impact due to mining activities. Conversely, although they got the environment impacts information from newspapers, friends, NGOs and local government units, it is believed that it's not become their mainly concern. Therefore, only few of the miners pointed out that environment assignment is the priority in tin mining activities. Not only have the miner's perception itself, local government also paid minimal attention to the need for maintaining environment degradation in ASM sectors, it is worsen by most of the reclamation attempts by government and large companies have failed due to weak of law enforcement.

ASM's environmental impacts such as erosion, biodiversity loss and water pollution from dumped tailings, alluvial river damage, acid rock drainage, and river siltation in Bangka Barat are become dangerous (Bangka Post, 2016). Post-mining areas–(ex-excavation areas) are not reclaimed and become a source of disease, such as dengue fever and malaria. To sum, it is also have effects on community health such as contaminated drinking water and reducing water surface.

5. Conclusions

The negative environmental and socioeconomic impacts of tin mining operation are expression of informality. These negative responses have also been predominant focus of the Bangka Belitung media, via local newspaper (i.e. Bangka Post), which massive and frequent depict the entire ASM sector in an extremely negative statements.

The negative statements of ASM activities cited in those local newspaper in terms of environmental, health and social issues, including pollution and destruction of water bodies, the degradation of the environment, arable farmland and fishery catchment, as well as the negative health impact, mineworkers died and accident in mine sites, and sweep action which conduct by military groups.

The negative environmental impacts experienced by tin miners and their communities demonstrate the need for comprehensive, networked-up local plans that realize ASM's development potential. Therefore, there is a need to educate miners about laws and regulations as well as supporting them to meet these requirements, to improve their efficiency and mitigate the environmental impacts of their operations.

Local governments have paid minimal attention to the need for maintaining environmental degradation in ASM areas. Therefore, the action of local governments were limited to the raid action through military or armed groups. This neglect has undoubtedly exacerbated the environmental problem throughout the region.

Most of the reclamation attempts by governments and large companies in Bangka Barat Regency at post-mining have failed. Giving sanctions on the companies is one of the effort could be taken by governments. Reclamation system in which companies have to deposit money for reclamation in the post-mining stage has scarcely changed. Therefore, the funds and law enforcement has been not working properly.

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Comparative SWOT Analysis of the Key Stakeholders for Assessing Irrigation Governance in Cambodia

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Abstract

This study assesses the governance of irrigation through SWOT analysis of the relevant key stakeholders who are engaged with the irrigated agriculture at two sites in Cambodia. As a background of this study, as water demands in Cambodia rapidly increase due to population and economic growth, it is natural that the agricultural sector should be urged to improve efficiency of their water use. In the managerial context of the irrigation scheme, for better irrigation governance, some previous research have pointed out coordination among different stakeholders is important. Therefore, this study considers the characteristics of both internal and external environment of each stakeholder through SWOT analysis as a methodology. As a result, this analysis reveals the presence of Farmers Water Users Community (FWUC) is very weak because FWUC is a newly established organization, and thus, the trust by farmers is relatively low. In contrast, farmers are reliable with the heads of local governments (village and district) since they are elected by votes. However, due to the lack of budget and human capacity, it is not possible that their condition of irrigation get improved. Finally, in conclusion, this paper assesses irrigation governance in Cambodia still has some challenges and also suggests that the national government should have more initiative to build closer relationship among FWUC, local, and national government.

Keywords: Irrigation governance/ Cambodia/ SWOT analysis/ stakeholder assessment

1. Introduction

Water demands in Cambodia is steadily increasing because of both population and economic growth. Cambodia is still one of Leased Developed Countries as of 2015[1], but according to the World Development Indicators[2], total population has risen up from 9 million in 1990 into 12.2 in 2000 and 15.6 in 2015, GDP from 2.53 billion USD in 1990 into 3.65 in 2000 and 18.05 in 2015, and GNI per capita from 300 USD in 2000 to 1,070 in 2015, for example.

As the economy in Cambodia expands, the industrial sector grows up, whereas the agricultural sector shrinks, relatively based on GDP (Table 1). However, agricultural production has been rapidly increasing and underpinning the economic growth of the country. As shown in Figure 1, the amount of rice export, which is one of the most popular agricultural crops, is dramatically rising. These data remind us of the fact that not only industrial sector but also agricultural sector needs more and more water, and thus, securing enough amount of water and improving efficiency in water utilization are the urgent challenges Cambodia is facing.

But in fact, water use in agriculture is not efficient from technological and social aspects. Regarding the first point, it is clear that farmers at local level need more sophisticated infrastructure and better-organized plan for securing water (Chem et al., 2011). In terms of the latter viewpoint, we can easily find several causes of inefficient water use, for instance, the lack of education or training for farmers, uncoordination among different stakeholders, the lack of management leadership and capacity at local level, and so on (Nang et al., 2011).

[1]United Nations Department of Economic and Social Affairs (UNDESA) Website: http://www.un.org/en/development/desa/policy/cdp/cdp_ldcs_countryfacts.shtml.
[2]World Bank website: http://databank.worldbank.org/data/views/reports/ReportWidgetCustom.aspx?Report_Name=CountryProfile&Id=b450fd57.

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From the viewpoint of the cooperation among various stakeholders at irrigation scheme level, in recent years, donor agencies have introduced the concept of Participatory Irrigation Management (PIM), and this encouraged the central government to establish water users associations (WUAs) which consist of farmers and to transfer the authority of water management to WUAs. In Cambodia, too, the national government has organized several Farmers' Water Users Communities (FWUC) and recommended them to manage water (Figure 2).

Table 1. Transition of economic indicators in Cambodia

	Year (% of GDP)		
	1900	2000	2015
Agriculture (value added)	47	38	28
Industry (value added)	13	23	29
Service, etc. (value added)	40	39	42
Exports of goods and services	16	50	68

Source: country profile provided by World Bank website

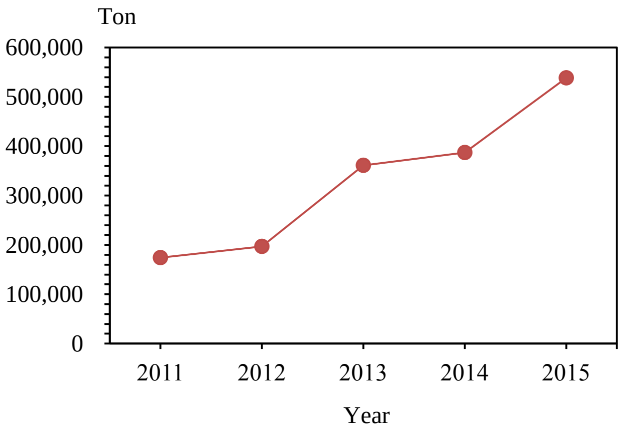

Figure 1. Transition of rice export
(Source: data from Cambodia Rice Federation website: http://www.crf.org.kh/?page=front&lg=kh&lg=en)

In the context of PIM, FWUC should have responsibility for the operation and management (O&M) of water courses and gates, collection of water fee, and liaison and coordination between the government and farmers. But in reality, water and agricultural management at local site is not good or effective in general, and the different understanding and overlap of the FWUC role are thought to be one of the biggest challenges for efficient water management[3].

Therefore, the objectives of this research is to consider what is the key to success of the water and agricultural management at each irrigation scheme by assessing them on the basis of SWOT analysis and comparing them in two locations.

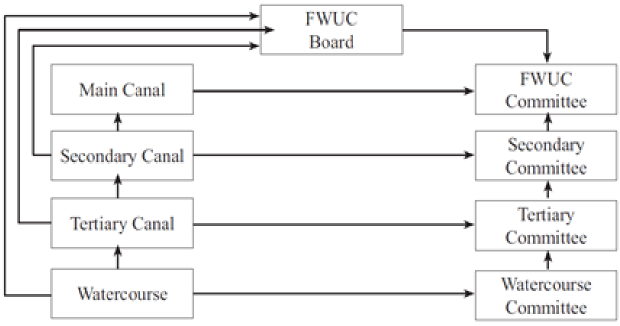

Figure 2. FWUC Committee Structure (Sinath, 2002)

2. Methodology

This study is basically based on qualitative approach, which means the authors had in-depth interviews with various key stakeholders who are engaged with irrigation management (shown in Figure 3) by taking advantage of semi-structured questionnaire sheet. The key stakeholders for this study were selected through stakeholder assessment (Susskind et al., 2006) as shown in Table 2. The number of check marks describes how strong/weak the interest of each stakeholder is in each major issue related to irrigation management.

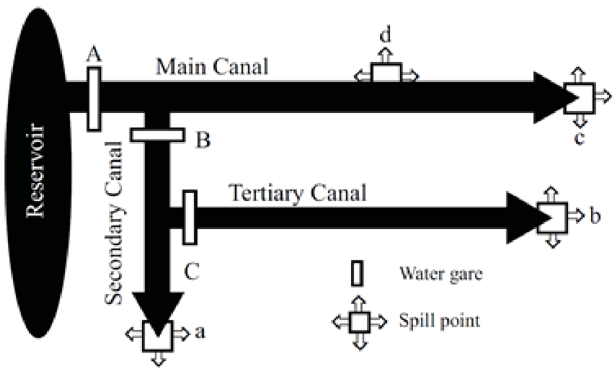

Figure 3. Common structure of irrigation scheme in Cambodia. Source: Nang, et al. (2011)
Note: Provincial Department of Water Resources and Meteorology (PDOWRAM) controls water in the reservoir and the main canal (A), FWUC controls the secondary canal (B) and Farmers Water Users Group (FWUG) is responsible for the tertiary canal (C).

[3]This hypothesis is based on the interviews with some individual farmers, a few board members of FWUCs and chiefs of districts, which were held by the authors in February and March, 2016.

Table 2. Stakeholder assessment in relation to irrigation management in Cambodia's case

		major issues					
		water allocation	water fee	management of reservoir	O&M	rice price	rice yield
key stakeholders	farmers	✓✓✓	✓✓✓	✓✓	✓✓✓	✓✓✓	✓✓✓
	fisherpersons	✓	✓	✓✓✓	✓	✓	✓
	retailers	✓	✓	✓	✓	✓✓	✓
	FWUC	✓✓✓	✓✓✓	✓✓	✓✓✓	✓✓	✓✓✓
	commune chief	✓✓✓	✓✓✓	✓✓	✓✓✓	✓✓	✓✓✓
	district director	✓✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
	PDOWRAM	✓✓	✓	✓✓✓	✓✓	✓	✓
	MOWRAM	✓✓	✓	✓✓✓	✓✓	✓	✓

Source: The authors have made the above table by reference to Susskind et al. (2006)

Our key informant interview survey was conducted in two locations (Figure 4), which are 1) Kirivong District in Takeo Province, and 2) Baray District in Kompong Thom Province. In each site, we made a fece-to-face semi-structured interview with selected three farmers, heads of FWUCs, local government agencies as interviewees on the basis of the above stakeholder assessment.

The former is the case whose management is not so good, meaning that their water course is not well-organized, there is a big gap of water availability between the near field and the far one, and the collection ratio of water fee and farmers' satisfaction are relatively low. On the other hand, the latter can be kind a good practice, indicating their irrigation scheme is far better designed because Asian Development Bank funded its construction, and thus, collection ratio and farmers' satisfaction are high, in general.

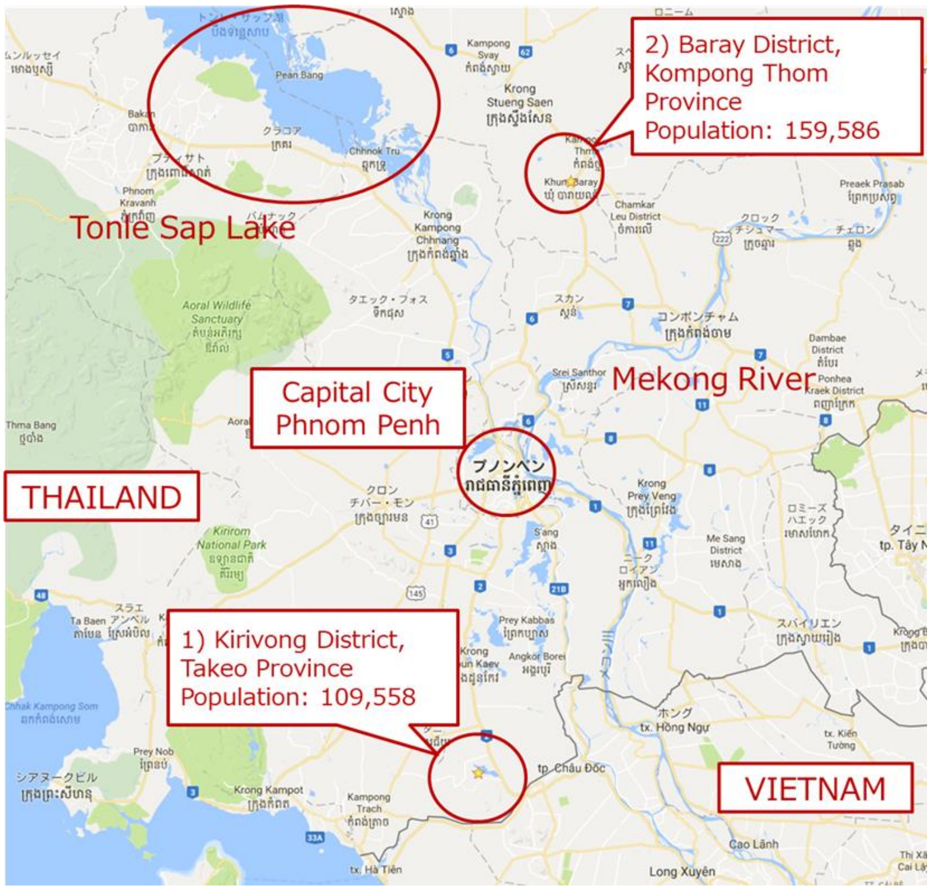

Figure 4. Location of case study sites

As a methodology for assessing stakeholders' roles on the basis of the information which were collected through in-depth interviews, the authors have decided to adopt SWOT analysis. "SWOT" is the abbreviation for strength, weakness, opportunities, and threats and its definition "is a qualitative examination that pinpoints internal (Strength and Weakness) and externals (Opportunities and Threats) factors at play in a specific environment that helps in understanding the status and formulates follow-up strategies" (Rachid et al., 2013; Kajanus et al., 2012).

The topics of interview survey according to SWOT analysis are shown in Table 3. The below questions in Table 3 are common in each interview as a structured part, and some additional questions related to their answers are also asked by the authors.

Table 3. Topics of interview survey

Strength/Weakness
• What kind of characteristics in terms of water management do you have?
• Do you have enough money (budget/income) to improve the situation of water?
• Do you have enough skills/capacity to use/manage water?
Opportunities/Threats
• What kind of chances/threats in terms of water do you have?
• How much water do you have in the districts?
• Are the communications among stakeholders good/bad?

3. Results and Discussion

The results of SWOT analysis about the district governments and FWUCs in two sites are shown in Figure 5 and Figure 6.

As a result of SWOT analysis from the information collected through interviews, commonly in both cases in Takeo and Kompong Thom Provinces, farmers, FWUCs, and district governments have limited power or presence to ask upper-level government agencies (central or provincial level) to reflect their requests or opinions, while they have power and money for constructing new infrastructure and irrigation schemes, but they don't care about individual farmers so much. Basically, this results from the weak authority or presence of FWUCs, and thus, FWUCs are not able to connect or bridge individual farmers with government agencies, in general, in spite of PIM. Because the concept of PIM and management by FWUCs is new, the authority and presence of FWUCs in irrigation governance may be weak and limited.

However, especially in the case of the Baray District, Kompong Thom Province as a good practice, through comparing the good and not-good case in two different sites, we could find out that farmers there seemed to have higher consciousness of self-reliance because our interview revealed because the leader of FWUC has a strong leadership, they often have meetings and dialogues among farmers, FWUC, and the district government, and they usually try to find the way they themselves can solve their problems, sometimes with the help of the district government.

Basically, this is because FWUC in Kompong Thom Province is very active and communicates with farmers for improving their irrigation management. These frequent communication and dialogue between farmers and FWUC can be thought to enforce their mutual trust and confidence building, and can contribute to improving the governance of irrigation management, efficiency of water use, and higher agricultural productivity. In fact, as evidence of this abductive inference, in the case of Takeo Province, we couldn't find any positive activities by FWUC or communication between FWUC and farmers.

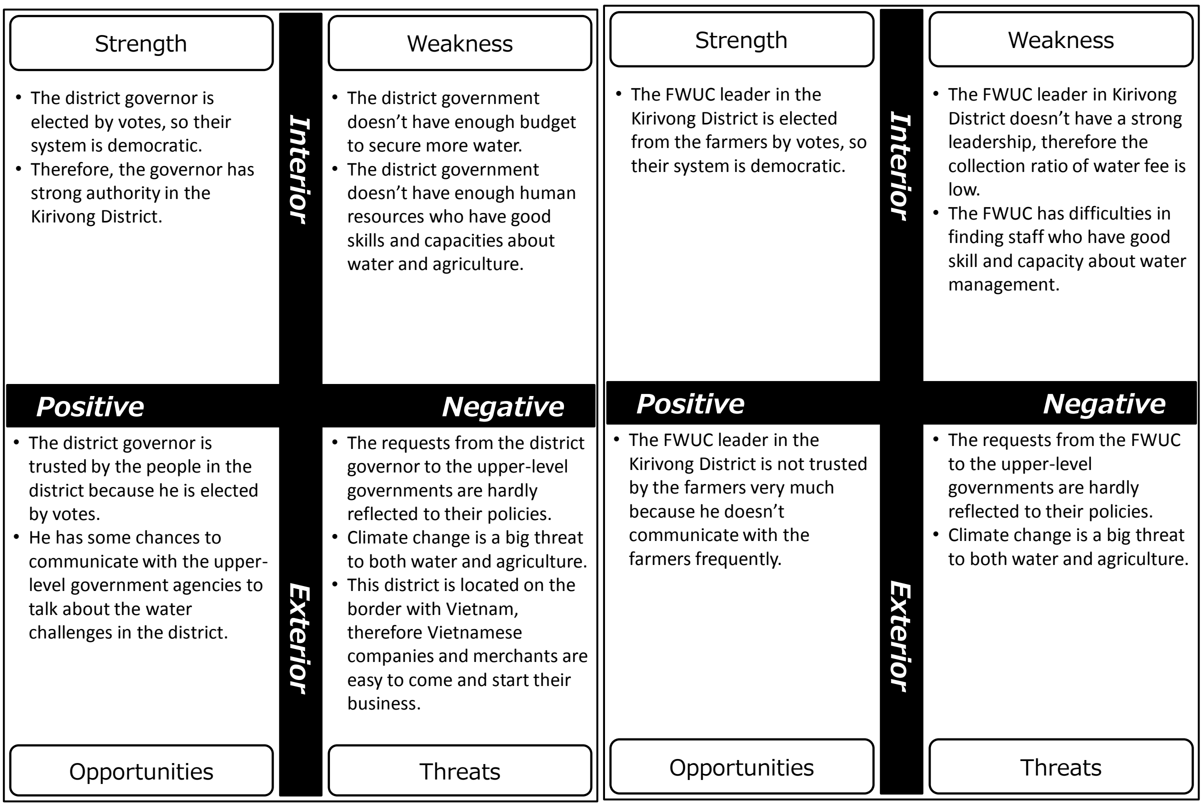

Figure 5. Result of SWOT analysis about the district government (left-hand side) and FWUC (right-hand side) in the Kirivong District, Takeo Province

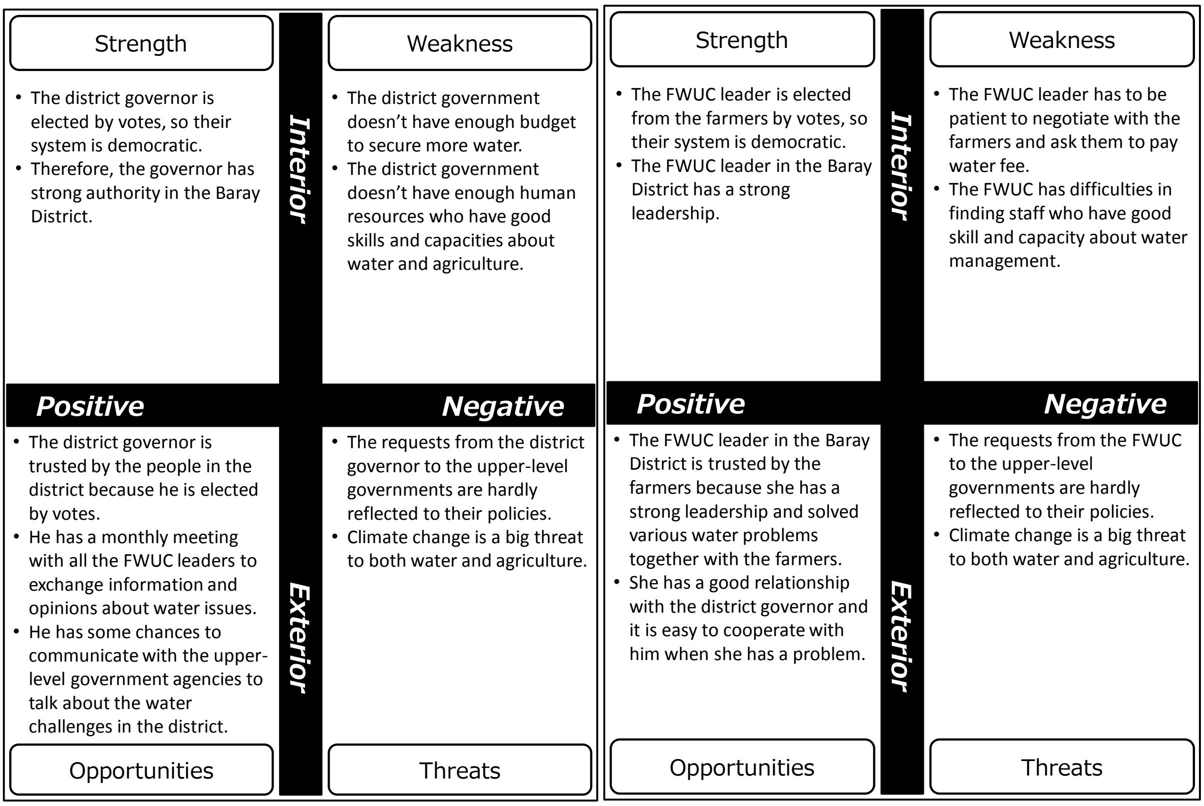

Figure 6. Result of SWOT analysis about the district government (left-hand side) and FWUC (right-hand side) in the Baray District, Kompong Thom Province

4. Conclusions

Finally, as a conclusion, this paper discovered the low and limited power and presence of district governments and FWUCs for reflecting the requests and opinions from individual farmers and community, compared with the upper-level government agencies such as provincial and central governments. On the other hand, our survey figured out the key to success of water and agriculture management is a leadership of FWUC and communication and dialogue among stakeholders for building mutual trust and confidence. The comparison of the cases in two sites shows that the strong leadership of FWUC in Baray District of Kompong Thom Province made the farmers active and help them cooperate with the district government to solve their problems. Therefore, this paper can conclude that the leadership or initiative of FWUC is important in the irrigation governance.

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Livable City - Liveable Life: Assessment of Happiness of Citizens of Danang, a City in the Central of Vietnam - Research for Social and Sustainable Development

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Abstract

To study and increase the quality of life, social development towards sustainability, focusing only environmental quality and physical life are not enough, the integration of quality of mental health of the people is needed to study in-depth and consider. This paper addresses the issues of living environment development for the people of Danang City, located in the central of Vietnam, where the area has been considered as the most livable city of the country, people have positive attitudes towards life and fell happier be themselves.

According to the study of Veenhoven Ruut (1942), states that "Happiness is the love of life we live in", which including 8 main factors affecting human well-being, e.g. 1) spousal relationship; 2) family relationship (parents/ siblings); 3) children; 4) work; 5) health; 6) self-confidence; 7) social relationships (colleagues/ friends); and 8) income.

In this paper, researchers applied 29 items of happiness (from 1 point to 6 points), in which applied from the study of Michael Argyle and Peter Hills (2002), and developed as questionnaire for survey form 378 staff who is working in the University of Da Nang.

The results show that the staff are quite happy (level 5), from the self-confident factor. Whereas, factors that make them unhappiness are factors like relationships within the family and personal health status. While, factors that could increase their happiness are linked to the psychological well-being (i.e., feeling stressed, sad or angry.); joining group activities such as yoga; conversation club for the staff, etc.

Keywords: Happy/ Happiness/ Woman/ Life/ Mental health/ Confident/ Social environment

1. Introduction

Danang: a city in the central of Vietnam, is the most livable city in Vietnam. It has implemented a wide range of social policies to become a livable city including 5 No, 4 Safe and 3 Yes. 5 "No" means No extremely poor household, No dropout students, No beggars, No drug addicts in the community and No murderers for robbery. 4 "Safe" includes Safe security, Safe traffic, Safe food and Safe society. 3 "Yes" means Yes for enough accommodation, Yes for enough jobs and Yes for civilized lifestyle.

By 2020, Danang has set the targets of having a population not exceeding 2 million, being a civilized and humanitarian urban area, having natural fresh air and high quality of social life, developing an intellectual economy and becoming a friendly, peaceful, attractive and livable city.

Together with the city to create a better living environment, in addition to developing the infrastructure, preserving the landscape, we focus on the quality of life of people in the area. Research on the level of happiness of the people who are the staff of the University of Da Nang to analyze the status of happiness of the people and at the same time to propose to the city leaders measures to enhance happiness for the community through activities.

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There are many types of research about happiness and definitions of happiness: from 1850, Spencer published the book "Social Statics or the Conditions Essential to Human Happiness'. McCall 1975) defines quality-of-life as "necessary conditions for happiness", and (Terhune, 1973) defined it as subjective satisfaction itself. Likewise, Colby (1987) described wellbeing as "adaptive potential', whereas Jolles and Stalpers (1978) defined it as 'basic comments to life". Elsewhere I have listed fifteen definitions of happiness (Veenhoven, 1984). Lane (1994) made this distinction clear by emphasizing 'quality of persons'. Musschenga (1994) discerned "quality of conditions for living from 'the quality of being human". Veenhoven's current research is on subjective quality of life. Major publications are: 'Conditions of happiness' (1984), 'Happiness in nations' (1993), 'The four qualities of life' (2000) and 'Greater happiness for a greater number: Is that possible and desirable?' (2010).

With Veenhoven, the expression of life happiness is *livability of the environment; life-ability of the individual; the external utility of life and inner appreciation of life.* With Robert Holden, happiness has 10 items*: Self; Relationships; Work; Attitude; Gratitude; Forgiveness; Health; Humor; Spirituality and Present.*

Shaver and Freedman (2005) Three A's of Happiness is *Acceptance; Affection and Achievement.* Meltzer and Ludwig (1985), the happiness of the people are *family, marriage, good health, and the completed work.* Research of Sears (1989) also concluded, satisfaction with their lives normally derived from happy family life, and then career.

Brickman et al. (1978), Diener (1984) and Isen and Stevenson (2008) show that, the manifestations of people happy are *laughing a lot; waking up feeling rested, being interested in other people; being satisfied with everything in the life; having a cheerful effect on others, warm feelings towards almost everyone…* many time each day.

Ross et al. (1997), Diener (2000), Diener (2006), Stevenson and Wolfers (2008) - there are many situations in the life make people stressed, worried or unhappy - relationship with husband/ with friends; with jobs; and health.

And we can make our life happier by finding amusing things; always thinking that the world is a good place; having good friends and being confident about sharing everything … Robinson and Godbey (1999); Kimball and Willis (2006) in *Women's Education and Family Behavior: Trends in Marriage, Divorce and Fertility.*

And in Vietnam, there aren't studies on specifically happiness, satisfaction with the life, Ho Si Quy is one of the first researcher on happiness, who accepted that: Happy life is happy Years (Happy life years) and living happily (Well-being: cheer Existence , happiness, pleasant life). The data presented in this paper is derived from the research results of the University of Danang: "Study on happiness: index of staff of Da Nang University code B2016-DN03-05".

In this article, the concept of happiness is defined as the positive emotional state of a person when they are satisfied with the elements of their life (including spouses, children, self-confidence, wellbeing, good payment, positive social relationships, and good work), thereby giving people motivation to work more effectively.

2. Research implementation

The research population is 378 staff members of Da Nang University, of which 65.03% lecturers 34.97% administrative staff, Male is 46.7%; Female 53.3%; By income level: 61.4% of staffs earn from 3-5 million VND per month. From 10 million or more only 26 people, comprising 4.71%. 162 peoples now earn 5-10 million. In terms of age: from 25-30 years old22.64%, 30-35 accounts 34.24%, 35-40 24.09% and more than 45 years old 19.03% (Table 1).

3. Methodology

The study used a questionnaire consisting of 4 methods.

3.1 Method 1: The happiness test

The test designed by Peter Hills, Michael Argyle (2002), The Oxford Brookes was studied for many years and was published in Personality and Individual Differences in 2002 in the University's Happiness Project., Oxford Brookes, United States. SPSS software is used to calculate the validity and reliability of the test for the staff of the University of Danang.

Table 1. Research samples and characteristics of samples

Staff	Percentage	Income (VND)	Percentage	Age	Percentage
Lecturers	65.03%	Under 3 mill	4.53%	25-30	22.64%
Administration staff	34.97%	3-5 mill	61.4%	30-35	34.24%
Male	46.7%	5-10 mill	29.3%	35-40	24.09%
Female	53.3%	More than 10 mill	4.71%	45-50	13.59%
				50-55	3.99%
				55+	1.45%

The test consists of 29 items, the staff can read each sentence and the selection of 6 points, with different level of "not true", "partly correct", "really correct", "very correct". The average score of the sentences is in seven intervals, corresponding to the happiness levels of the subjects (Table 2). When calculating the point for the opposite point for 14 sentences (2, 5, 6, 7, 9, 12, 13, 18, 19, 22, 24, 26, 27 and 28).

Table 2. Level of happiness

Level	Point	Level of happiness
1	Between one - two points	Not happy
2	Between two - three points	Somewhat unhappy
3	Between three - four points	Neutral – not really happy or unhappy
4	Four points	Somewhat happy or moderately happy - satisfied
5	Between four - five points	Rather happy; pretty happy
6	Between five - six points	Very happy
7	Six points	Too happy

3.2 Method 2: Assessment of happiness (expression, factors of influence, influence...) including 9 questions. Assessment of happiness through questionnaires related to factors affecting happiness such as: relationship with spouse/ partner (1); Parent/ Sibling Relationship (2); child problems (3); work (4); good health (5); self-confidence (6); have positive and positive social relationships (7); good income (8). The questionnaires of the perspectives of the object changes with happiness and related factors, affecting happiness. The subject will mark the degree of disagree, partly agree, agree and strongly agree, with a score of 1 to 4 points.

3.3 Method 3: Personal information (sex, age, job, income, family circumstances). Personal information is considered to calculate the correlation between characteristics of sex, age, education, job, income, family circumstances.

3.4 Method 4: The study used questionnaires for in-depth/ structured interviews with 40 staff members of Da Nang University to find out more about happiness concept, expression, influence and solutions to increase happiness.

Survey results were processed by SPSS software; the reliability of the test was Cronbach Alpha>0.8 and the coefficient of test KMO=0.768 Bartlett's test at significance level (p=0.000). From the test, the results of each item show that the indexes of the 29 items are consistent with the measurement structure and are reliable. This result allows us to use in the official investigation.

4. Results

4.1. The level of staff's happiness

The result of research from 378 staff in Danang, Vietnam show that: There are 10.2% of the staff feel not happy; 9.3% feel somewhat unhappy; Neutral - not really happy or unhappy (11.2%); Somewhat happy or moderately happy - satisfied (17.9%); Rather happy; pretty happy (31.3%); Very happy (15.1%) and too happy 3.6%. Grade point average's study group is 4.4, to the extent Rather happy; pretty happy. The average score for the whole class was 4.4 and the standard deviation was 0.23.

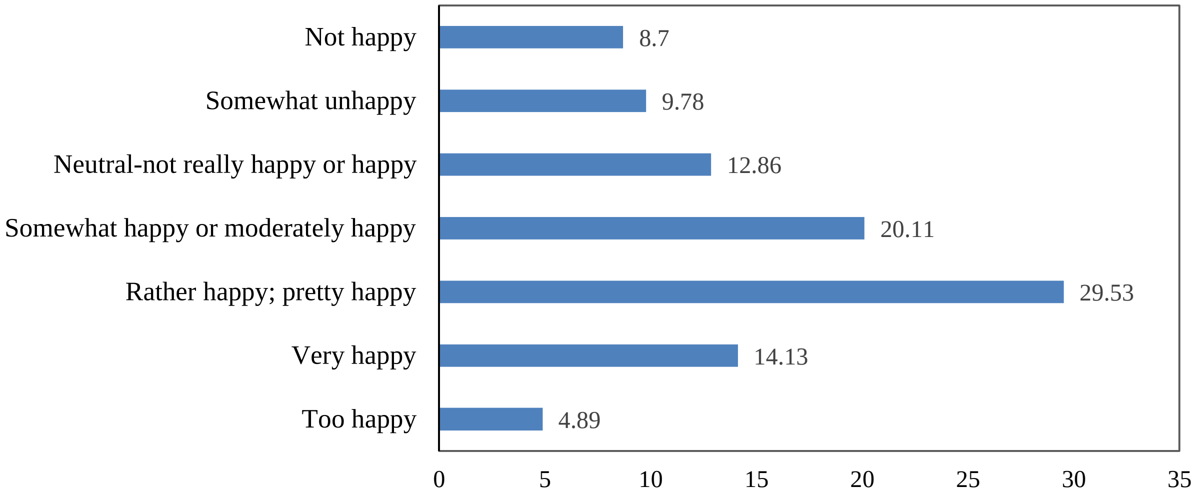

Figure 1. The level of staff's happiness

Of the 40 people interviewed for happiness, 12 were happy, and 21 were unhappy, and only seven said that their lives were considered happy at the level medium.

Compared to the age range of 50 years, meanwhile, the mean age of the population was 35-45 years (mean=3.5) (p=0.05). worried about her husband, health care, study work ... Ms TUS, 54 years old said: *I feel stable, my job is to practice good health and care for children, this is fun.*

Compared to the income level, those who earn between 3 and 5 million per month are the happiest people in the age group of 25-27. Mr. T.H.Q said: *"I am temporarily satisfied with my monthly salary of 4 million, it will increase by 4.5 million total incomes. Not much but I find it enough for life now".*

There is a correlation between academic degrees and degrees of happiness. The group of the happiest 35-40-year-old doctors, at 6 ($p<0.05$). Staff who were under 35 years old were the least happy, at 2 ($p<0.05$).

4.2 Manifestations of happiness of the staff

The results in 29 items showed that, the most expression of happiness of the staff is: *feel that life is very rewarding*, mean=5.03; *laugh a lot*, mean=4.69; *find beauty in some things*, mean=4.68; *intensely interested in other people*, mean=4.48; *Think the life is good* mean=4.43; *always committed and involved*, mean=4.28; *very happy*, mean=4.11; *well satisfied about everything in life*, mean=4.08 (Table 3).

Table 3. Manifestation of happiness of the staff

The manifestation of happy of the staff	Mean	SD	Order
1. I don't feel particularly pleased with the way I am.	3.34	1.54	18
2. I am intensely interested in other people.	4.48	1.19	4
3. I feel that life is very rewarding	5.03	1.36	1
4. I have very warm feelings towards almost everyone.	4.08	1.27	9
5. I rarely wake up feeling rested.	2.83	1.39	26
6. I am not particularly optimistic about the future.	2.95	1.61	8
7. I find most things amusing.	3.81	1.40	17
8. I am always committed and involved.	4.28	1.32	6
9. Life is good.	4.43	1.27	5
10. I do not think that the world is a good place.	2.32	1.34	27
11. I laugh a lot.	4.69	1.29	2
12. I am well satisfied with everything in my life.	4.08	1.42	10

Table 3. Manifestation of happiness of the staff (cont.)

The manifestation of happy of the staff	Mean	SD	Order
13. I don't think I look attractive	3.33	1.59	20
14. There is a gap between what I would like to do and what I have done.	3.39	1.59	18
15. I am very happy.	4.11	1.40	8
16. I find beauty in some things.	4.68	1.30	3
17. I always have a cheerful effect on others.	4.14	1.10	7
18. I can fit in (find time for) everything I want to.	3.96	1.28	13
19. I feel that I am not especially in control of my life.	3.01	1.52	22
20. I feel able to take anything on.	3.53	1.43	16
21. I feel fully mentally alert.	3.75	1.24	14
22. I often experience joy and elation.	4.01	1.37	11
23. I don't find it easy to make decisions.	3.56	1.61	15
24. I don't have a particular sense of meaning and purpose in my life	2.30	1.38	27
25. I feel I have a great deal of energy.	4.05	1.47	10
26. I usually have a good influence on events.	2.93	1.24	22
27. I don't have fun with other people.	2.86	1.54	24
28. I don't feel particularly healthy.	2.87	1.65	23
29. I don't have particularly happy memories of the past.	1.75	1.32	28
Mean	3.06	0.58	

4.3 Elements of happiness

Based on the rationale, we have developed a group of eight factors that influence the level of well-being of staff, including the relationship with spouse/partner (1); Parent/ Sibling Relationship (2); child problem (3); work (4); good health (5); self-confidence (6); positive and positive social relationships (7); good incomes (8). The question of the perspective of the object changes with happiness and related factors, affecting happiness.

Survey results show that the factor "self-confidence" is the most important factor, occupying the first place making the individual happiest (with average score=3.35). Self-confidence includes self-esteem; completing the assigned tasks; confident, creative; be trusted; enthusiatic and energetic.

The second factor that affects the perception of happiness is the close family relationship, including parents, siblings (average score=3.28) - that is, good relationship with parents, brothers and sisters; respect, mutual love. There are no conflicts, disagreements, conflicts between parents and children.

The third factor that makes employees happy is good health (mean=3.22). "If we are in good health, we can do a lot of things for our families, for our society," says the researcher. "It means that you can feel stable, healthy.

The relationship between husband and wife means that you are comfortable with your relationship with your spouse or partner (feel safe, love, respect ...). The results show that this is also a weak group ranked third in the level of happiness of people in Da Nang.

Table 4. Elements of happiness

Elements	Mean	SD	Order
1. Feel happy when I think about my husband/my darling/ spouse/partner	2.96	0.302	8
2. Feel happy when I think about family (parents)	3.25	0.481	4
3. Feel happy when I think about children	3.32	0.462	2
4. Feel confident my self	3.35	0.346	1
5. Have good health	3.22	0.328	6
6. Have good job	3.24	0.331	5
7. Have good relationships	3.28	0.215	3
8. Have good financial	3.08	0.336	7

The fourth factor that affects happiness is having a good job, working in a safe, friendly, trusting environment supported by everyone in the organization.

The fifth factor is the children, the sixth is the positive social relationship and the seventh is the good income.

4.4 The measures to increase happiness, reduce stress for the staff

Research about the measure to increase happiness in life, on of the staff shares, *when I'm worried or stressed, I'll look forsomeone for sharing, I have a close friends, they can hear me all days;* - this is the most chosen option, (with mean=3.36); and the second chosen option is going to the temple (mean=3.32). Another staff say that: *when I feel unhappy, I'll go to the Temple for praying, I believe that God can help me overcome all bad things in my life.*

Some other measures are *Looking for fortuneteller; Giving up, ignoring everything; Going out of the house, going somewhere to wish for a miracle; Joining yoga club, entertainment, sports*; *Traveling.*

Table 6. The measures to increase happiness

Methods	Level (%)				Mean	Order
	Never	Rarely	Sometimes	Often		
Accept the situation	26.1	4.3	10.1	59.5	2.65	8
Use of stimulants (beer, alcohol, ...)	50.8	26.1	2.9	13.0	2.23	9
Go to Pagoda, Church, praying ...	42.8	10.9	16.2	30.1	3.34	3
Talk, share with friends, consulting center	52.3	2.7	32.9	12.1	3.51	1
Find fortune tellers	48.7	12	11.4	35.9	3.21	5
Ignore everything	1.6	10.8	48.6	38.9	3.27	4
Go somewhere out of the house	2.2	5.9	49.2	42.7	3.27	4
May a miracle	4.9	7.0	53.5	34.6	3.18	6
Join clubs, yoga classes, entertainment, sports; conversation club, café shop ...	3.2	3.2	50.3	43.2	3.42	2
Travel	3.8	4.9	53.5	37.8	2.86	7

Happiness is an important value in life, this is the true value everybody looks for all their life… but many people don't know that the happiness reside beside them, in their life, on their ways. Happiness is all the thing they meet everyday, their family members; parents, wife/husband; children; neighbors; colleagues; in helping another; in each morning we get up.

5. Discussions

The mental health of a person is very important in model life when people feel they are mentally healthy, they will work and have a stable life, but when they are uncomfortable, unhappy, their lives are affected. Research on human psychological life and other social issues will help society to develop better.

This research aims to show the level of happiness of people are average, (the citizen who are the staff of the University of Da Nang, Da Nang University). This result suggests some measures for improving the quality of life. Research should be continued with the implementation of psychological activities support for the citizen.

6. Conclusions

The result of research from 378 staff in Danang, Vietnam shows that: the staff at the University of Danang is pretty good (level 5).

Expressions of a happy persons include some positive actions and feelings about life such as laughing a lot; assume that life is good and meaningful; willing to help others; I feel very much meaningful at work, in life.

The factors that create happiness for the staff of Danang University is belief in themselves; good health; good relationships; good job, etc. To improve the well-being of our staff, we have some suggestions based on the happiness from the factor make happiness for staff.

Our research will continue in the future with how to improve the well-being of the staff.

7. Acknowledgements

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Using Animals such as Buffaloes in Thai Tourism Industry: An Analytical Review of Animal Ethics

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Abstract

Human beings have utilized animals for different reasons from times immemorial. In the present society, animal or wildlife tourism is increasing its popularity in the global tourism market and many animals are being used in various ways for animal - based attractions of the tourism industry. However, only limited research or studies were conducted till date regarding the ethical characteristics of animal usage in Thailand's tourism industry and this is evident because of its scarcity in the tourism literature. Animal Ethic is therefore a topic under dispute. This led researchers, societies and social scientists to be concerned, which led them to initiate to review, discuss and study the rationale for using animals in tourism business or otherwise and consider deeply the ways and treatment meted to animals usage in order to find views and opinions of the general public on the issues under dispute. Therefore, the purpose of this paper is to overcome these shortage in written works by reviewing from various available literature from various sources relating to the ethics of using animals in tourism industry. The present article begins by providing introduction on tourism and on ethical concern with regard to using animals in tourist entertainment in particular, along with the significance of the study with overcoming the deficit in the available research studies. The paper is organized in the following way as: introduction, objectives of the study, description of the methods used in the study, practical and theoretical output obtained from the review of literatures, along with reviewing and describing animals, used in Tourism Industry of Thailand such as buffaloes and finally the article is summarized and concluded.

Keywords: Ethical issue/ Animal ethics/ Tourism industry/ Using animals in tourism

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Sustainable Low-Carbon Community Development: A Study Based on a Royal Project for Highland Community Development in Thailand

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Abstract

Development of community for sustainability needs to be balanced in all dimensions. Global warming and climate change from human emissions can affect the development of communities including highland communities in Thailand in terms of human life, growth and yield of plants and animals due to scarcity of water from global warming and climate change. For this reason, the Royal Project Foundation, Highland Research and Development Institute (Public Organization) and Mahidol University unite together to carry out the Royal Project for Community Development Project with aims to reduce greenhouse gas emissions by becoming a low-carbon sustainable community. The objective of this research is to study and develop the prototype of sustainable low carbon highland communities, based on the Community capital framework for sustainable development using Ethnographic Delphi Future Research (EDFR) methodology for collecting data for standardization from a panel of 15 community experts belonging to different strata, professions and organizations in Thailand. The evaluation level of the 12 communities low carbon development project evaluation levels is estimated by comparing the development standards of four parts in a period of 12 months. The results of this research can be used as a guideline for a sustainable low carbon community development. The four dimensions of development that the project takes into account are: (1) environmentally friendly agriculture; (2) forest restoration and conservation; (3) community health management; and (4) community strength to support change. It also evaluates other criteria, indicators, as well as development steps for preparing and evaluating community development levels for certification of community development standards into low carbon sustainable communities.

Keywords: Low Carbon/ Royal Project Foundation/ Sustainable Community/ Highland

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Solid Waste Management in a Secondary School in Ubon Ratchathani Province

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Abstract

Solid waste management is a national agenda that has announced the management of solid waste in the year 2015. Sources of solid waste in the community include homes and educational institutions. In Ubon Ratchathani province, the amount of solid waste coming from educational institutions was 25% per day. For this reason, this research aims to investigate solid waste management in a secondary school in Ubon Ratchathani province by interviewing 11 persons involved in solid waste management. Moreover, an opinion of solid waste management from 151 representative samples using the questionnaire, which was developed by the researcher, was based on the study of relevant data and theories. Data were analyzed using descriptive statistics.

The results of the solid waste management study revealed that there was 600 kg of waste per day during the regular semester. The sources of solid waste mostly come from a cooperative shop in schools, classrooms and cafeterias. The most common type of waste is plastic, organic waste 46.32% and 44.21%, respectively. The collection process is carried out using plastic containers and bamboo containers for 50 containers. For solid waste disposal was collected by the municipality every day. From the interviews with the personnel and the students found that overall opinion of solid waste management of samples were in strongly agree. However as the study result found some issues in solid waste management included there was no solid waste container by category, not enough waste bin, lack of awareness and wrong disposal. So the school should consider organizing a campaign to raise awareness for reducing waste generation reuse and recycle include providing solid waste container by category, and encourage students to dispose of the type in order to be properly managed.

Keywords: Solid waste management/ Solid waste/ Secondary School

1. Introduction

Currently, waste in Thailand, there are 1.14 kg per person per year. In year 2016, there were 27.04 million tons of wastes (74,073 tons per day) (Pollution Control Department, 2016). Situation of waste in Ubon Ratchathani province, the amount of waste is approximately 1,188.49 tons per day. There are 102 municipal solid waste disposal organizations. The amount collected was about 560.76 tons per day. There are 3 sanitary waste disposal areas including Warin Chamrab municipal (Office of Natural Resources and Environment Ubon Ratchathani, 2015). According to the municipality's data, solid from educational institutions amounted to 21 tons per day (Ubon Ratchathani, 2016).

A secondary school located in Warinchamrab district Ubon Ratchathani province, there were 2,947 students and 143 teachers and staffs. (Office of the Basic Education Commission, 2016) If there is no proper waste management, it could lead to environmental problems within the school in the future. Therefore, the researcher studied the waste management and an opinion of solid waste management from students, teachers and staffs of a secondary school in Warin Chamrab District Ubon Ratchathani province. The result of this study should provide some suggestion for proper waste management of a secondary school.

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Objectives:

1. To study solid waste management in a secondary school in Warin Chamrab District Ubon Ratchathani province.
2. To study an opinions of students, teachers, and staff in a secondary school in Warin Chamrab District Ubon Ratchathani province.

2. Methodology

2.1 Study design

This study was a cross-sectional descriptive study which study solid waste management in a secondary school in Warin Chamrab District Ubon Ratchathani province and study an opinions of students, teachers, and staff in a secondary school in Warin Chamrab District Ubon Ratchathani province. The data collection process was conducted from August 2016-December 2016.

2.2 Sample size

Solid waste management information was collected from 11 relevant persons on waste management in a secondary school. In a secondary school while the opinion of waste management was collected from 151 representative of 3 ,0 9 0 persons of a secondary school which consist of students teacher and staff. The sample was calculated from the population size estimation formula. As following

$$n = \frac{[NZ^2_{\propto/2}P(1-P)]}{[e^2(N-1)+Z^2_{\propto/2}P(1-P)]}$$

$$n = \frac{[(3{,}090)(1.96^2\ )(0.72)(1-0.72)]}{[(0.07^2)(3{,}090-1)+(1.96^2)0.72(1-0.72)]}$$

$$n = 150.41$$

2.3 Tools

2.3.1 Solid waste management information consists of 2 parts:

1) Analysis of physical composition of solid waste using quartering techniques which conducted by researcher

2) Waste management information use a questionnaire which consist of general information of solid waste management, quantity of solid waste, source of solid waste, waste container, solid waste collection and solid waste management issue.

2.3.2 Solid waste management's opinion information consists of 4 parts:

1) General information such as gender, age, education, salary and information perception.

2) Solid waste collection's opinion consists of 7 items. 5 level of rating scale, totally agree (5 points), agree (4 points), uncertain (3 points), disagree (2 points) and strongly disagree (1 point).

3) Solid waste carrying's opinion consist of 5 items. 5 level of rating scale, totally agree (5 points), agree (4 points), uncertain (3 points), disagree (2 points) and strongly disagree (1 point).

4) Solid waste disposal's opinion consists of 4 items. 5 level of rating scale, totally agree (5 points), agree (4 points), uncertain (3 points), disagree (2 points) and strongly disagree (1 point).

The questionnaire was try out with 30 representatives of another secondary school in Warinchamrab Ubon Ratchathani province this justified of solid waste management's opinion questionnaire reliability with score 0.72.

2.4 Data analysis

The quantitative data was analyzed using descriptive statistics.

2.4.1 Solid waste management information was presented by frequency, percentage, median, minimum and maximum.

2.4.2 Solid waste management's opinion information analyzed by using maximum score minus with minimum score then divided by 3 level.

1) Solid waste collection's opinion

Score 22-30 mean strongly agree
Score 15-23 mean agree with moderate level
Score 8-16 mean agree with low level

2) Solid waste carrying's opinion

Score 20-25 mean strongly agree
Score 12-19 mean agree with moderate level
Score 5-11 mean agree with low level

3) Solid waste disposal's opinion

Score 15-20 mean strongly agree
Score 9-14 mean agree with moderate level
Score 4-8 mean agree with low level

4) Overall solid waste management opinion analyzed by using overall score divided by 3 level.

Score 55-75 mean strongly agree
Score 36-56 mean agree with moderate level
Score 15-35 mean agree with low level

3. Results and Discussion

3.1 Solid waste management information

1) Physical composition of solid waste**:**

Result found most of solid waste was plastic type for 46.32%, organic waste for 44.21% and paper waste for 4.91% of total waste respectively same as a study of Wittawas Photilukha (2013) who studied about solid waste management in Khon Kaen school that found most of solid waste in school was plastic and paperrelevant with Nut Chaimraungjarus (2010) who studied about waste composition of community waste in a municipal that found most of waste were organic waste and recycle waste. It may cause from most of people use/ bought water and snack from many shop to consume in school, as show in table 1.

Table 1. Physical composition of solid waste

Waste type	Proportion (%)
Paper	4.91
Plastic	46.32
General waste	3.16
Organic waste	44.21
Foam	1.40
Total	100

2) Solid waste management information Information from 11 solid waste responsible persons found quantity of solid waste approx. 600 kg per day. Most of solid waste found at cooperative stores in schools, classrooms and cafeterias. Solid waste container is made from plastic and bamboo. There were 50 solid waste containers. As data found there was no waste sorting process prior to waste disposal. Solid waste disposal by municipal every day.

For solid waste management issues was not enough solid waste containers and lack of awareness same as a studied of Thitiya Sureechatyanthi (2010) who studied about a study of prototype management participatory waste disposal of civil and environmental engineering students that found issue of waste management was no sorting process and not enough waste containers.

3.2 Solid waste management opinion

1) General information of samples.

Most of samples are female (73.51%). Age average was 16 years (SD=6.51 years). Average salary was 2,604.50 baht (SD=9.289 baht), Most of sample got solid waste information from leaflets (35.76%) followed by internet source (33.77%).

2) Solid waste management opinion.

Result of solid waste management opinion of samples as classify by part found as follow.

Solid waste collection part found most of totally agree were solid waste should be sorted or separate by type of waste (77.48), solid waste containers should be covered to prevent rain or animal (72.85%) and school should promote to sorting the waste prior disposal (66.89%) respectively. The information is based on the theory of waste management. A separate garbage container must be provided. (Department of Local Administration, 2009).

Solid waste carrying part found most of totally agree were solid waste carrying vehicle should be closed while carrying (73.51%), prevent the leakage of water from solid waste (71.53%) and recycle waste carrying vehicle should separate from general waste carrying vehicle (50.99%).

Solid waste disposal part found most of totally agree were plastics, papers, able to recycle (72.19%), waste from food, vegetable and fruit should produce bio-fermented water or compost (56.29%) and should provide a place to build a fermentation plant. (46.36%). Therefore the school should provide knowledge about composition or fermentation of bio-waste for all persons. The school should promote some activity about reuse and recycle plastic waste as well. Moreover the school should take some action and encourage all staff to raise up waste reduction awareness which will be benefit of all people and environment (Pollution Control Department, 2013) as show in Table 2.

Result of solid waste management opinion of samples as classify by part found all 3 part were in strongly agree which lead overall opinion were in strongly agree (98.70 %)as well, as show in table 3 and table 4.

Table 2. The number and percentage of Solid waste management opinion of samples (n=151)

Solid waste management opinion of sample	Number (Percent)				
	Totally agree	Agree	Uncertain	Disagree	Strongly disagree
<u>Solid waste collection part</u>					
1. Provide solid waste collection area sufficiently for the amount of waste.	87 (57.62)	54 (35.76)		8 (5.30)	2 (1.32)
2. Sufficient space for solid waste.	40 (26.49)	68 (45.03)	40 (26.49)	3 (1.98)	
3. There are four types of waste that are promoted: waste, organic waste, hazardous waste and recyclable waste.	101 (66.89)	42 (27.81)	7 (4.64)	1 (0.66)	
4. There are four categories of waste containers: general waste, organic waste, hazardous waste and recyclable waste. To be easy to gather.	117 (77.48)	31 (20.53)	3 (1.99)		
5. Provide hazardous waste container.	100 (66.23)	45 (29.80)	5 (3.31)	1 (0.66)	
6. Provide knowledge and understanding about waste collection.	85 (56.29)	58 (38.41)	7 (4.46)	1 (0.66)	
7. Solid waste containers should have a cover can protect the rain or animals.	110 (72.85)	34 (22.52)	7 (4.64)		
<u>Solid waste carrying part</u>					
1. Housekeepers should be provided to collect waste that has fallen during carrying.	55 (36.42)	84 (55.63)	10 (6.62)	1 (0.66)	1 (0.66)
2. Recycle waste carrying vehicle should separate from general waste carrying vehicle.	77 (50.99)	58 (38.41)	14 (9.27)	1 (0.66)	1 (0.66)
3. Prevent the leakage of water from solid waste.	108 (71.53)	42 (27.81)	1 (0.66)		
4. Proposed to the municipality to provide more than one waste collection service per day.	72 (47.68)	53 (35.10)	22 (14.57)	4 (2.65)	
5. Solid waste carrying vehicle should be closed while carrying.	111 (73.51)	34 (22.52)	4 (2.65)	2 (1.32)	
<u>Solid waste disposal part</u>					
1. Should provide a small landfill in schools.	51 (33.77)	49 (32.45)	38 (25.17)	6 (3.97)	7 (4.64)
2.Should provide a place to build a fermentation plant.	70 (46.36)	52 (34.44)	22 (14.57)	4 (2.65)	3 (1.99)
3. Plastics, papers, able to recycle.	109 (72.19)	39 (25.83)	2 (1.32)		1 (0.66)
4. Waste from food, vegetable and fruit should produce bio-fermented water or compost.	85 (56.29)	52 (34.44)	13 (8.61)		1 (0.66)

Table 3. The number and percent of Solid waste management opinion of samples by part (n=151)

Opinion by part	Number(Percent)		
	Strongly agree	Agree with moderate level	Agree with low level
1. Solid waste collection part	88 (56.96)	61 (40.40)	4(2.64)
2. Solid waste carrying part	139 (92.05)	12 (7.95)	
3. Solid waste disposal part	129 (85.43)	21 (13.91)	1 (0.66)

Table 4. Level of solid waste management opinion of samples (n=151)

Level of Solid waste management opinion	Number	Percent
Strongly agree (Score 55 – 75)	149	98.70
Agree with moderate level	2	1.30

4. Conclusions

Solid waste management in a secondary school in Ubon Ratchathani province found there was 600 kg of waste per day during the regular semester. By the source of solid waste mostly come from a cooperative shop in schools, classrooms and cafeterias. The most common type of waste is plastic, organic waste. The collection process is carried out using plastic containers and bamboo containers. For solid waste disposal was collected by the municipality every day.

Overall, the samples were in strongly agree with the solid waste management of the school.. However, the study result found some issues in solid waste management included no solid waste container by category, not enough waste container, lack of awareness and wrong disposal. Therefore, the school should consider organizing a campaign to raise awareness for reducing waste generation reuse and recycle include providing solid waste container by category, and encourage students to dispose of the type in order to be properly managed.

5. Acknowledgements

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Using Contingent Valuation Method for Estimating the Willingness to Pay for Mangrove Forest: A Study in West Lombok, Indonesia

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Abstract

Mangrove ecosystem provides a range of market and non-marketed goods and services. It serves as a natural protection from storm, flood and abrasion. It also plays an important role in reducing the impact of land pollution to the sea. However, the total value of mangrove products is not easily recognized, thus they often neglected. As a result, mangrove forests often be converted in order to generate directly marketable products, such as aquaculture that might lead to massive economic lost for the surrounding community once natural disaster such as storm happen in the location.

This study estimated the monetary value of mangrove forest. A mangrove ecosystem in West Lombok Indonesia was considered as the case study. Respondent for this study were tourists, local communities and fishermen who were selected using purposive sampling technique. By employing Contingent Valuation Method (CVM), this study estimated respondents' willingness to pay (WTP) for the mangrove forest. This study found that the average WTP of tourist is IDR10, 500 (USD 0.75) per visit. While the local community and fishermen are willing to pay for a monthly contribution of IDR 8,500 (USD 0.61) and IDR 9,500 (USD 0.68) respectively. The WTP for mangrove forest of tourist was influenced by socio demography characteristics, facility, frequency of visit, and travel cost. For local community, the determinants of WTP for mangrove forest were socio demography characteristics, facility and preservation participation. While WTP for mangrove forest of fishermen was influenced by socio demography characteristics and household assets. The estimated WTP is expected to be utilized as useful information for setting up the entrance fee to the mangrove forest as well as for setting up a voluntary contribution of the local communities and fishermen for mangrove conservation.

Keywords: Mangrove forest/ Contingent valuation method/ Willingness to pay/ Ecosystem services/ Dichotomous choice

1. Introduction

1.1 Background of the study

The existence of mangrove ecosystems is beneficial to human life for suppling marketed goods and non-marketed environmental services, including provisioning, regulating, and cultural services (M. Brander, L., J. Wagtendonk, A., S. Hussain, S., McVittie, A., Verburg, P. H., de Groot, R. S., and van der Ploeg, 2012; Mendoza-González, G., Martínez, M. L., Lithgow, D., Pérez-Maqueo, O., and Simonin, 2012). The value of the mangrove ecosystem is not easily recognized by most people thus it impacts are often neglected (Hamilton, L. S., Dixon, J. A., and Miller, 1989; Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., and Raskin, 1997; Gunawardena, M. and Rowan, 2005). People lack of awareness and will usually realize the presence of mangroves when the mangroves are already extinct and disasters happened in nearby locations (Badola, R., Barthwal, S., and Hussain, 2012; Schwerdtner Máñez, K., Krause, G., Ring, I., and Glaser, 2014).

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This situation might not occur if people value the existence of such environmental services provided by a mangrove forest. The value of mangrove forests might be acknowledge more when it's have been converted into other forms such as aqua culture. The presence of economic valuation methods for environmental goods and services that do not have a market system has given awareness of the importance of mangroves for human life (Spaninks, Frank and van Beukering, 1997). This kind of valuation method can contribute to a more appropriate decision-making process regarding the use of mangrove forests, whether mangrove forests will be conserved or converted into other forms.

In West Lombok Indonesia, a mangrove forest has become one of tourist attractions. With the natural beauty offered by mangrove forests in this place, many domestic and foreign tourists come. In addition, the concept of education tourism was developed where the existence of this forest is used as a laboratory for the development of science. The local community also indirectly get benefit from the mangrove forests in the form of storm protection. In addition, fishermen who live in the surrounding area of mangrove forest also benefited from the variety of fish thanks to the existence of the mangrove forest (Authors' survey, 2018).

Ironically, the condition of this mangrove forest continues to damage. Based on data provided by the Forestry Service of West Nusa Tenggara (NTB) Province, until 2014 there was damage of around 65% of the total area of mangrove forests in this location. Similar conditions also occur in almost all mangrove forests in West Lombok.

Mangrove forest damage that continues to occur needs to be stopped to prevent wider damage that can have a negative impact on the life of people living around the area. Economic valuation methods can contribute to this matter by estimating the mangrove forest value from the perspective of the related stakeholders, including communities, fishermen and tourists. By knowing the value of mangroves, further policy can be formulated to protect the mangrove forests in the long term.

Economic valuation technique provides a quantitative environmental goods and services value for these kind of goods and services that do not have market price. An economic valuation approach can produce an estimate economic value of these types of goods and services. Prices that emerge from a natural resource often do not reflect actual costs and benefits, resulting in inaccuracies related to policies for the maintenance of these resources (Panayotou, 1993). The presence of valuation methods has also helped land pricing or also the cost of mitigating the impact of environmental damage as a result of a development process (Loomis, J., Kent, P., Strange, L., Fausch, K., and Covich, 2000).

As the mangrove forest in West Lombok is threatened by deforestation, a scientific study that evaluates the economic value of this forest is needed. This study is important as a reference for the related policy maker about the economic value of this mangrove forest. This present study aims to fill the literature gap by estimating the economic value of the mangrove forest through a contingent valuation method.

2. Methodology

2.1 Study area

This study took place in West Lombok, West Nusa Tenggara Province of Indonesia. The area was selected following a mapping using Geographic Information Systems conducted by (Saptutyningsih, E. and Diswandi (2018). Their study clarifies a decreasing of mangrove area in West Lombok as reported by the forestry agency in this province. The area was selected as the case study site.

2.2 Sampling procedure

The subjects of this study were communities living around the Lembar Mangrove Forest, fishermen and tourists who visited the mangrove forest. The reason for choosing these subjects was because they were well-informed about the mangrove forest so that they could help to elicit information on the value of it. The sample size of the study was determined after consideration had been given to the availability of resources including funds, the number of enumerators that could be recruited and the time it would take to complete the survey. Based on these considerations, it was decided that 300 people consisting of 100 tourists, 100 local communities, and 100 fishermen were selected as samples. The sampling technique used was purposive sampling technique. Among them, 30 people were selected for pilot survey, to find the average respondents' willingness to pay.

2.3 Design of the questionnaire

The questionnaire for this study was set into three sections. Section A collected information on socioeconomic characteristics of respondents including family size, age, and sex. Section B enquired the environmental awareness of the respondents (i.e. mangrove forest conditions, facilities in the mangrove forest, and effort for mangrove conservation). Finally, section C consisted of Contingent Valuation Method (CVM) questions to estimate willingness to pay of respondents for mangrove forest conservation. In this study, respondents were firstly asked whether they would be willing to pay for their experiences regarding the mangrove forest site. Respondents with "yes" answer were asked to give a monetary value for their experiences which is reflecting their willingness to pay for forest conservation.

Data was collected through survey. The respondents were approached by enumerators who firstly introduced him/her-self and the aims of the study. Then, respondents were asked whether they were willing to participate in the survey. If a respondent did not want to participate, then the enumerator approached the next available visitor. There was approximately 14% of visitor who refuse to join the survey. If a visitor was willing to participate in the survey, the questionnaire was given to him or her to fill in. The questionnaire was collected by the surveyor once it was done onsite. Similar onsite survey method has been used in some previous contingent valuation studies such as conducted by Lee (2002), and Togridou, A., Hovardas, T., and Pantis (2006).

2.4 Method of analysis

A Contingent Valuation Method (CVM) was employed to estimate the willingness to pay of the local communities, tourists and fishermen for mangrove forest conservation. CVM is considered as one of the most widely technique that used for conducting ecosystem services valuation (Gunawardena, M. and Rowan, 2005; Gupta, V. and Mythil, 2007; Stone, K., Bhat, M., Bhatta, R. and Mathews, 2008; Yacob, M.R., Radam, A. and Shuib, 2009; Binilkumar, 2010; Ekka, A. and Pandit, 2012). This method is called "contingent" valuation method, due to the technique that ask people about their willingness to pay or willingness to accept, a certain hypothetical situation of environmental goods and services (Brookshire, D. and Eubanks, 1978).

The CVM in this study was conducted through a survey of selected respondents. The survey asked some questions as mentioned in the previous section, that also consisting of a by-designed hypothetical market condition regarding the mangrove forest to capture the respondents' WTP (Loomis, Bair and González-Cabán, 2002). Through the CVM, stated preferences of the respondents were collected that reflecting the direct value of the quality of related environmental goods or services. This information was captured by asking the respondents about their willingness to pay (WTP) for an improved condition of mangrove forest.

CVM is an important tool in environmental economics that has been used by some number of studies (Bateman, I.J., Willis, K.G., Garrod, G., Doktor, P. and Turner, 1992; Stevens, T.H., Benin, S. and Larson, 1995; Oglethorp, D.R. and Miliadou, 2000; Wattage, P. and Mardle, 2008). This technique was also used in valuation of wetlands by Binilkumar (2010) and mangroves by Ekka, A. and Pandit (2012). The present study follows and adopts the method employed by Bann, (1999), Lal (2003), Gunawardena, M. and Rowan (2005), Gupta, V. and Mythil (2007), Stone, K., Bhat, M., Bhatta, R. and Mathews (2008), Yacob, M.R., Radam, A. and Shuib (2009) and Sathya and Sekar (2012).

This study also assesses factors influencing the WTP. This is done by using multiple regressions analysis with WTP as the dependent variable and some other related variables as the explanatory variables, including socioeconomic characteristics, neighbourhood characteristics, and environmental awareness.

2.5 Variables and definition

As mentioned above, the dependent variable in this study is willingness to pay for conserving mangrove forest. The WTP is estimated to be influenced by socioeconomic characteristics, neighbourhood characteristics and environmental awareness characteristics. The lognormal WTP function for the *i*th respondent can be written as

$$\log(WTP\ ) = \quad ' \quad + \quad + \quad + \quad ,$$

Yi is a vector of social demography characteristics; Z is vector of neighbourhood characteristics; X is vector of environmental awareness characteristics and $\varepsilon \sim N(0,\sigma^2)$. If the respondent's true valuation, *WTPi*, is known to lie within the interval (*ti*, *ti+1*), then log(*WTPi*) will lie between log(*ti*) and log(*ti+1*). Each pair of individual thresholds for log(*WTPi*) can then be standardized to state the probability that respondent *i* will select *ti* as

$$
\begin{aligned}
\Pr(\ ) &= \Pr((\log \quad - \quad ' \quad)/ \quad < \quad < (\log \quad _{+1} - \quad ' \quad)/ \quad) \\
&= \Phi((\log \quad - \quad ' \quad)/ \quad - \Phi(\log \quad _{+1} - \quad ' \quad)/ \quad) \\
\Pr(\ ) &= \Pr((\ og \quad - \quad)/ \quad < \quad < (\ og \quad _{+1} - \quad)/ \\
&= \Phi\log \quad - \quad _{ii}\beta\sigma - \quad (\log \quad + 1 - \quad)
\end{aligned}
$$

where z_i is the standard normal random variable and Φ is the cumulative standard normal density function. With the assumed lognormal distribution of valuations, the median of an individual's conditional WTP distribution was estimated as the anti-log of that individual's predicted log(*WTP*) (Cameron, T. A. and Huppert, 1991). The mean of *WTP*, for each individual, was obtained by scaling the median by $\exp(\sigma^2/2)$. The mean WTP per individual for the mangrove forest conservation was estimated by averaging across all respondents of first survey in the sample.

Table 1. The definition of explanatory variables

Variables		Definitions
Dependent variable		
WTP conservation		Willingness to pay (WTP) for conserving mangrove forest
Independent variables		
Socioeconomic characteristics	AGE	Age of household head
	SEX	Sex of household head (1 if male; 0 if female)
	FAM_SIZE	Family size
	EDUC	Years of schooling
	INCOME	Income per month
Neighbourhood characteristics	DISTANCE	Distance from home to mangrove forest
	ACCESS	Transportation access (=1 if good; =0 if otherwise)
	FACILITIES	Facilities condition (=1 if good; =0 if otherwise
Environmental awareness characteristics	CONSERVATION	Perception of need for mangrove forest's conservation (=1 if conservation is needed; 0=if otherwise)
	PARTICIPATE	Willingness to participate in the mangrove forest conservation (=1 if willing to participate; 0=if otherwise)

Environmental awareness in this study refers to perception of respondents for a need of mangrove forests conservation and willingness to participate in the conservation. In this case, the environmental awareness reflects people's behaviour related to the value of mangrove forest that can be recorded through CVM. As described in Table 1, the conservation indicator is perception of need for mangrove forest's conservation that measured using a dummy variable. It is valued as 1 if the respondents believe that conservation is needed and valued as 0 if otherwise. The participate variable defined as a value of 1 if respondents are willing to participate in the mangrove forest conservation and 0 if otherwise.

3. Result and Discussion

Before the survey, interviews with 35 respondents for each group of respondents (tourists, local communities, and fishermen) were conducted to find an average value of the WTP in each group. The respondents were asked for their maximum WTP for conserving the mangrove forest, with a bidding game technique. This technique produced an average of maximum amount of money that the respondents were willing to pay for conserving the mangrove forest, which was IDR 10,500 for tourist, IDR 8,000 for local communities, and IDR 9,500 for fishermen. This amount of money was then used as a value of WTP to define whether the respondents were willing to contribute or not. This question was asked through the questionnaire to the rest of respondents. The survey result indicates that 70%; 71%; and 77% of tourists, local communities, and fishermen, respectively implied that they were willing to pay for the mangrove forest conservation (see Table 2.).

Table 2. Willingness to pay for adapting climate change

WTP	Tourist		Local Communities		Fishermen	
	Yes	No	Yes	No	Yes	No
Amount (person)	70	30	71	29	77	23
Percentage (%)	70%	30%	71%	29%	77%	23%

Among variables that categorized as socioeconomic characteristic, age has positive and significant influence in the local communities' WTP for mangrove forest conservation at significant level of 90%, but it is not significantly influence the WTP of tourists and fishermen. Male has higher WTP than female in the local communities at significant level of 95%. Married people in the local communities also has higher WTP than unmarried people at significant level of 99%. This was expected given that married people would think about their future generation. Meanwhile, people who has longer years of schooling has greater WTP than those who has shorter years of schooling at significant level of 99%, 95% and 90% for tourist, local communities, and fishermen respectively. An economic variable, income, has positive and significant influence to WTP of tourist only.

Regarding the neighbourhood characteristics, the transportation access and facilities condition has positive and significant influence on the WTP of tourists and communities at significant level of 99% and 95% respectively.

This study indicates that among the environmental awareness characteristics, only willingness to participate in the mangrove forest has a positive and significant influence on the WTP at significant level of 90%, 95% and 99% for tourist, local communities, and fishermen respectively. Meanwhile, this study found that the perception of need for mangrove forest's conservation is not significantly influence the WTP. Most respondents perceive that mangrove forest's conservation is needed.Detail of the regression result is presented in Table 3.

Table 3. Regression results

Variables		Odd ratio		
		Tourist	Local communities	Fishermen
Constant		.104	0.000	0.000
		(2.941)	(2.692)	(4.292)
Age of respondent	(AGE)	-	1.057*	1.125**
			(.031)	(.049)
Sex of respondent	(SEX)	-	.208**	-
			(.682)	
Marriage status	(MARRIAGE)	1.204	7.986***	30.937***
		(.655)	(.729)	(1.228)
Years of schooling	(EDUC)	.595***	1.350**	1.766*
		(.178)	(.137)	(.304)
Income per month	(INCOME)	1.000**	1.000	-
		(.000)	(.000)	
Distance to mangrove forest	(DISTANCE)	1.004	.947	-
		(.054)	(.428)	
Transportation access	(ACCESS)	5.869***	1.481	.720
		(.633)	(.473)	(.530)

Table 3. Regression results (Cont.)

Variables		Odd ratio		
		Tourist	Local communities	Fishermen
Facilities condition	(FACILITIES)	2.571 (.671)	2.143** (.369)	1.300 (.791)
Perception of need for mangrove forest's conservation	(CONSERVATION)	-	-	-
Willingness to participate in the mangrove forest conservation	(PARTICIPATE)	2.724* (.577)	5.061** (.670)	15.922*** (.801)
Nagelkerke R Square		.382	.488	.688

Dependent variable: WTP for conserving mangrove forest
Number in bracket ( ) shows standard error.
*significant at α 10%; **significant at α 5%; ***significant at α 1%

This study confirms the finding of some previous studies concerning the link between socioeconomic characteristics and WTP for improving environmental quality. This study suggests that income has a positive and significant effect on the WTP of mangrove forest conservation. This implies that respondents with higher income will be willing to pay more for the conservation of mangrove forests than those with lower income. Some contingent valuation studies have similar results to this study. For instance, (Boyle, K. and Bishop, 1987) suggest that there is a positive effect of income on WTP on the conservation of endangered species.

Similarly, (Carson, R. T., Flores, N. E., Martin, K. M., and Wright, 1996) found that some individuals are less willing to pay to preserve quasi-public goods as income increases. (Loomis, J. and Larson, 1994) also found that individual WTP to increase the number of environmental goods is associated with socioeconomic factors including income. Whereas (Hadker, N, Sharma, S, David, A and Muraleedharan, 1997) in his study found that individuals with higher incomes tend to have a willingness to do conservation than those with lower incomes. This study also supports (Verbic, M. and Slabe-Erker, 2009) who employed the classical contingent valuation method for assessing the economic valuation of the landscape development and protection area in Slovenia. The respondents' WTP for improving that area was positively affected by income.

Regarding the years of schooling, this study suggests that there is a positive and significant effect on WTP for conserving mangrove forest. This finding confirms (Hema, 2013) who found that the education level (years of schooling) was positively related to WTP of the residents to offer labour for replanting efforts and conservation of the existing mangrove stand. A positive sign of the coefficient of education indicates that the probability of saying "yes" to WTP questions increases with the increase in the year of schooling. This is understandable because the longer the time for schooling, the more one's knowledge about social, political, economic and environmental issues. Also, education will help a person to understand the issue of the environmental effects of economic development and also, on the other hand, the effect of environmental conservation on the economy. This study suggests a similar finding with several studies related to contingent valuation that found a relationship between education level and WTP for mangrove forest conservation. For example, (Whitehead, 1992) has found that education levels are positively related to WTP. Likewise, research by (Pate J and Loomis, 1997) illustrates a positive relationship between education and WTP conservation of wetland and salmon in California. Higher education provides better awareness about the necessity of the mangrove conservation for their well-being. Better education may also facilitate better jobs and higher income. Hence, the importance of mangrove conservation and the economic value attached to mangroves by fishermen are highly influenced by their income from the major livelihood activity. The studies by (Hadker, N, Sharma, S, David, A and Muraleedharan, 1997), (Binilkumar, 2010) and (Ekka, A. and Pandit, 2012) also have reported similar results.

Another important variable that influences people's WTP is age. Age is positively influencing people's WTP for the conservation of mangrove forests. This implies that older people tend to say "yes" to WTP questions than those who are younger. An older people would have more experience than the youth so that they would consider the need for mangrove forest conservation due to a bequest motivation for their future generations.

4. Conclusions

This study assessed people's willingness to pay for mangrove forest conservation. For this purpose, surveys of tourists, local communities and fishermen in Lembar mangrove forest area in West Lombok was conducted.

This study found that the average WTP of tourist was IDR 10,500 (USD 0.75) per visit. While the local community and fishermen were willing to pay for a monthly contribution of IDR 8,500 (USD 0.61); IDR 9,500 (USD 0.68) respectively. The amount of WTP is influenced by socioeconomic characteristics, neighbourhood characteristics, and environmental awareness characteristics. This influence slightly differences among the type of respondents. For local communities, the WTP for conserving mangrove forest was influenced by sex, age, marriage status, years of schooling, facilities condition and willingness to participate in mangrove forest conservation. The WTP of tourist was determined by years of schooling, income, transportation access and willingness to participate in mangrove forest conservation. Meanwhile, for fishermen, their WTP was influenced by age, marriage status, years of schooling and willingness to participate in mangrove forest conservation.

This study has several limitations that need to be solved through further studies. Since this study is only conducted in one district, the result might not be applicable in other location with different socioeconomic, neighbourhood, and environmental awareness characteristics. Another study with broader location and variety characteristics is necessary to produce a more general result, to provide evidence that those characteristics can determine an individual's WTP for conserving the mangrove forest.

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Ecosystem Services Provided by Potential Wild Edible Plant Species in the Cordillera Region, Philippines

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Abstract

Wild edible plant species are included in the provisioning ecosystem services that have direct and positive implications to human well-being. This study focuses on the significance of wild edible plant species (WEPS) as an ecosystem service, frequency of ES uses, month of harvest, and the socio-demographic profile of its beneficiaries in the Cordillera Region. Information was obtained with (n=648) farmers as key informants using a semi-structured survey questionnaires, and focused group discussion. A total of 85 WEPS were documented and 14 identified significant ecosystem services (ES) like food, income, sharing of blessing, recreational activity, handicraft materials, etc. Among these WEPS, 52 are indigenous or endemic fruits, 12 are utilized as tea, and 21 are used as condiments or spices which are collected in and consumed throughout the year. Respondent's sex, age, education, and cultural factors explain the importance, knowledge and spatial distribution of WEPS in the area. Tapping and recognizing this nature's gift will enhance economic and nutritional security of the rural communities. WEPS potential contribution to the local food basket and livelihood may raise public awareness and strengthen local policies on ecosystem conservation and management.

Keywords: Edible wild plant species/ Endemic/ Indigenous/ Ecosystem services/ Cordillera/ Rural communities

1. Introduction

Researches continue to escalate after the Millennium Ecosystem Assessment highlighted the concept of ecosystem services (MA, 2005). Ecosystem services (ES) as defined by TEEB Foundation (2010), as those direct and indirect contributions of ecosystems to human well-being. Supported by MA (2005); Costanza et al. (1997); and Daily, (1997) recognized both natural and human-modified ecosystems as sources of ES and using the term "services" to encompass tangible and intangible benefits which are sometimes separated into ''goods" and "services".

The WEPS are included in the provisioning ES that have direct and positive implications to human well-being. Gathered and consumed traditionally in the form of fruits, vegetables (shoot, stem, flower), tubers, medicinal, tea, spices and or condiments that were utilized as a dietary component and sustenance to rural households (Cruz-Garcia and Price, 2014). The significance of WEPS has been meeting the nutritional needs of the local residents in terms of proteins, carbohydrates, vitamins, phytochemicals and minerals (phytonutrient) to a greater extent (Sundriyal and Sundriyal, 2004). Despite their values, WEPS are not represented and identified contributory in mapping ES studies, probably due to its perceived minimal importance (Ego et al., 2008) and the absence of data to quantify this service (Maes et al., 2011). It was also excluded in the official statistics on economic values of natural resources (Bharucha and Pretty, 2010) and information on the quantities of wild food collected hardly exist on literatures (Schulp et al., 2014).

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A considerable wild food collection is performed for household consumption and served as additional income for the family. But, notably absent data regarding on the contribution of WEPS, and neglecting the role of traditional ecologicalknowledge systems that sustain these food chains (Pilgrim et al., 2008). Though currently, there is an emerging literatures worldwide on the ethno-botanical studies of wild edible plants (Turreira-Garcia et al., 2015; Leal et al., 2018; Chua-Barcelo, 2014). The objectives of the study are to identify significant ES provided by WEPS, frequency of ES uses, month of harvest, and the socio-demographic profile of its beneficiaries in the Cordillera Region.

1.1 Framework of the study

WEPS as ecosystem services are defined as plants gathered in the wild, inexpensive and a rich source of antioxidants, vitamins, fiber, and minerals (Shaheen et al., 2017; Reyes-Garcia et al., 2015). The food value of WEPS is not only for subsistence oriented economies but also consumed in rural and urban areas in developed countries due to income, nutrition, food security and recreational activities (Bharucha and Pretty, 2010; Schulp et al., 2014). The study focused on the identification of ES provided by WEPS, frequency of ES, month of harvest, and importance to its beneficiaries. The noble output of this study illustrate WEPS's contribution to the local food basket that may raise public awareness and strengthen local policies on ecosystem conservation and management. ES emphasized the anthropocentric approach not only on the direct provisions of ES, but also on the underlying ecosystem functioning, contributing to make visible the role of biodiversity and ecological processes (MA, 2005). The Economics of Ecosystems and Biodiversity (TEEB) was a follow up activity by MA (2005) elucidating the linkage between the ecosystems provision to human well-being (Figure. 1). This study followed the cascade approach that was firstly introduced by Haines-Young (2009) and was adopted by Schulp et al. (2014) and de Groot et al. (2010) in their framework.

The figure shows, that ES are generated by ecosystem functions, which is the capacity to supply these ES to human well-being (de Groot et al., 2010). This cascade paved a way for WEPS supply and production seasonally. The ecosystem service supported by the definition of ecosystem properties as the ecological conditions determining the ES to be provided (Schulp et al., 2014) such as the production and availability of WEPS. Supply of ES is actually the WEPS that are gathered and consumed by humans. WEPS gatherer, consumer, and seller benefitted in the form of food, income, medicine, and others. In response, WEPS gatherer-seller (respondents which gathered WEPS and sell to the local markets) and gatherer-consumer (respondents which gathered WEPS for household consumption) somehow determine the socio-demographic profiles of the ES beneficiaries. Noticed, in the framework, there is no conservation aspect (ecosystem management) that supposedly influences the ecosystem function and properties that sustained ES supply. Instead, in the perspective of gatherers, WEPS are free to be exploited which is naturally occurring from the ecosystems and agro-ecosystems.

However, when these WEPS are boosted as complementary staple agricultural foods, poverty alleviation, perceived health-benefits, and vanishing cultural significance (Soukand, 2016), and the disappearance of biodiversity which directly affecting ecosystem functions that underpins "services" for human well-being (Braat and de Groot, 2012), leads to public awareness and strengthen local policies towards ecosystem conservation and management.

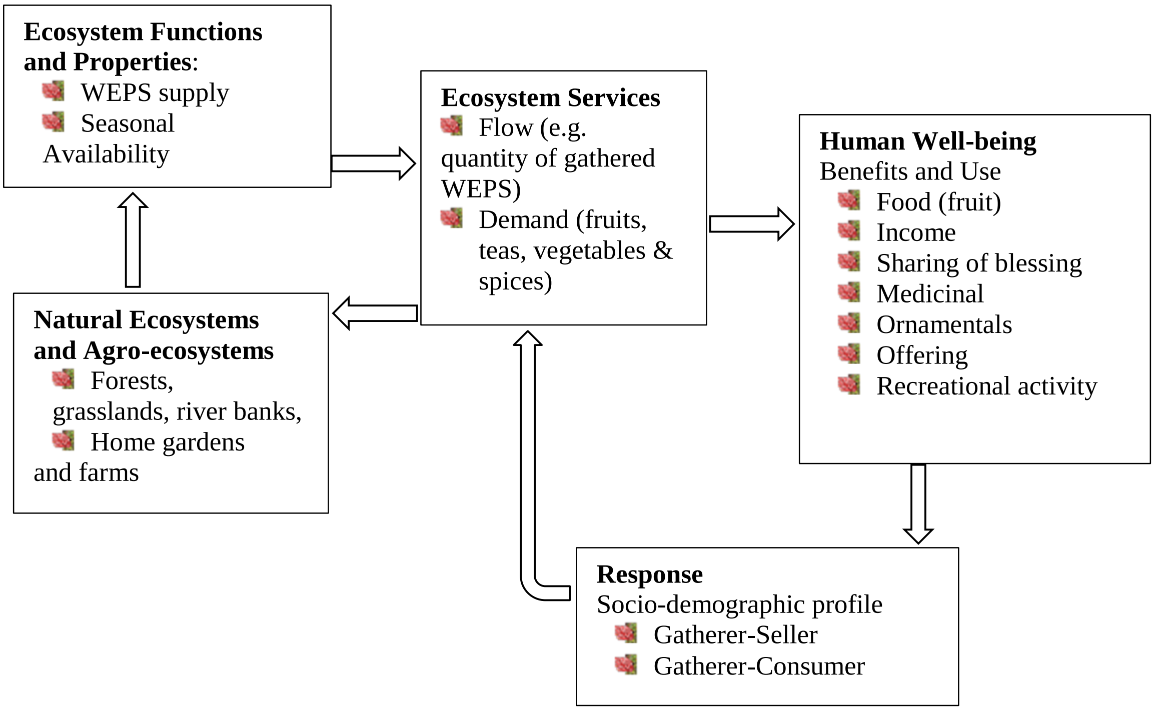

Figure 1. The relationships between WEPS supply as ES to human well-being

2. Methodology

2.1 Study sites

The research was conducted in the two provinces of Cordillera Administrative Region, Northern Philippines (Figure 2). This landlocked region bounded on the north by the provinces of Ilocos Norte and Cagayan, south by the provinces of Pangasinan and Nueva Vizcaya; east by Cagayan Valley; west by the Ilocos Region. It has a total land area of 18,293.70 square kilometers which is about 16% of the total land area of the Philippines.

The region is dominated by a mountainous topography, characterized by very steep slopes and high elevation. More than three-fourths of the region is classified as high elevation (500-2,000 meters above sea level). This gives the region its generally cool climate which nurtures many unique and rare biological species (DILG-CAR, 2018).

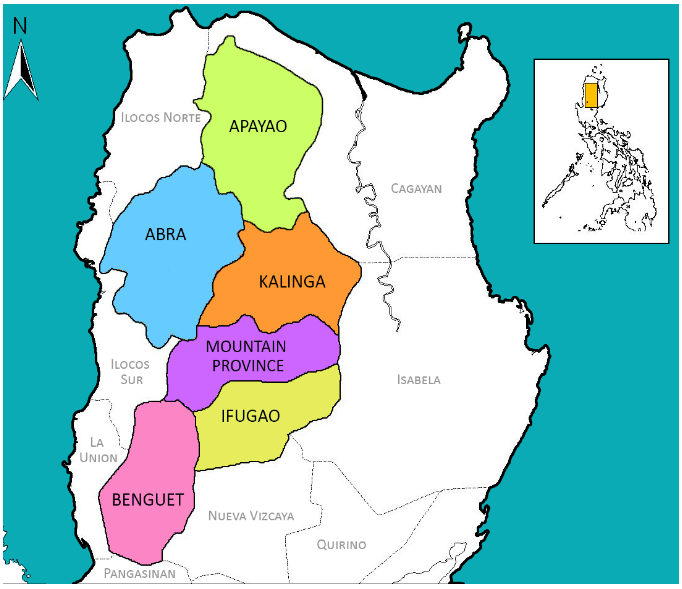

Figure 2. Map of the Cordillera Region.

2.2 Sampling and data collection

The project teams of two or three interviewers and including the author, conducted field surveys from November 2017 to May 2018. Collaboration was made through the Local Government Units (LGUs) of the Municipal and Barangay Level where project objectives were presented and as a requirement for the issuance of permit to conduct survey in the community. A multidisciplinary approach was used during the data collection by combining semi-structured interviews, focused group discussions with the key informants which were completed for about 30 to 60 min interaction. Initial list of respondents were subsequently enlarged using a "snowball technique" whereby interviewed informants were asked to contact new informants (Camps-Calvit et al., 2016).

The household was used as sampling unit wherein one from the household was counted as one key informant. Assisted by the community organizer, research assistant, and student assistants, we randomly selected an approximately 20 households per village. The study covered 4 village (Barangay) per municipality saturating 4 municipalities each of the 2 provinces of Benguet and Ifugao. A total of 648 key informants interviewed 293 female and 355 male. ES were identified using a free listing technique (Beiling et al., 2014) where respondents were asked to list benefits and uses of WEPS to human well-being.

A field notes were used to record respondents answers, this were subsequently matched with stated benefits of ES categories established by MA (2005). To conclude this survey, data gathered include sex, age and education of respondents. This information was used to define the socio-economic profile of the respondents by means of descriptive statistics.

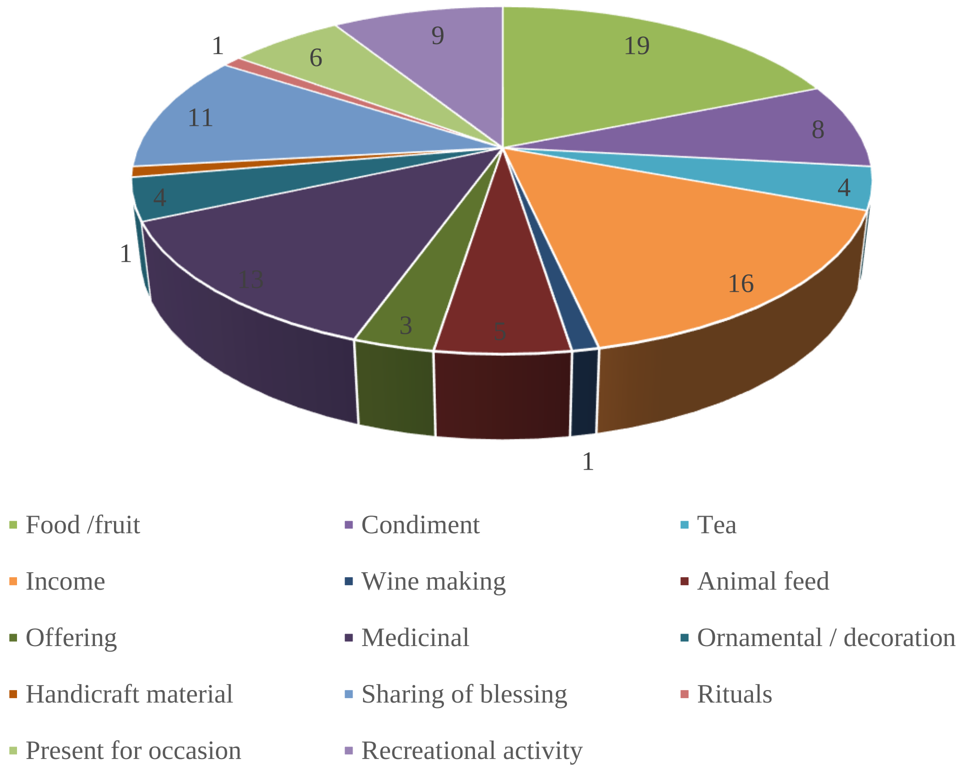

Figure 3. Identification of ES provided by WEPS. (Results based on 648 in-depth interviews with local communities in the Cordillera Region, Philippines)

Table 1. Ecosystem services by WEPS in the Cordillera Region

Ecosystem services	Frequency		Total No. of Species
	Benguet	Ifugao	
Food (fruit)	281	271	52
Condiment	74	133	21
Tea	106	69	12
Income	28	24	15
Wine making	28	9	4
Animal feed	46	14	20
Handicraft material	6	30	2
Offering	2	12	3
Medicinal	75	82	41
Ornamental / decoration	7	10	7
Sharing of blessing	5	6	25
Rituals	4	8	2
Present during occasion	6	3	5
Recreational activity	5	2	8

*Frequency is the number of times that WEPS was referenced by the respondents.

3. Results and discussion

3.1 Ecosystem services provided by WEPS

These wild foods have long been supplementing farmers a “hidden harvest”, since it co-evolved growing around their farms which deliver nutritious food and income (Grivetti and Ogle, 2000). The identified significant 14 ES provided by 85 WEPS was categorically under the provisioning and cultural ES by MA (2005). About one half (54%) of WEPS belonging to provisioning ES which include food (fruits), income, condiment, animal feed, tea, handicraft material, and wine making. WEPS cultural ES (46%) are medicinal, sharing of blessing, recreational activity, ornamental/ decoration, present during occasion, offering, and rituals (Figure 3). Provisioning services are ecosystem services that describe the material or energy outputs from ecosystems while cultural services relate to non-material benefits people obtain from the ecosystem (TEEB, 2010).

Table 1 shows the frequency of ES provided by the total number of WEPS in the Cordillera Region. Frequency is the number of times WEPS was referred by the respondents. Like in Benguet Province, the ES provision of food (fruits) has an occurrence of 281 while 271 in Ifugao Province. Meanwhile, 52 total number of WEPS were identified to provide ES in the form of food such as fruits. Though interestingly, these 52 WEPS has either once or were referred by farmers to have multiple uses, like it is consumed as fresh fruits simultaneously used as an offering during Sunday masses, or as a present to valued guest during special occasion.

3.2 WEPS as sustainable foods

Wild edible plants have great importance in supplying benefits at different places to provide nutrients throughout the year. Bharucha and Pretty (2010) cited forest ecosystems which provide livelihoods and food for some 300 million people in the form of non-timber forest products (NTFPs) which naturally intertwined with the rural communities. According to the key informants, majority of WEPS (85%) grow naturally in the forest, grassland, riverbank ecosystems while (15%) were found in agro-ecosystems. Majority of WEPS’ growth habit are trees (41%), herbaceous (24%), vine (18%), shrubs (15%) and fungi (1%).

Of the 85 WEPS, 45 are endemic or indigenous fruits, 3 are edible leaves, 2 are edible flowers, 2 are edible stem; 12 are utilized as tea and 21 are used as condiments. The edible parts were harvested and consumed depending on species, growth stage (Pinela et al., 2017), and preference of the local folks. The study of Chua-Barcelo (2014) documented 36 wild edible fruits while Lirio et al. (2007) surveyed 49 indigenous vegetables in the same region. Women and elderly were the main gatherers of WEPS in which they knew the month of harvest season.

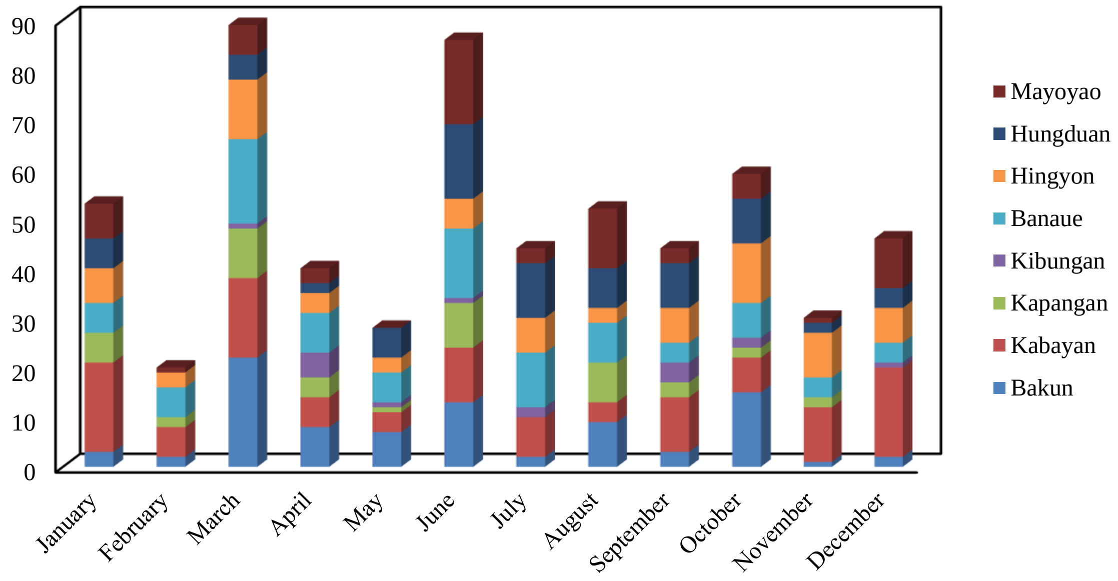

Figure 4. WEPS availability and harvest month per municipality in Benguet and Ifugao province. (Abundance of WEPS was not included)

In Figure 4, WEPS are available throughout the year for food consumption, wherein majority of WEPS (89%) were harvested in the month of March (summer), while other fruits were also available in June (86%) rainy season, October (59%), January (53%) and August (52%). The legend refers to the 8 municipalities (4 each) in Benguet and Ifugao Province. WEPS were eaten as snack or occasionally (mostly by children and shepherds) and not as regular food, but the nutritional value was perceived (Menendez-Baceta et al., 2012). Other WEPS are aromatic in which they used as condiments or spices for flavoring and seasoning traditional dishes (Table 1). They are also used as herbal teas drunk hot or cold according to the season and due to perceived health benefits. It is also a part of recreational activity for the family, parents or elderly summons their children to gather WEPS during harvest season. Pinela et al. (2017) mentioned that people still continue reserving some time for collecting these wild foods, which are rich in micronutrients and highly treasured additives for traditional recipes.

3.3 Socio-economic and WEPS diversity

Regarding the profile of ES direct beneficiaries (the farmers) from the provision of WEPS, include 45% female and 55% male informants. Their ages ranged between 21 years to 80 years old and are distributed as follows: 21-30 years (21%), 31-40 years (18%), 41-50 years (20%), 51-60 years (19%), 61-70 years (15%), and 71-80 years in above (8%). These were considered during the survey to represent reliable information across ages. As mentioned in the study of Schulp et al. (2014), age influence wild food gathering because elderly people are more likely to have witnessed times when collecting wild food was a necessity and they are also familiar where the WEPS are to be gathered.

Among these documented WEPS, 52 are indigenous or endemic fruits, 12 are utilized as tea, 21 are used for condiments or spices which are collected in and consumed throughout the year. The most frequently consumed WEPS in this study were fruit trees, which were supported by the study of Cruz-Garcia & Price (2011); Chua-Barcelo (2014); Turreira-Garcia et al. (2015). Thirty-one percent of the total respondents had received basic educational foundation, while 38%, 12%, and 19% had finished secondary, vocational courses and tertiary education respectively. Respondents age, gender and level of education are important factors in gathering, because women and elderly were identified as gatherers and sellers of WEPS and in determining diversity (Caballero-Serrano et al., 2016). Interestingly, WEPS are gathered not only for subsistence but according to the key informants, they were satiated with their daily foods from their farms or gardens. WEPS have significant role in providing ES to the community, where in a subsistence economy, food security stratagems are necessary priority and there must be a common goal to protect cultural diversity, ecological systems and healthy consumption of traditional foods (Kuhnlein, 2014).

4. Conclusions

The survey of WEPS and the ES contribution to the Cordillera Region serves as a reference and a useful compilation of knowledge to the younger generation. Diversity of WEPS significantly contributed to local food basket and boosts nutritional security in the Cordillera Region. WEPS are good sources of phytochemicals, vitamins, and minerals (phytonutrients) though dependent on the available and different seasonal harvest of each species. They are also ecologically resistant to pest and diseases, climate change and thrive on marginal areas. Rural folks acknowledged the WEPS highly abundance before, but the mere fact these species are still gathered and consumed today. Production potential of WEPS can be sustained through an *in situ* or *ex situ* conservation which ultimately help augment the socio-economic condition of the rural communities. Finally, these study might contribute to local sustainable development and innovative options for local policies that would be suitable for the rural communities, simultaneously promoting the main goals of United Nations' sustainable socio-economic growth and healthy lives for all well-being (European Commission, 2016).

5. Acknowledgements

My deepest gratitude to the rural communities and LGU's of Benguet and Ifugao Provinces for their kind participation during the survey. Thanks to the fieldwork assistance from the Benguet State University-BS Environmental Science (BSU-BSES) students Ronnel, Jessica, Mari Grace, Arlene, CJ, Roxanne, and Melchor, and the administrative support of Benguet State University. This study was supported by Neys-van Hoogstraten Foundation, Hague, the Netherlands and with partial foreign grant from the Commission on Higher Education-the Philippines.

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Valuation of Irrigated Water Used in Crop Production: A Case of Rice and Sugarcane in Nakhon Pathom Province

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Abstract

Water is a significant resource in agricultural production. Albeit a renewable resource, an appropriate management is vital. How to efficiently and equitably allocate it has become a concern, therefore understanding the values of irrigated water is crucial. This paper presents an economic analysis of the value of irrigated water used in crop production in the central plain of Thailand. The objectives of this research are to identify the economic value of irrigated water for rice and sugarcane production in Nakhon Pathom and study the factors affecting the value. Residual valuation technique was employed for the study. Input for valuation were collected from secondary sources from corresponding authority. The results of the study shows that the average value of irrigated water from 2012 - 2016 for first crop rice (wet season rice), second crop rice (dry season rice), and sugarcane were 1.61 Baht/m^3 (\$0.046/m^3) 0.91 Baht/m^3 (\$0.026/m^3) and 0.4 Baht/m^3 (\$0.011/m^3) respectively. This paper could provide an alternative to water allocation decision for more sustainable and more efficient use of water in agricultural sector in the future.

Keywords: Residual valuation/ Irrigation/ Water value/ Rice/ Sugarcane

1. Introduction

Water resources have long been experiencing intense pressure from a growing demand. Despite the fact that competition for freshwater is intensifying, global freshwater resources has been treated as an almost free resource (Turner et. al., 2004). Following 1992 International Conference on Water and the Environment, water has an increasing interest both physically and economically as one of the principles under integrated water resources management was "water has an economic value in all its competing uses and should be recognized as economic goods" (Solanes & Villarreal, 1999). In Thailand, agricultural sector has long been important to the nation's development as it plays a crucial role for economic growth as well as generating wealth for nation. The country has its total area of 320.70 million rai (≈ 51.31 million ha) and 149.24 million rai (≈ 23.88 million ha) or 46.54 % of total area has been utilized for agriculture. Required water for all sectors is 151,750 million m^3 and for agricultural sector only is as high as 113,960 million m^3 or 75% of total water required among every sectors. Thailand agricultural production has long played an important role in country's socio-economic development. Hence, water resources are inevitably crucial for crop production for such important commodities and its value should not be overlooked. However, the current knowledge about the value of water is limited and the absence of water market cause difficulties in addressing economic values of irrigated water (Ziolkowska, 2015; Muchara et. al., 2016). Under the circumstance that farmers do not have to pay for irrigation, it can lead to inefficient use of water. Therefore, it is crucial to estimate the actual value of water for irrigation as to enhance demand management and also help conserve water resources (Sacolo, 2013). Residual valuation method is among the most widely used method for evaluating water used in agriculture (Hellegers & Davidson, 2010; Berbel et. al., 2011; Ziolkowska, 2015; Kiprop et. al., 2015; Muchara et. al., 2016). Here, water is seen as an intermediate input to the production along with other traditional input (land, labor, capital).

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Nakhon Pathom is a province situated in the central plain of Thailand and considered having intense agricultural activities. Over half (58.78%) of total area is agricultural area (Nakhon Pathom Provincial Office, 2016). Majority of crop production in Nakhon Pathom is supported with irrigation. Rice and sugarcane are among vital important crops. Both crops contributed great significance to the province's economy therefore being selected as a site for the study. The main objective of this study was to estimate the value of irrigated water as an input in rice and sugarcane production.

2. Methodology

2.1 Study Area

Nakhon Pathom is a province located on the central region of Thailand. It is set around the Tha Chin river basin amongst the central plain between 13˚45'10'' N and 100˚4'28''W. The total area is 2,168.327 km^2 or 1,355,204 rai (Figure 1). Elevation is between 2 and 10 meter above mean sea level (MSL). Tha Chin river flow passed the province from north to south. Nakhon Pathom experiences a mean annual temperature of 27.6°C and 1,009.2 mm of mean annual precipitation. Irrigated water is employed in 75% of the total area.

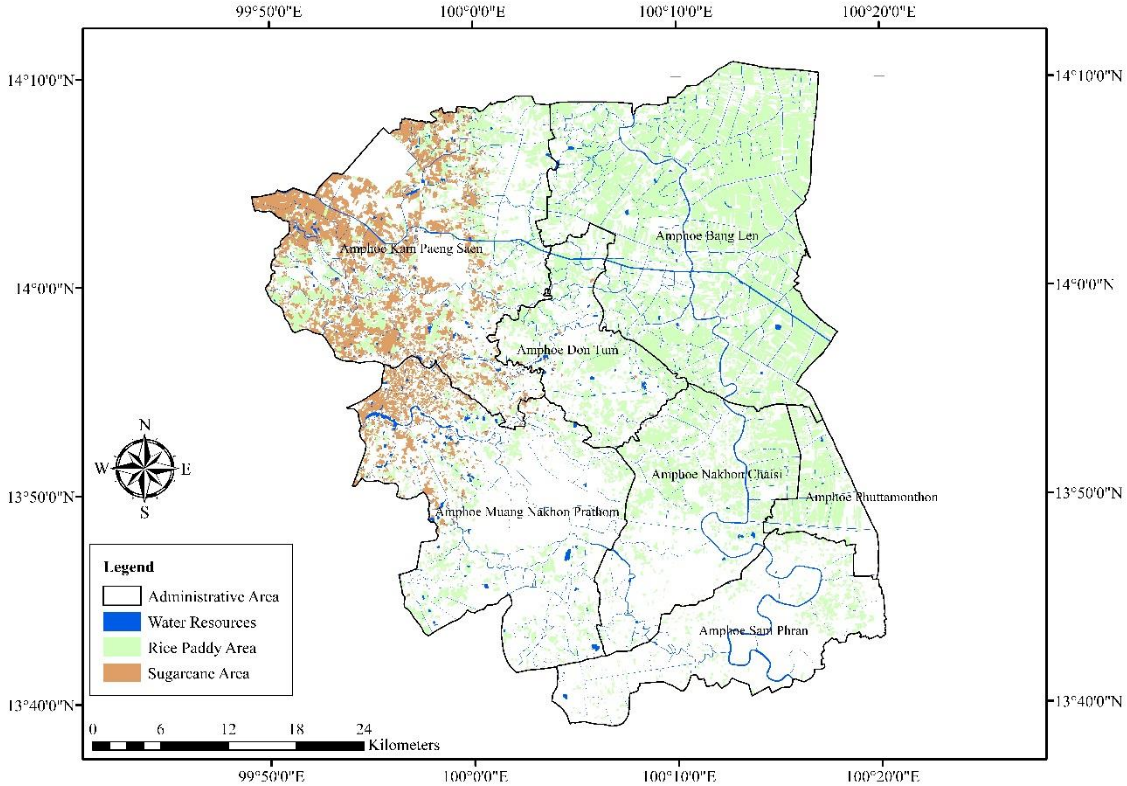

Figure 1. Map of Nakhon Pathom

2.2 Residual Valuation Method

Young (1996) proposed a residual valuation method (RVM) to value water resource used in agricultural production. RVM is based on the production function that represents the relationship between inputs and outputs in crop production. An implementation of residual valuation approach is as following. The production costs were first subtracted from the net profit to obtain the residual revenue. Then, the residual revenue was divided by the quantity of irrigated water employed to rice and sugarcane production. The results of the residual value of irrigated water per cubic meter of irrigated water employed were derived. According to the formula for the residual valuation, this method yielded the average value of irrigated water. This method of estimating the average value of irrigated water is grounded in theory that the value of output produced is equal to the summation of the value of inputs used to producing it. The residual value of irrigated water is represented using the following formula;

$$Y = f(R,L,K,W)$$

Where; Y is net value, R is land resource, L is labor, K is capital, W is irrigate water

If competitive factor and product market can be assumed. It may be written as this equation;

$$TVP_Y = (P_R \times Q_R) + (P_L \times Q_L) + (P_K \times Q_K) + (P_W \times Q_W)$$

On the assumption that all variables are known except P_W, that mathematical expression can be solved for that unknown value as;

$$P_W = \{TVP_Y - [(P_R \times Q_R) + (P_L \times Q_L) + (P_K \times Q_K)]\}/Q_W$$

Where; P_W is value of irrigated water (Baht/m^3), TVP_Y is total value of product Y (Baht), P_i is price of resources i (Baht), Q_i is quantity of resource i, and Q_W is quantity of irrigated water (m^3).

2.2.1 Data Collection

Data used in analysis (crop budgets, crop yields, cultivated area, and irrigated water used) were collected from Nakhon Pathom Agricultural Office, Office of Agricultural Economics (OAE), Office of Cane and Sugar Board (OCSB), and the Royal Irrigation Department (RID). Data used in study was from 2012-2016 to illustrate the trend and changes.

The production costs were based on the crop budgets in a provincial level. The production costs included fixed costs and variable costs. Fixed costs included land rents, depreciation, and opportunity costs. Variable costs included both handed labor and machineries in land preparation, cultivars, fertilizer, pesticides, maintenance costs, harvest, and management costs. It must be noted that in case of sugarcane production cost, records were not available on the provincial level, an average regional production costs were employed in this study. Crop prices were based on at farm gate price. Irrigated water demand is based on crop evapotranspiration concept (FAO's penman-monteith method) provided by RID.

3. Results

The calculation results and residual value of irrigated water are shown in Table 1. For all analyzed crops, the value of irrigated water for first crop rice production is the highest. However, the amount of irrigated water used are greatly varies. For net returns of sugarcane in 2016 have negative values and eventually impact the residual value. This indicates unprofitability in a crop production in that given year (Ziolkowska, 2015). The highest average value of 1.61 Baht/m^3 (\$0.046/m^3) was found in first crop rice, while the lowest was found in sugarcane of 0.4 Baht/m^3 (\$0.011/m^3)

Table 1. Value of irrigated water for crops production in Nakhon Pathom

Crop	Net Returns (Baht)	Irrigated Water (m^3)	Residual Value (Baht/m^3)
First crop rice			
2012	505,068,811	282,223,274.19	1.79
2013	1,320,514,689	283,631,603.97	4.66
2014	178,821,097	278,833,912.50	0.64
2015	93,057,927	251,684,754.75	0.37
2016	134,346,029	233,703,725.97	0.57
Avg.	446,361,710	266,015,454.28	1.61
Second crop rice			
2012	1,607,751,374	788,427,797.47	2.04
2013	1,471,535,247	790,779,889.45	1.86
2014	161,626,787	770,335,227.88	0.21
2015	85,745,951	482,636,528.97	0.18
2016	105,210,282	384,682,905.33	0.27
Avg.	686,373,928	643,372,469.82	0.91
Sugarcane			
2012	326,203,954	162,266,559.68	2.01
2013	41,777,408	161,213,081.92	0.26
2014	35,401,786	163,813,855.14	0.22
2015	12,441,289	166,091,226.18	0.07
2016	-97,421,378	167,032,384.62	-0.58
Avg.	63,680,611.9	164,083,421.51	0.4

Source: Author's Calculation

When comparing residual value of irrigated water in any given year, it is apparent that the value is directly determined by the net returns. Comparability of residual value limited due to the fact that several factors included in the production function and cost and revenue budget are vary in different places. However, in the presented results are comparable with other studies analyzing the residual value at national level. Prombut (2007) examined the value of irrigated water in the Mae Kuang and Huai Saneng irrigation projects in Chiang Mai and Surin Province respectively. For Mae Kuang, the average value for wet season rice was 1.31 Baht/m^3 and the average value for dry season rice was 0.13 Baht/m^3. For Huai Saneng, the average value for wet season rice was 0.03 Baht/m^3 while for dry season rice was 0.13 Baht/m^3. A study by Kaenchan & Gheewala (2017) for sugarcane production in Thailand using three different methods to derive economic value of water which including residual valuation method, production function approach, and value-added method. The value ranged from 1.04-1.77 Baht/m^3, 3.47-5.01 Baht/m^3 and 1.70-2.90 Baht/m^3 respectively. Variability of residual value is largely depends on crop market that varied mainly due to the fluctuation in weather conditions, such as drought.

Net return of most crops were higher prior to 2013 which resulted in higher residual value for most studied crops. Due to drought in the following period along with uncertainty in crop price, caused cultivated area to decrease especially for first crop rice and second crop rice (Table 2) which consequently resulted in lower return.

Table 2. Crop cultivated area, crop yield, and crop price in Nakhon Pathom

Crop	Cultivated Area (Rai)	Yield (kg/Rai)	Price (Baht/kg)
First crop rice			
2012	403,597	549	11.99
2013	405,611	748	11.22
2014	398,750	755	7.24
2015	359,925	739	7.00
2016	334,211	725	6.95
Second crop rice			
2012	408,277	775	11.99
2013	409,495	769	11.45
2014	398,908	763	7.20
2015	249,927	758	7.03
2016	199,203	741	7.03
Sugarcane			
2012	83,792	12,180	1.07
2013	83,248	11,450	0.99
2014	84,591	11,390	0.95
2015	85,767	11,240	0.90
2016	86,253	8,900	0.88

Source: Author's presentation based on Office of Agricultural Extension (OAE), Nakhon Pathom Agricultural Office, Office of Cane and Sugar Board (OCSB)

Decreasing amount of precipitation and declining in water supply in major dams had direct impact to second crop rice production which it heavily relied on irrigated water during the dry season. Consequently, the cultivated area for second crop rice has decreased drastically. To cope with drought and to increase productivity of agricultural product, government has intervened by promoting farmer to shift cultivation from unproductive rice area to other economic crops. This explains the continuous increase in sugarcane cultivated area.

Once considering residual value of irrigated water using residual valuation method, the value is heavily dependent on climatic and economic condition. Majority of the period of study which covered 2012 to 2016 was plague with drought which can be observed in declining in net returns for most crops. Ideally, water value can help ensure efficient allocation of water by minimizing use on low value crops and shifting to high value crops. However, crop with the lowest value is not always the one to be sacrificed because despite having the highest value, it does not implies that farmer should shift their cultivation into the highest value crop because there are couple other limitation such as, land suitability, topographical difference, lack of know-how for other cropping pattern, familiarity to a single production, and unwillingness to bare risk, which prevent farmers from shifting from a particular crop to one another. Farmers may choose to go less risky way by growing less profitable crops (Hellegers & Davidson, 2010). To fully utilize the economic valuation approach in decision making process, other crops should also be considered to investigate a representative value of water used in agricultural production as a whole (Kaenchan & Gheewala, 2017).

4. Conclusions

The value of irrigated water was estimated using residual valuation method as an attempt to assist the decision making on water allocation. Nakhon Pathom, a province in the central plain of Thailand was the case studied. The results showed average value of irrigated water as an input to first crop rice, second crop rice, and sugarcane production were 1.61 Baht/m^3 (\$0.046/m^3) 0.91 Baht/m^3 (\$0.026/m^3) and 0.4 Baht/m^3 (\$0.011/m^3) respectively.

The results of this study should have practical implication for the Royal Irrigation Department and Department of Water Resources as the value of irrigated water should be considered in the allocation of the resources. Their 20-year strategic framework states the need to build water security in agricultural production seeking to balance between demand side and supply side, also, seeking to minimize the use of water resource and increase the value of irrigated water. Direction towards those target maybe supported by the results derived from using this technique in this study.

4.1 Recommendations for Future Research

The economic valuation in this study only estimated the economic benefit of water used for rice and sugarcane. Other crops could also be considered to evaluate the value of irrigated water used in agriculture as a whole. A study analyzing linear relationship among production factors, environmental attributes and value of water can potentially help inform better water management practices as well

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Monitoring Plant Species Diversity and Carbon Storage in a Recovery Dry Dipterocarp Forest on Volcanic Rock at Huai Hong Khrai Royal Development Study Center, Northern Thailand

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Abstract

Plant species diversity and carbon storage in a secondary dry dipterocarp forest on volcanic rock was assessed in 2010 and monitored in 2017 at the Huai Hong Khrai Royal Development Study Center in Chiang Mai province, northern Thailand. Twelve permanent plots, each of size 40 × 40 m, were used for vegetation survey in the forest from altitude 404 m to 564 m MSL. In each plot, stem diameters at breast height (DBH, 1.3 m above ground) and tree heights of all species with height over 1.5 m were measured. The plant characteristics, plant biomass and stored carbon amounts derived from carbon contents in bark, stem, branch, leaf and root of all species were measured. The forest included two stands based on the most dominant species: *Shorea obtusa* and *Shorea siamensis*. The number of species, genera and family were increased: 70 to 83 species (+13), 60 to 73 genera (+13) and 30 to 34 families (+4). Shannon-Wiener index (SWI) of species diversity was increased from 3.66 to 3.81 (+0.15), but tree density was decreased from 3,450 trees/ha to 2,642 trees/ha (-808) caused by death of mainly small saplings. Forest condition index was increased from 2.70 to 3.16 (+0.46). Average amount of plant biomass was increased from 83.65±13.55 to 97.27±17.70 mg/ha (+13.62). As a result, the average amount of carbon stored in plant biomass during 2010 and 2017 was increased from 41.33±6.69 to 48.07±8.75 mg/ha with the net increase of 6.74 mg/ha (2.30% per year) or only 0.96 mg/ha/yr. The annual increment rates of plant biomass and carbon storage were relatively slow, and the restoration by enrichment planting of some appropriate tree species is important.

Keywords: Carbon storage/ Dry dipterocarp forest/ Monitoring/ Plant diversity/ Volcanic rock

1. Introduction

Temporal changes of plant communities in the tropical forests are influenced by either natural or human causes. Many agents influencing plant communities occur in the forests such as forest fires, selective tree cutting, non-wood product harvest, competition, violent storms, deceases and others. The plant communities observed today reflect processes of recovery from disturbances which occurred at different times in the past (Crawley, 1986). These changes usually vary among forest types, subtypes or stands (Oliver and Larson, 1996). In Thailand, most forests had been suffered from forest concession, illegal cutting by some people, and selective uses by local people particularly in the utilization community forests (Khamyong et al., 2014). These activities occur in both of national reserved forests and protection forests inside the national parks and wildlife sanctuaries. Thus, most of the dry dipterocarp forest (DDF) are a secondary forest at the variable stages of natural succession which have the different forest conditions. Since this forest usually composes of many forest sub-types (Pongkhamphuanh et al., 2015; Khamyong et al., 2016), changes of their community structures are therefore complicated. These include plant species composition, richness, diversity, ecological roles and biological production. The physical structures involve plant growth forms and population structures, spatial distribution of plant populations, vertical and horizontal structures, etc.

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The different levels of disturbance cause the variable stages of succession in subtype communities within the same forest. Many environmental factors particularly the altitude, topography, soil, parent rocks and forest fires have also affected variable the temporal changes of forest communities. However, forest fires are minimized in the forests inside the protection forests controlled by more strict laws compared to the general national reserved forests.

Measuring plant ecological parameters during a period will provide information of plant community changes in the forest as a monitoring study (Burley and Gauld, 1994). The DDF usually covers on xeric sites in the north, northeast and central of Thailand. The soil in this forest varies from very shallow to moderate or deep, and is normally unfertile soil having various soil types classified into Order Entisols to Inceptisols, and Ultisols (Wattanasuksakul, 2012). This forest covers on extensive areas from a peneplain of about 150 m altitude in the central plain to slopes and rides of up to 1,300 m in the northern highland (Smithinand, et al., 1980). Four dipterocarp species are recognized as dominant trees in the forest; *Shorea obtusa*, *Shorea siamensis*, *Dipterocarpus obtusifolius* and *Dipterocarpus tuberculatus*. The parent rocks vary from sedimentary rocks (sandstone, limestone) to igneous rocks (granite, rhyolite and andesite). The biomass production and stored carbon of standing tree species are the ecological parameters which can be used to identify community changes. Carbon sequestration by forest ecosystems through the process of photosynthesis and subsequently storage of the carbon as carbohydrate in plant tissues of different organs including bark, stem, branch, leaf and root is the important process of reducing atmospheric CO_2 and global warming (Landsberg and Gower, 1997; Waring and Running, 1998). Then, a part of carbon amount is moved to the forest floor and accumulated in the soil system. The potentials of carbon storage are usually different among forest types, subtypes and stages of forest succession after human disturbance. The DDF in this research area before establishing the Huai Hong Khrai Royal Development Study (HHKRDS) Center was very poor in 1982 caused by over timber harvesting, and become the recovery forest at the present resulted from forest protection. The parent rocks under the forest include volcanic rock (rhyolite, andesite) and sedimentary rock (sandstone and limestone), and are considered as an important factor of different plant communities in the DDF. Forest protection from tree cutting and forest fire has been taken since 1982, and some studies were taken in this forest. The assessment of plant species diversity and carbon storage in the DDF on sandstone was conducted by Khamyong et al. (2016), and the monitoring study was reported by Sutthawan et al. (2016).

The research objective is to apply a method of plant community analysis for monitoring plant community changes of species diversity and carbon storage in a recovery DDF on volcanic rock in the HHKRDS Center between the year 2010 and 2017 as the basis information for forest and watershed management.

2. Methodology

2.1. Study area

The research was conducted in the Huai Hong Khrai Royal Development Study (HHKRDS) Center, Doi Saket district, Chiang Mai province in northern Thailand. The Center is located on the upland about 27 km to the north of Chiang Mai city, on the road from Chiang Mai to Chiang Rai province. It is established in 1982 by the initiation of the King (Rama 9) to be the place for study of people in the northern region about integrated watershed management; forestry at the upstream, fishery at the downstream and agriculture in the between areas. It covers an area of about 1,360 ha with an altitude range between 350 and 591 m MSL.

Many activities of forest and wildlife management, agriculture and fishery are demonstrated in the Center for the study of officers and people for extension. Foreign visitors also come here for learning.

Meteorological data recorded between the year 1985 and 2011 using instruments in the Center could be reported: average annual rainfall, 1,328.9 mm, average maximum and minimum air temperatures, 32.2ºC and 18.9ºC, and average water evaporation, 1,222.6 mm per year (Khamyong et al., 2016). The two deciduous forests including the dry dipterocarp forest and the mixed deciduous forest distribute in most area of the Center. The DDF on volcanic rock covers in the eastern part of the Center whereas that on sandstone appears on the western part. The volcanic rock is igneous rocks which are cooled on the earth surface after volcanic explosion. In this area, it composes of mainly andesite and rhyolite. The andesite and rhyolite are intermediate and acidic soil-forming rocks, respectively (Fisher and Binkley, 2000). They are glassy to fine-grained igneous rocks. The andesite consists of plagioclase with feldspar, phenocrysts, augite, hornblende and biotite whereas the rhyolite had orthoclase, quartz, orthoclase, phenocrysts, accessory biotite, hornblende, apatite and zircon. Weathering processes of these rocks are difficult or very slow rates, and the soil in this forest is very shallow having 10 cm to 40 cm depth containing almost rocks, and classified to be the embryonic soil (Order Entisols) having a profile of A/C/R and the shallow soil (Order Inceptisols) with A/B/C/R profile.

2.2 Plant community study

A method of the plant community analysis was applied for vegetation survey in 2010 and 2017. Twelve permanent sampling plots, each of size 40 × 40 m, were arranged randomly over the forest. All trees in each plot were made the sequent number on their stems. The stem girths at breast height (gbh, 1.3 m above ground) and tree heights of all tree species with height over 1.5 m were measured. All plots were located using the GPS. The recorded field data of all tree species were calculated for the parameters implying their ecological influences in 2010 and 2017 including the plant frequency, density, dominance and importance value index (IVI), and the species diversity of plant communities using Shannon-Wiener Index (SWI) as given by Krebs (1985).

Forest condition index (FCI) within the 12 plots was calculated using an equation studied by Seeloy-Ounkeaw et al. (2014) which used for only the 40 × 40 m plot. For immature trees, the stem girth class of tree species was divided into 25 cm intervals for stem-girth up to 100 cm. The 100 cm interval was applied for mature trees with the DBH over 31.85 cm (girth over 100 cm). This assumed that the greater importance of the mature trees was due to their stem sizes (which gave merchantable timber for concession in the past for Thailand as well as their high ecological influence). The larger number of big trees existed in the plot can result in the higher FCI value.

$$FCI = \sum n_1.10^{-4} + n_2.10^{-3} + n_3.10^{-2} + n_4.10^{-1} + 1(n_5) + 2(n_6) + 3(n_7) + \ldots\ldots$$

When:
n_1 = number of tree individuals having girth <25 cm
n_2 = number of individuals, girth 25 to <50cm
n_3 = number of individuals, girth 50 to <75 cm
n_4 = number of individuals, girth 75 to <100 cm
n_5 = number of individuals, girth 100 to <200cm
n_6 = number of individuals, girth 200 to <300 cm
n_7 = number of individuals, girth 300 to <400 cm

2.3 Estimation of standing plant biomass

The recorded data of stem-DBH and tree heights of all tree species in 12 plots were also used for the calculation of biomass amounts in stem, branch, leaf and root organs using allometric equations studied in the deciduous forests in Thailand by Ogino et al. (1967).

$$W_S = 189\ (D^2H)^{0.902}$$
$$W_B = 0.125Ws^{1.204}$$
$$W_L = (11.4/ws^{0.90}) + 0.172$$

When: W_S = stem biomass in kilogram
W_B = branch biomass in kilogram
W_L = leaf biomass in kilogram

The unit of stem diameter (D) and tree height (H) was in meter. The root biomass was calculated using an equation of Ogawa et al. (1965).

$$W_R = 0.026\ (D^2H)^{0.775}$$

Units used here were in kilogram for root biomass (W_R), centimeter for stem diameter (D), and meter for tree height (H).

2.4 Estimation of carbon storages in plant biomass

The calculation of carbon amounts stored in standing plant biomass of all tree species in the forest were taken by multiplying the biomass amounts with average carbon contents in plant tissues of tree species in Thailand investigated by Tsutsumi et al. (1983). The average carbon contents in stem, branch, leaf and root organs of 62 tree species were reported to be 49.90%, 48.70%, 48.30% and 48.20%, respectively.

3. Results

3.1 Changes in plant community structures

3.1.1 Species richness and composition

Appendix 1 shows a species list of plant species in the DDF on volcanic rocks in the year 2010 and 2017. Based on Smitinand (2014), a total of 70 species (60 genera, 30 families) were existed in 2010, and increased to 83 species (73 genera, 34 families) in 2017. These implied to the changes of species richness and composition in this forest. The new 11 species (11 genera and 4 families) were occurred in the forest by a result of natural succession including *Goniothalamus laoticus, Protium serratum, Albizia lebbek, Caesalpinia godefroyana, Hiptage Bengalhensis, Bombax anceps, Sterculia villosa, Toona ciliata, Breynia glauca, Ventilago denticulata* and *Brucea javanica*. These species were those in the adjacent mixed deciduous forest which normally distributes along the valley sites. Two saplings of the horticultural fruit tree species, *Tamarindus indica* and *Dimocarpus longan*, were observed as the result of natural generation from dropped seeds in the forest by some visitors. An exotic leguminous fast-growing tree species, *Leucaena leucocephala*, was introduced to grow in the Center, and now become the invasive species. The number species per plot in most plots of 12 plots was increased from 2010 to 2017, three species on average.

3.1.2 Plant population and characteristics

The structures of a plant community involve species richness and composition, and relative abundance of all species. The vertical and spatial arrangements of these species provide the vertical and horizontal structures. However, this study focuses mainly on changes of the population structure of plant species, and their roles on plant quantitative features. The total number of tree individuals of all species within 12 plots in 2010 and 2017, and the net changes was given in Appendix 1. Changes of the quantitative plant features in the DDF on volcanic rock between 2010 and 2017 including plant frequency, density, stem basal area, relative frequency, relative density, relative dominance and relative IVI are given in Appendix 2 and 3.

<u>Plant frequency</u>: The frequency value of tree species is used to imply the spatial distribution in the forest. Most dominant tree species still had the same frequency values, and only small changes were observed for some species between 2010 and 2017. Eight species of *Shorea obtusa*, *Shorea siamensis*, *Terminalia alata*, *Phyllanthus emblica*, *Dalbergia assamica*, *Semecarpus anacardium*, *Morinda coreia*, and *Gardenia obtusifolia* had 100% frequency in 2010, and the seven species except for *Gardenia obtusifolia* (92) had 100% frequency in 2017. These species thus distributed over the forest. Other common species had unchanged frequencies: *Dalbergia oliverli* and *Pterocarpus macrocarpus* (92), *Xylia xylocarpa* and *Canarium subulatum* (83) while those of *Hardina cordifolia* and *Chukrasia velutina* had a small increase. The exotic species, *Leucaena leucocephala*, had the small decrease value. The increased frequencies of species indicate to the wider spatial distribution in the forest areas in opposite to the decreased values.

<u>Tree density:</u> The population structure of plant community in the DDF was changed as some trees were died and the appearance of tree individuals of successional species. In Table 3, the average tree density in 2010 was 3,450 trees/ha, and decreased to be 2,642 trees/ha in 2017. Many dead trees of mainly small saplings were observed for the dominant species. *Shorea obtusa* had the highest number of dead individuals, 279 trees/ha, followed by *Terminalia alata* (94), *Shorea siamensis* (74), *Gardenia obtusifolia* (43), *Dalbergia assamica* (42), etc. The population of some species were increased: *Chukrasia velutina* (68), *Canarium subulatum* (65), *Xylia xyloxarpa* (51), *Leucaena leucocephala* (44), etc. Most dead individuals were small saplings having the gbh below 25 cm, and the number was decreased for the bigger trees.

The changes of plant population in two stands between 2010 and 2017 were different (Appendix 3 and Fig. 1). In the *Shorea obtusa* stand, many dead saplings having gbh below 50 cm, were observed, and resulted in the decrease of average tree density. Some increases of the bigger trees in this stand were occurred. The density in this stand was 2,904 trees/ha in 2010 and decreased to 2,696 trees/ha in 2017, with the net decrease of 208 trees/ha. As for the *Shorea siamensis* stand, the density was 2,659 trees/ha in 2010 and increased to 2,859 trees/ha in 2017, the net increase of 200 trees/ha. Total tree individuals measured within 12 plots in 2010 were 5,497 trees and then decreased to 5,228 trees in 2017. The causes of changes involved the death of

778 tree individuals and appearing 509 saplings of the existing and new coming species with the net death of 269 individuals.

Tree dominance: Though many tree individuals were died during the period between 2010 and 2017, the average stem basal area of the DDF was increased from 16.65±2.68 m^2/ha to 18.23±2.79 m^2/ha with the net change of 1.58 m^2/h (Appendix 3).

The relative dominance of each species was calculated from its stem basal area to the all species. Dominance values of the most dominant species within 10 plots, *Shorea obtusa*, were slightly decreased (-2.44%). These caused by death of many saplings (-208 individuals). In contrast to *Shorea siamensis* (2 plots), it had the net increase (+2.03%) because of its increasing population (+200 individuals). However, the average dominance value within 12 plots of the two species was the net negative (-7.76%) caused by the death of trees (-140 individuals). This implies to decline of dominance of the most dominant species as increasing species diversity in the forest influenced by the new coming species (Appendix 4).

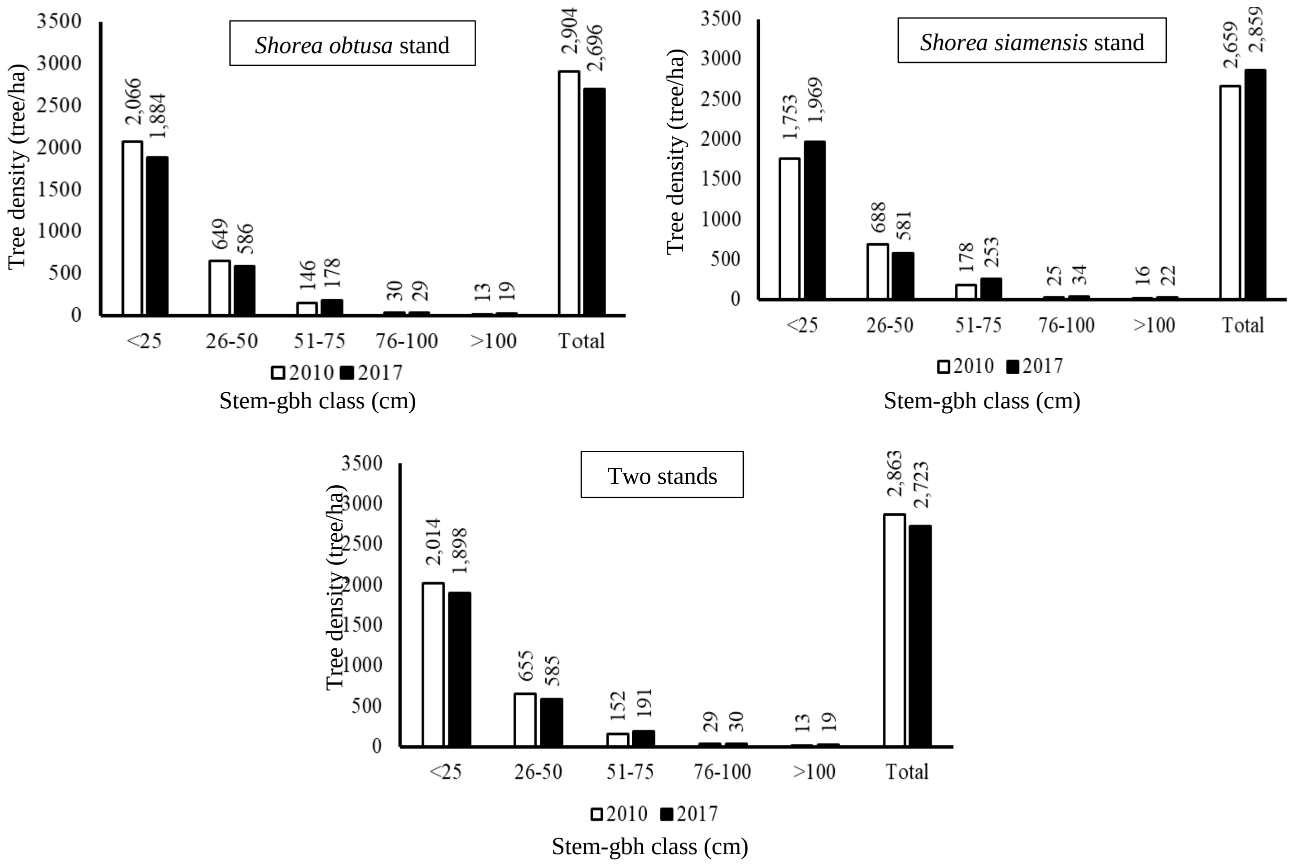

Figure 1. Changes of plant population based on stem-girth classes in DDF during 2010 and 2017 (Positive and negative values of dead trees were given in Appendix 4)

Importance value index (IVI) This index combines the relative frequency, relative density and relative dominance into a measure that can be used to indicate the ecological influence of each species in the forest. Using 12 plots, *Shorea obtusa* had the net decreased IVI value (-1.97%). The net increase IVI was the highest for *Shorea siamensis* (+2.64%), followed by *Canarium subulatum* (+1.42%), *Dalbergia oliverli* (+1.22%), *Pterocarpus macrocarpus* (+0.88%), *Semecarpus anacardium* (0.42%), etc. The net decreases for other species included *Gardenia obtusifolia* (-1.02%), *Dalbergia cultrata* (-0.70%), *Terminalia alata* (-0.49%), *Spathlolobus parviflorus* (-0.38%), *Catunaregum spathulifolia* (-0.36%), etc. The small changes were observed for the remained species. Among the two stands, net differences of the relative IVI values of the most dominant tree species in the *Shorea obtusa* and *Shorea siamensis* stands between 2010 and 2017 were calculated to 2.90% and -0.82%, respectively. The values indicated that the decreased ecological influences of the most dominant species (-2.55% on average) in the forest occurred as the species richness and diversity increased. The newly species had some contributions of ecological influences on environmental factors such as light condition, temperature, soil, moisture, etc., as indicated by the IVI value.

3.1.3 Plant species diversity and forest conditions

Plant species diversity: The species diversity here is not considered from only the species richness, but also the relative abundance of all species. The Shannon-Wiener Index (SWI) was used to determine species diversity according the combined concepts of species richness and heterogeneity (Krebs, 1985), and the data were given in Appendix 5. The mean values of the SWI in 2010 and 2017 were 3.66±0.42 and 3.81±0.44, respectively, with the net change of +0.15. This implied to a little increase of plant species diversity in the DDF during this period. Some differences were observed among the two stands. The SWI in nine plots of the *Shorea obtusa* stand varied between 2.71 to 4.24 (3.65±0.46 on average) in 2010, and the values were changed to the values between 2.69 and 4.45 (3.82±0.48 on average) in 2017, with net increase of 0.17. The average value in the *Shorea siamensis* stand was 3.73 in 2010 and 3.77 in 2017, the net increase of 0.04.

Forest condition: Appendix 6 and 7 show the number of tree individuals having different stem-gbh classes used for the calculation of the FCI values within 12 plots of two stands in the DDF. The mean values of the FCI in 2010 and 2017 were 2.70±1.74 and 3.16±1.87, respectively, with net change of +0.46. Thus, the DDF on volcanic rock had a little increase of forest condition during 2010 and 2017. Some differences of the FCI values among two stands were observed. In the *Shorea obtusa* stand, the FCI in nine plots varied between 0.38 and 4.37 (1.43±0.68 on average) in 2010, and the values were changed to be a range of 0.58 to 4.81 (1.51±1.27 on average) in 2017, with net increase of 0.59. In the *Shorea siamensis* stand, the FCI in two plots were 3.32 and 5.96 (2.64 on average) in 2010 and the values changed to 4.08 to 6.88 (2.80 on average) in 2017, the net increase of 0.16.

3.1.4 Temporal change of standing plant biomass

Standing plant biomass in the DDF on volcanic rocks could be separated into the bark, stem, branch, leaf and root organs as given in Table 5 and Fig. 2. The average biomass amount was increased from 83.65±13.55 mg/ha in 2010 to 97.27±17.70 mg/ha in 2017, the net increase of 13.62 mg/ha. The annual increment was calculated to be 1.95 mg/ha (2.22% per year). Some small variations of biomass production in the two stands were occurred. In the *Shorea obtusa* stand, biomass amounts within ten plots in 2010 varied between 56.14 mg/ha and 104.32 mg/ha, 83.01 mg/ha on average. In 2017, the amounts varied in a range of 70.79 to 133.51 mg/ha, 95.68 mg/ha on average. The net increase was measured to be 12.67 mg/ha (1.81 mg/ha/year). As for the *Shorea siamensis* stand, the amounts within two plots in 2010 were 80.59 mg/ha and 93.16 mg/ha, 86.88 mg/ha on average. In 2017, they varied in range of 89.88 to 120.63 mg/ha, 105.26 mg/ha on average, with the net increase of 18.38 mg/ha (2.63 mg/ha/year). These amounts of standing plant biomass did not include litterfall and dead trees.

3.2 Change of carbon amount in plant biomass

Amounts of carbon stored in the standing plant biomass including bark, stem, branch, leaf and root components in the DDF on volcanic rocks were given in Table 6 and Fig. 2. The average carbon amount in the forest was increased from 41.33±6.69 mg/ha in 2010 to 48.10±8.74 mg/ha in 2017, the net increase of 6.77 mg/ha. The annual increment of carbon storage was only 0.97 mg/ha/year (2.30% per year). The carbon amounts in ten plots of the *Shorea obtusa* stand in 2010 varied between 27.74 mg/ha and 51.55 mg/ha, 46.03 mg/ha on average. In 2017, the carbon amounts varied between 34.98 mg/ha and 66.00 mg/ha, 59.64 mg/ha on average. The net increment was measured at 13.61 mg/ha (1.94 mg/ha/year). As for the *Shorea siamensis* stand, the carbon amounts in 2010 were 39.83 mg/ha and 46.03 mg/ha, 42.93 mg/ha on average. In 2017, the amounts changed to 44.42 mg/ha to 59.64 mg/ha, 52.03 mg/ha on average. The net increment in this stand was measured at 9.10 mg/ha or 1.30 mg/ha/year.

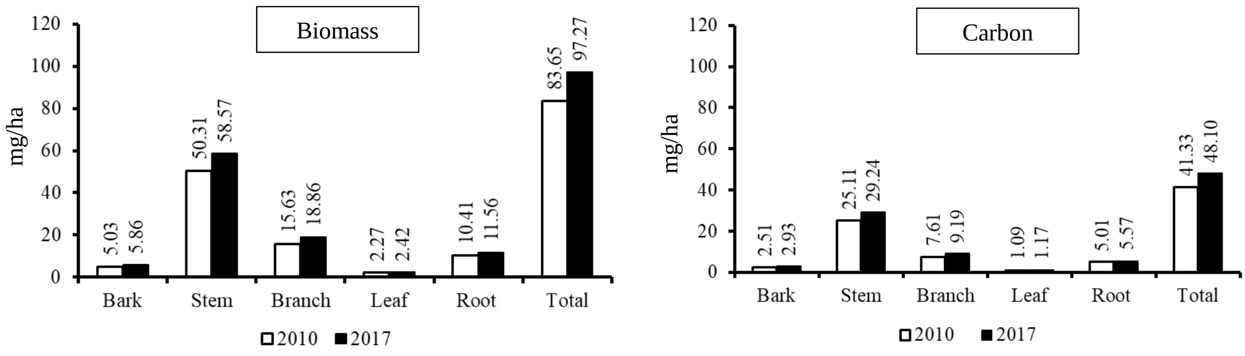

Figure 2. Changing plant biomass and carbon storages in the DDF during 2010 and 2017

4. Discussion

4.1 Plant species composition, richness, diversity and forest condition

During 2010 and 2017, plant communities in the secondary DDF on volcanic rock which composed of two stands had some changes of species composition, richness, diversity population structure, forest condition, biomass production and carbon storage. The species richness, family richness, species diversity (SWI) and forest condition index (FCI) were increased. The 13 newly species were mainly saplings of those species immigrated from the adjacent mixed deciduous forest. Two horticultural fruit tree species were found as the result of natural regeneration from dropped seeds by the visitors, and an existing introduced exotic fast growing tree species was become invasive species. The saplings of these successional species changed species composition and richness in the forest. Since forest fire was strongly protected in the Center, most seedlings did not suffer from the fire, and the site moisture might be improved and more suitable for regeneration of successional plant species. However, the death of many small saplings of existing species particularly the dominant species were observed, but it varied greatly among sampling sites. It is assumed to be the effects of variable competition among sites for the growing space and environmental factors such as light, moisture, nutrients, etc., either by intraspecific or interspecific competition (Kimmins, 2004). Since the DDF had opened canopy, unfertile shallow soil and very dry site during summer, the competition for nutrients and moisture might be the main causes of sapling death. As for the lower montane forest, it had the closed evergreen canopy, deep fertile soil, and intermediate moist site during summer, the competition for light might be the main factor of death of seedings and saplings (Khamyong and Anongrak, 2016); however, the topographic condition particularly the relative altitude and slope convexity were reported to be important factors affecting habitat differentiation of Lauraceae species in this lower montane forest (Sri-Ngernyuang et al., 2012). The microclimate especially light, moisture, air temperature, etc. might be altered by the topographic condition. Some different results were reported by Sutthawan et al. (2016) that the DDF on sandstone consisted of four stands. The species richness, family richness, tree density and the SWI in 2010 and 2015 were decreased from 60 to 53 species (-7), 31 to 27 families (-4), 3,865 trees/ha to 2,780 trees/ha (-1,085), and 3.17 to 3.06 (-0.11), respectively, but the FCI value was increased from 1.94 to 2.27 (+0.38). Thus, the species richness and diversity were decreased in the DDF on sand-stone. These might be influenced by different moisture conditions between the DDF on two areas having different parent rocks.

4.2 Changes of plant population structures

The population structures of tree species in the forest were changed as some tree individuals were died versus recruitment of new saplings of existing and successional species. The death of tree individuals was high for most of common species in the forest, and caused them had the decreasing populations. *Shorea obtusa* had the highest number of dead tree individuals, followed by *Terminalia alata*, *Shorea siamensis*, *Gardenia obtusifolia*, *Dalbergia assamica*, etc. In contrast, the population of some tree species were adversely increased including *Chukrasia velutina*, *Canarium subulatum*, *Xylia xylocarpa*, *Leucaena leucocephala*, *Haldina* *cordifolia*, *Pterocarpus macrocarpus*, etc. The number of dead and newly established saplings of tree species varied greatly among the species and sampling plots or sites. Some plots had the strong net death of tree individuals while the others had the strong net recruitment, and the remained plots were weak or intermediate. Most dead tree individuals were mainly those having the stem gbh class below 25 cm. Therefore, the changes in population structures of tree species in the forest was then occurred. The similar results were reported by Sutthawan et al. (2016) that the death of many tree individuals was the main cause of population changes in the DDF on sandstone.

4.3 Amounts of plant biomass and stored carbon

Since most of the DDF are the disturbed forest, the standing biomass is usually lower than other forests in Thailand. In this study, the biomass amount in 2017 was 97.27 mg/ha whereas the DDF on sandstone in this Center had the amount of 90.65 mg/ha in 2015 (Sutthawan et al., 2016). The biomass amounts of the DDF in other locations varied between 62 mg/ha and 159 mg/ha (Phongkhamphanh et al., 2015). As for the lower montane forest, biomass amount was higher (132 mg/ha to 256 mg/ha) (Khamyong et al., 2014), and it was very high for the upper montane forest located at the highest mountain in Thailand, Doi Inthanon summit (703.8 mg/ha) (Khamyong and Anongrak, 2016). Although many dead trees versus lesser recruitment of newly established saplings in the forest resulted in decreasing tree density, the amount of standing plant biomass was found to be increased from 2010 to 2017. The remained existing trees could grow slowly, and provided a little net annual increment of plant biomass in the forest. The net increment between 2010 and 2017 was measured to be 13.62 mg/ha, or 1.95 mg/ha/year (2.30% per year). This included the net increase of above-ground biomass at 1.78 mg/ha/year. However, this value did not include the biomass losses in dead trees, litterfall and grazing. The slow growth rates of plants in the DDF were influenced by the combined effects of poor soil and dry site. The soil under this forest on volcanic rocks was very poor and shallow which contained many fragmented rocks and gravels. The slow growth rates of plants further affected the biomass production. According to Sutthawan et al. (2016), the average amount of plant biomass in the DDF on sandstone had increased from 83.74±12.35 to 90.65±11.36 mg/ha (1.36 mg/ha/year). Sahunalu (1994) studied 52 stands of the DDF in Thailand which were classified into six subtype communities, and reported that average gross primary production of the forest was 11.25±2.50 mg/ha/year, divided into the net primary production (NPP) of 5.77±1.16 mg/ha/year (51.30%), and the rate of respiration loss at 5.48±1.42 mg/ha/year (48.70%). The NPP included the net increment of plant biomass, dead trees, litterfall and grazing loss. The data indicated that most of the primary production was lost from the DDF ecosystem, and therefore the net increment of plant biomass was so very small. As for the climax montane forest at Mt. Inthanon reported by Sunpalee et al. (2015), the amount of standing above-ground biomass was measured at 372.70 mg/ha. The annual biomass increment was a little higher than the DDF, 2.02 mg/ha/year. However, death of some old big trees in each year was occurred in this climax forest, and thus the annual biomass increment compared to its standing biomass amount was not high, only 0.5% per year. In contrast to the secondary DDF, dead tree individuals were mainly small saplings, and the net biomass increment compared to its standing biomass (83.65 mg/ha in 2010) was calculated to 2.3% per year, higher than the climax montane forest.

The carbon amount stored in the standing plant biomass depends upon biomass production of the forest. The small increase of carbon amount stored in the plant biomass was also observed with the net increase of 6.77 mg/ha, and the annual increase was only 0.97 mg/ha. As compare to data investigated by Sutthawan et al. (2016), the carbon amount stored in plant biomass in the DDF on sandstone were in-creased from 41.59 mg/ha in 2010 to 44.79 mg/ha in 2015, a net increase of 3.20 mg/ha or 0.64 mg/ha/year (1.50% per year). This implies to the more rapid rate of carbon storage in the DDF on volcanic rocks than the same forest on sandstone.

5. Conclusions

The DDF on very shallow soil derived from volcanic rock was severely disturbed through over harvesting before establishment of the HHKRDS Center, and later protected from selective tree cutting and forest fire. It is become the recovery forest developed to be the more good conditions. The plant species diversity and biomass carbon storage in the forest was assessed in 2010 and monitored in 2017 to evaluate changes of plant communities, and found that they had the slow rates. This DDF included two stands based on the most dominant tree species: *Shorea obtusa* and *Shorea siamensis*. The number of species, genera and family were increased as well as the Shannon-Wiener index (SWI) as indicating species diversity as well as the forest condition index (FCI) were slightly increased, but its tree density was adversely declined caused by death of mainly small saplings. The amounts of plant biomass and stored carbon were increased at the slow rates, only 0.96 mg/ha/year (2.30% per year). Thus, enrichment planting of some appropriate tree species in the forest is considered as an important technique to reduce time of forest development to be the more abundant forest.

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Supplementary data

Appendix 1: Species list and number of tree species in 12 plots of the DDF in 2010 and 2017

Family	Scientific name	Life form	Total individuals		
			2010	2017	Net
1. Anacardiaceae	1. *Buchanania lanzan* Spreng.	BT	19	20	+1
	2. *Lannea coromandelica* (Houtt.) Merr.	BT	7	9	+2
	3. *Semecarpus albescens* Kurz	MT	75	58	-17
	4. *Spondias pinnata* (L.f.) Kurz	BT	2	3	+1
2. Annonaceae	5. *Goniothalamus laoticus* (Finet & Gagnep.) Ban	ST	0	1	+1
3. Apocynaceae	6. *Amphineurion marginatum* (Roxb.) D.J. Middleton	C	28	34	+6
	7. *Cryptolepis dubia* (Burm.f.) M.R. Almeida	C	33	32	-1
4. Bignoniaceae	8. *Dolichandrone serrulata* (Wall. ex DC.) Seem.	ST	10	6	-4
	9. *Orcxylum indicum* (L.) Benth. ex Kurz	ST	20	24	+4
	10. *Stereospermum cylindricum* Pierre ex Dop.	MT	3	3	0
	11. *Stereospermum neuranthum* Kurz	MT	10	7	-3
	12. *Stereospermum tetragonum* DC.	MT	10	7	-3
5. Burseraceae	13. *Canarium subulatum* Guillaumin	ST	180	245	+65
	14. *Protium serratum* Engl.	ST	0	1	+1
6. Celastraceae	15. *Celastrus paniculata* Willd.	C	9	8	-1
7. Combretaceae	16. *Combretun punctatum* Blume	C	2	2	0
	17. *Terminalia alata* B. Heyne ex Roth	BT	353	259	-94
	18. *Terminalia chebula* Retz.	MT	12	10	-2
8. Dilleniaceae	19. *Dillenia obovata* (Blume) Hoogland	ST	2	2	0
9. Dipterocarpaceae	20. *Dipterocarpus tuberculatus* Roxb.	BT	72	70	-2
	21. *Shorea obtusa* Wall. ex Blume	BT	1492	1213	-279
	22. *Shorea siamensis* Miq.	BT	530	456	-74
10. Ebenaceae	23. *Diospiros ehretioides* Wall. ex G. Don	ST	12	13	+1
11. Fabaceae	24. *Albizia lebbeck* (L.) Benth.	MT	0	1	+1
	25. *Albizia odoratissima* (L.f.) Benth.	MT	16	20	+4
	26. *Bauhinia variegata* L.	ST	1	2	+1
	27. *Caesalpinia godefroyana* Kuntze	C	0	1	+1
	28. *Cassia fistula* L.	MT	1	1	0
	29. *Dalbergia cultrata* Graham ex. Benth.	ST	225	183	-42
	30. *Dallbergia oliveri* Gamble	MT	264	261	-3
	31. *Dallbergia velutina* Benth.	MT	3	5	+3
	32. *Leucaena leucocephala* (Lam.) de Wit	ST	103	147	+44
	33. *Millettia extensa* (Benth.) Baker	C	4	3	-1
	34. *Millettia pachycarpa* Benth.	MT	3	4	+1
	35. *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne	MT	5	32	+27
	36. *Pterocarpus macrocarpus* Kurz	BT	184	215	+31
	37. *Spatholobus parviflorus* (DC.) Kuntze	C	63	31	-32
	38. *Temarindus indica* L.	BT	0	1	+1
	39. *Xylia xylocarpa* Taub. var. *kerrii* Nielsen	BT	347	398	+51
12. Salicaceae	40. *Casearia gallifera* Tathana	MT	1	1	0
	41. *Flacourtia indica* (Burm.f.) Merr.	ST	3	3	+1
13. Hypericaceae	42.*Cratoxylum formosum* (Jacq.) Benth & Hook F.ex Dyer	ST	39	92	+53
14. Lamiaceae	43. *Tectona grandis* Linn.f.	BT	12	39	+27
	44. *Vitex peduncularis* Wall. ex Schauer	MT	13	27	+14
15. Lauraceae	45. *Litsea glutinosa* (Lour.) C.B.Rob.	MT	2	3	+1
16. Lecythidaceae	46. *Careya sphaerica* (Roxb.	ST	1	1	0
17. Lythraceae	47. *Lagerstroemia macrocarpa* Wall. ex Kurz	BT	14	10	-4
18. Malpighiaceae	48. *Hiptage benghalensis* (L.) Kurz	S	0	1	+1

Appendix 1: Species list and number of tree species in 12 plots of the DDF in 2010 and 2017 (cont.)

Family	Scientific name	Life form	Total individuals		
			2010	2017	Net
19. Malvaceae	49. *Decaschistia siamensis* Craib	MT	1	1	0
	50. *Bombax anceps* Pierre	BT	0	2	+2
	51. *Sterculia villosa* Roxb.	MT	0	1	+1
	52. *Berrya mollis* Wall. ex Kurz	MT	1	1	0
	53. *Colona flagrocarpa* (C.B.Clark) Craib	ST	39	49	+10
20. Meliaceae	54. *Azadirachta indica* A.Juss.	MT	1	1	0
	55. *Chukrasia tabularis* A. Juss.	MT	67	139	+72
	56. *Toona ciliata* M.Roem.	BT	0	1	+1
21. Moraceae	57. *Artocarpus gomezianus* Wall. ex Trecul	MT	1	1	0
22. Myrtaceae	58. *Syzygium cumini* (L.) Skeels	MT	2	2	0
23. Ochnaceae	59. *Ochna intergerrima* (Lour.) Merr.	ST	12	10	-2
24. Opiliaceae	60. *Meilentha suavis* Pierre	ST	7	11	+4
25. Phyllanthaceae	61. *Antidesma sootepense* Craib	S	3	3	0
	62. *Antidesma ghaesembilla* Gaertn.	S	4	5	+1
	63. *Antidesma acidum* Retz.	S	2	1	-1
	64. *Breynia glauca* Craib	S	0	1	+1
	65. *Bridelia retusa* (L.) A.Juss.	ST	42	46	+4
	66. *Phyllanthus emblica* L.	ST	315	328	+13
26. Rhamnaceae	67. *Ventilago denticulata* Willd. C	S	0	3	+3
27. Rubiaceae	68. *Catunaregam spathulifolia* Tirveng.	S	229	66	-163
	69. *Dioecrescis erythroclada* (Kurz) Tirveng.	ST	10	10	0
	70. *Gardenia obtusifolia* Roxb. ex Gordon	S	129	86	-43
	71. *Mitragyna rotundifolia* (Roxb.) Kuntze	MT	169	195	+26
	72. *Ixora cibdela* Craib	S	9	7	-2
	73. *Morinda coreia* Ham.	ST	83	86	+3
	74. *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre	ST	18	19	+1
	75. *Vangueria pubescens* Kurz	S	2	1	-1
28. Sapindaceae	76. *Schleichera oleosa* (Lour.) Merr.	BT	7	10	+3
	77. *Dimocarpus longan* Lour.	MT	0	1	+1
29. Sapotaceae	78. *Madhuca esculenta* H.R. Fletcher	ST	44	42	-2
30. Simaroubaceae	79. *Brucea javanica* (L.) Merr	S	0	1	+1
31. Loganiaceae	80. *Strychnos nux-vomica* L.	S	41	47	+6
32. Ulmaceae	81. *Ulmus lancaefolia* Roxb. ex Wall.	ST	1	2	+1
33. Primulaceae	82. *Embellia sessiliflora* (C.B. Clarke) Mez	C	29	34	+5
34. Unknown Unidentified species	83. *Climber*	C	44	50	+6
	Total		5497	5228	-269

Note: BT = big tree, MT = medium tree, ST = small tree, S = shrub, C = climber

Appendix 2: Frequency, density and basal area of species in DDF between 2010 and 2017

No.	Plant species	Frequency (%)			Density (trees/ha)			Basal area (m^2 /ha)		
		2010	2017	Net	2010	2017	Net	2010	2017	Net
1	*S. obtusa*	100	100	0	777	632	- 145	6.68	8.36	+1.69
2	*S. siamensis*	100	100	0	276	238	-39	1.59	3.60	+2.02
3	*X. xylocarpa*	83.33	83.33	0	217	207	-10	0.58	0.68	+0.09
4	*T. alata*	100	100	0	184	171	-13	0.61	0.45	-0.16
5	*D. oliveri*	91.67	91.67	0	117	136	+19	0.22	0.71	+0.49
6	*P.* emblica	100	100	0	164	135	-29	0.37	0.42	+0.05

Appendix 2: Frequency, density and basal area of species in DDF between 2010 and 2017 (cont.)

No.	Plant species	Frequency (%)			Density (trees/ha)			Basal area (m^2 /ha)		
		2010	2017	Net	2010	2017	Net	2010	2017	Net
7	*C. subulatum*	83.33	83.33	0	67	128	+60	0.16	0.52	+0.36
8	*D. cultrata*	100	100	0	150	102	-48	0.33	0.23	-0.10
9	*H. cordifolia*	83.33	91.67	+8.34	119	95	-24	0.31	0.41	+0.10
10	*P. macrocarpus*	91.67	91.67	0	44	77	+33	0.10	0.37	+0.27
11	*S. anacardium*	100	100	0	55	70	+16	0.15	0.29	+0.14
12	*M. coreia*	100	100	0	43	58	+15	0.14	0.25	+0.11
13	*C. velutina*	83.33	91.67	+8.34	39	48	+9	0.13	0.19	+0.06
14	*L. leucocepphala*	75	66.67	-8.33	72	45	-27	0.15	0.19	+0.04
15	*G. obtusifolia*	100	91.67	-8.33	106	45	-61	0.17	0.03	-0.14
Species 16-83		1,900	2,208	+308	434	537	+103	4.98	1.52	+0.57
	Total	3,292	3,600	+308	2,863	2,723	- 140	16.65	18.23	+1.58

Appendix 3: Changes in quantitative features of tree species in DDF between 2010 and 2017

Plant species	Relative value (%)											
	Frequency			Density			Dominance			IVI		
	2010	2017	Net	2010	2017	Net	2010	2017	Net	2010	2017	Net
1 *S. obtusa*	3.04	2.78	-0.26	22.52	23.91	+1.39	52.91	45.89	-7.03	26.16	24.19	-1.97
2 *S. siamensis*	3.04	2.78	-0.26	8.00	8.99	+0.99	12.57	19.76	+7.19	7.87	10.51	+2.64
3 *X. xylocarpa*	2.53	2.31	-0.22	6.29	7.85	+1.56	4.60	3.71	-0.89	4.47	4.62	+0.15
4 *T. alata*	3.04	2.78	-0.26	5.33	6.47	+1.14	4.80	2.44	-2.36	4.39	3.90	-0.49
5 *D. oliveri*	2.78	2.55	-0.24	3.40	5.15	+1.75	1.76	3.92	+2.16	2.65	3.87	+1.22
6 *P. emblica*	3.04	2.78	-0.26	4.76	5.11	+0.35	2.92	2.30	-0.62	3.57	3.39	-0.18
7 *C. subulatum*	2.53	2.31	-0.22	1.95	4.83	+2.88	1.25	2.85	+1.60	1.91	3.33	+1.42
8 *D.cultrata*	3.04	2.78	-0.26	4.35	3.84	-0.50	2.60	1.26	-1.34	3.33	2.63	-0.70
9 *H. cordifolia*	2.53	2.55	+0.01	3.46	3.61	+0.15	2.45	2.23	-0.22	2.81	2.80	-0.02
10 *P. macroca*	2.78	2.55	-0.24	1.27	2.90	+1.63	0.78	2.03	+1.25	1.61	2.49	+0.88
11. *S. anacard*	3.04	2.78	-0.26	1.58	2.66	+1.08	1.16	1.59	+0.43	1.93	2.34	+0.42
12 *M. coreia*	3.04	2.78	-0.26	1.25	2.21	+0.95	1.09	1.39	+0.30	1.79	2.12	+0.33
13 *C. velutina*	2.53	2.55	+0.01	1.13	1.81	+0.68	1.03	1.07	+0.04	1.56	1.81	+0.24
14. *L. leucoce*	2.28	1.85	-0.43	2.07	1.70	-0.38	1.18	1.06	-0.12	1.84	1.54	-0.31
15. *G. obtusifo*	3.04	2.55	-0.49	3.06	1.70	-1.37	1.35	0.15	-1.20	2.48	1.46	-1.02
Species 16-83	57.7	61.3	+3.63	29.5	17.3	-2.32	7.56	8.36	+0.80	31.6	28.9	-2.63
Total	100	100	0	100	100	0	100	100	0.00	100	100	0

Appendix 4: The relative value of the most dominant species in DDF between 2010 and 2017

Plot no.	Dominant species	Relative value for the most dominant species (%)								
		Density			Dominance			IVI		
		2010	2017	Net	2010	2017	Net	2010	2017	Net
1	*S. obtusa*	53.44	53.17	-0.27	49.56	46.06	-3.50	51.50	49.61	-1.89
2	*S. obtusa*	31.40	31.20	-0.20	43.00	40.40	-2.60	37.20	35.80	-1.40
3	*S. obtusa*	42.39	38.97	-3.42	77.36	77.57	0.21	59.87	58.27	-1.60
4	*S. obtusa*	40.48	35.80	-4.68	59.78	61.13	1.35	50.13	48.47	-1.66
5	*S. obtusa*	36.91	28.40	-8.51	60.01	57.44	-2.57	48.46	42.92	-5.54
6	*S. obtusa*	42.55	37.58	-4.97	61.51	61.72	0.21	52.03	49.65	-2.38
7	*S. obtusa*	14.93	13.88	-1.05	33.31	32.66	-0.65	24.12	23.27	-0.85
8	*S. obtusa*	12.94	12.21	-0.73	49.28	42.06	-7.22	31.11	27.13	-3.98
9	*S. obtusa*	15.42	12.39	-3.03	49.71	41.59	-8.12	32.56	26.99	-5.57

Appendix 4: The relative value of the most dominant species in DDF between 2010 and 2017 (cont.)

Plot no.	Dominant species	Relative value for the most dominant species (%)								
		Density			Dominance			IVI		
		2010	2017	Net	2010	2017	Net	2010	2017	Net
10	*S. obtusa*	26.59	19.84	-6.75	45.89	44.42	-1.47	36.24	32.13	-4.11
	Mean	31.71	28.34	-3.36	52.94	50.50	-2.44	42.32	39.42	-2.90
	S.D.	13.17	12.94	-0.22	11.56	12.84	1.28	10.96	11.36	0.40
11	*S. siamensis*	23.70	20.80	-2.90	47.80	51.10	+3.30	35.80	35.95	+0.15
12	*S. siamensis*	22.94	19.16	-3.78	31.28	31.51	+0.23	27.11	25.34	-1.78
	Mean	23.32	19.98	-3.34	39.54	5.14	-34.40	31.46	30.65	-0.82
	Mean (12 plots)	30.31	26.95	-3.36	50.71	42.94	-7.76	40.51	37.96	-2.55
	S.D.	12.42	12.22	-0.20	12.15	20.64	8.49	10.94	11.09	0.15

Appendix 5: Species richness, density, basal area and SWI of species in DDF between 2010 and 2017

Plot no.	Dominant Species	Species richness (species/plot)			Density (trees/ha)			Stem basal area (m^2/ha)			SWI		
		2010	2017	Net	2010	2017	Net	2010	2017	Net	2010	2017	Net
1	*S. obtusa*	27	24	-3	2,631	1,775	-856	20.55	20.25	-0.30	2.71	2.69	-0.02
2	*S. obtusa*	39	35	-4	2,950	1,944	-1006	18.05	18.59	0.54	3.87	3.85	-0.02
3	*S. obtusa*	29	32	3	2,094	2,069	-25	15.00	16.64	1.63	3.34	3.61	0.27
4	*S. obtusa*	39	49	10	3,381	3,038	-344	18.78	21.01	2.23	3.67	4.05	0.38
5	*S. obtusa*	23	31	8	3,031	3,631	+600	13.36	16.01	2.65	3.18	3.41	0.23
6	*S. obtusa*	30	37	7	2,013	2,063	+50	16.29	17.45	1.15	3.39	3.68	0.29
7	*S. obtusa*	32	32	0	2,219	2,206	-13	14.97	16.73	1.76	4.04	4.13	0.09
8	*S. obtusa*	31	33	2	3,713	3,256	-456	14.55	17.08	2.53	3.98	3.98	0.00
9	*S. obtusa*	35	39	4	3,081	2,975	-106	13.82	14.43	0.61	4.24	4.45	0.21
10	*S. obtusa*	48	51	3	3,925	4,000	+75	20.65	21.14	0.49	4.03	4.30	0.27
	Mean	33	36	3	2,904	2,696	-208	16.60	17.93	1.33	3.65	3.82	0.17
	S.D.	6.83	7.84	4	630	744	+114	2.58	2.14	0.94	0.46	0.48	0.03
11	*S.siamen*	42	47	5	3,875	4,088	213	20.02	24.33	4.31	3.86	3.83	-0.02
12	*S.siamen*	22	27	5	1,444	1,631	188	13.81	15.11	1.30	3.61	3.71	0.10
	Mean	32.0	37.0	5	2,659	2,859	200	16.92	19.72	2.80	3.73	3.77	0.04
	Mean	33.1	36.4	3.3	2,863	2,723	-140	16.65	18.23	1.57	3.66	3.81	0.15
	S.D.	7.5	8.2	4.0	7656	846	437	2.68	2.79	1.19	0.42	0.44	0.02

Appendix 6: Changes of tree number with stem-girth classes in DDF between 2010 and 2017

Plot no.	Dominant species	Number of tree individuals (trees/ha)											
		gbh <25 cm			26-50 cm			51-75 cm			76-100 cm		
		2010	2017	net	2010	2017	net	2010	2017	net	2010	2017	net
1	*S.obtusa*	1,569	831	-738	819	631	-188	213	263	+50	31	44	+13
2	*S.obtusa*	2,050	1,144	-906	738	606	-131	125	156	+31	25	-44	-69
3	*S.obtusa*	1,413	1,363	-50	525	500	-25	125	175	+50	13	6	-6
4	*S.obtusa*	2,363	2,038	-325	788	700	-88	206	275	+69	6	19	+13
5	*S.obtusa*	2,206	2,681	+475	744	806	+63	75	125	+50	6	13	+6
6	*S.obtusa*	1,275	1,356	+81	513	456	-56	131	138	+6	81	94	+13
7	*S.obtusa*	1,663	1,706	+44	344	300	-44	125	113	-13	69	69	0
8	*S.obtusa*	2,894	2,381	-513	669	681	+13	125	175	+50	13	19	+6
9	*S.obtusa*	2,413	2,344	-69	488	431	-56	150	156	+6	19	38	+19
10	*S.obtusa*	2,819	2,994	+175	863	744	-119	188	206	+19	38	38	0
	Mean	2,066	1,884	-183	649	586	-63	146	178	+32	30	29	-1
	S.D.	542	677	+409	162	151	+69	41	52	+25	25	35	+24

Appendix 6: Changes of tree number with stem-girth classes in DDF between 2010 and 2017 (cont.)

Plot no.	Dominant species	Number of tree individuals (trees/ha)											
		gbh <25 cm			26-50 cm			51-75 cm			76-100 cm		
		2010	2017	net	2010	2017	net	2010	2017	net	2010	2017	net
11	*S.siamen*	2,563	2,794	+231	1,075	888	-188	231	369	+138	6	31	+25
12	*S.siamen*	944	1,144	+200	300	275	-25	125	138	+13	44	38	-6
	Mean	1,753	1,969	+216	688	581	-106	178	253	+75	25	34	+9
	Mean plots)	2,014	1,898	-116	655	585	-70	152	191	+39	29	30	+1
	S.D.	607	705	+402	217	186	+73	45	73	+38	24	32	+23

Appendix 7: Changes of tree number with stem-gbh classes and FCI (continued from Table 6)

Plot no.	Dominant species	Number of tree individuals (trees/ha)								
		gbh >100 cm			Total for all gbh class			FCI		
		2010	2017	net	2010	2017	net	2010	2017	net
1	*S. obtusa*	0	6	+6	2,631	1,775	-856	1.10	2.23	+1.13
2	*S. obtusa*	13	81	+69	2,950	1,944	-1,006	2.75	3.27	+0.51
3	*S. obtusa*	19	25	+6	2,094	2,069	-25	3.51	4.48	+0.97
4	*S. obtusa*	19	6	-13	3,381	3,038	-344	0.59	0.88	+0.29
5	*S. obtusa*	0	6	+6	3,031	3,631	+600	0.38	0.58	+0.20
6	*S. obtusa*	13	19	+6	2,013	2,063	+50	3.61	4.81	+1.20
7	*S. obtusa*	19	19	0	2,219	2,206	-13	4.37	4.35	-0.02
8	*S. obtusa*	13	0	-13	3,713	3,256	-456	1.55	0.73	-0.82
9	*S. obtusa*	13	6	-6	3,081	2,975	-106	2.65	1.95	-0.70
10	*S. obtusa*	19	19	0	3,925	4,000	+75	4.08	4.09	+0.01
	Mean	13	19	+6	2,904	2,696	-208	2.46	2.74	+0.28
	S.D.	7	22	+22	630	744	+449	1.39	1.58	+0.67
11	*S.siamensis*	0	6	+6	3,875	4,088	+213	0.68	1.27	+0.60
12	*S.siamensis*	31	38	+7	1,444	1,631	+188	5.96	6.88	+0.92
	Mean	16	22	+6	2,659	2,859	+200	3.32	4.08	+0.76
	Mean	13	19	+6	2,863	2,723	-140	2.60	2.96	+0.36
	S.D.	9	21	+20	765	846	+438	1.70	1.91	+0.64

Appendix 8: Amounts of standing plant biomass in two stands of DDF between 2010 and 2017

Plot no.	Dominant species	Plant biomass (mg/ha)								
		Bark			Stem			Branch		
		2010	2017	net	2010	2017	net	2010	2017	net
1	*S. obtusa*	5.90	6.24	+0.34	58.98	62.42	+3.44	17.00	19.17	+2.17
2	*S. obtusa*	5.35	5.99	+0.64	53.54	59.94	+6.40	16.21	19.48	+3.27
3	*S. obtusa*	4.63	5.28	+0.65	46.33	52.79	+6.46	15.21	17.55	+2.34
4	*S. obtusa*	5.13	6.05	+0.92	51.34	60.55	+9.21	13.85	17.38	+3.53
5	*S. obtusa*	3.41	4.30	+0.89	34.10	42.99	+8.89	8.73	11.50	+2.77
6	*S. obtusa*	5.13	5.60	+0.47	51.30	55.98	+4.68	16.56	18.33	+1.77
7	*S. obtusa*	5.70	7.90	+2.19	57.04	78.98	+21.94	22.50	31.07	+8.56
8	*S. obtusa*	4.02	4.86	+0.84	40.18	48.61	+8.43	11.25	13.81	+2.56
9	*S. obtusa*	4.31	4.57	+0.26	43.14	45.70	+2.56	14.04	14.86	+0.82
10	*S. obtusa*	6.28	6.80	+0.51	62.82	67.90	+5.08	19.68	22.36	+2.68
	Mean	4.99	5.76	+0.77	49.88	57.59	+7.71	15.50	18.55	+3.05
	S.D.	0.85	1.03	0.52	8.49	10.31	5.20	3.73	5.11	1.98

Appendix 8: Amounts of standing plant biomass in two stands of DDF between 2010 and 2017 (cont.)

Plot no.	Dominant species	Plant biomass (mg/ha)								
		Bark			Stem			Branch		
		2010	2017	net	2010	2017	net	2010	2017	net
11	*S.siamensis*	5.66	7.33	+1.66	56.65	73.26	+16.61	15.51	21.46	+5.95
12	*S.siamensis*	4.83	5.38	+0.55	48.32	53.77	+5.45	17.03	19.33	+2.30
	Mean	5.25	6.35	+1.10	52.48	63.51	+11.03	16.27	20.39	+4.12
	Mean (all)	5.03	5.86	+0.83	50.31	58.57	+8.26	15.63	18.86	+3.23
	S.D.	0.80	1.05	0.54	7.99	10.45	5.41	3.43	4.73	1.99

Appendix 9: (continued from Appendix 8)

Plot no.	Dominant species	Plant biomass (mg/ha)								
		Leaf			Root			Total		
		2010	2017	net	2010	2017	net	2010	2017	net
1	*S. obtusa*	2.93	2.73	-0.21	12.39	12.50	+0.10	97.21	103.06	+5.85
2	*S. obtusa*	2.53	2.44	-0.09	11.07	11.74	+0.66	88.72	99.59	+10.88
3	*S. obtusa*	1.96	2.13	+0.17	9.17	10.27	+1.10	77.30	88.02	+10.72
4	*S. obtusa*	2.87	3.00	+0.14	11.27	12.68	+1.41	84.46	99.67	+15.21
5	*S. obtusa*	2.10	2.46	+0.36	7.80	9.54	+1.74	56.14	70.79	+14.65
6	*S. obtusa*	2.02	2.12	+0.11	10.02	10.82	+0.80	85.03	92.85	+7.82
7	*S. obtusa*	1.44	1.98	+0.54	12.02	13.59	+1.56	98.71	133.51	+34.80
8	*S. obtusa*	2.13	2.48	+0.35	8.71	10.35	+1.64	66.29	80.11	+13.82
9	*S. obtusa*	1.81	1.88	+0.07	8.58	9.05	+0.47	71.89	76.06	+4.17
10	*S. obtusa*	2.80	2.73	-0.07	12.74	13.29	+0.55	104.32	113.08	+8.76
	Mean	2.26	2.40	+0.14	10.38	11.38	+1.00	83.01	95.68	+12.67
	S.D.	0.48	0.34	0.22	1.67	1.52	0.54	14.49	17.70	+8.16
11	*S.siamensis*	3.09	3.47	+0.38	12.25	15.12	+2.87	93.16	120.63	+27.47
12	*S.siamensis*	1.53	1.62	+0.09	8.89	9.79	+0.90	80.59	89.88	+9.29
	Mean	2.31	2.55	+0.24	10.57	12.45	+1.88	86.88	105.26	+18.38
	Mean	2.27	2.42	+0.15	10.41	11.56	+1.15	83.65	97.27	+13.62
	S.D.	0.54	0.49	0.21	1.67	1.80	0.71	13.55	17.70	8.59

Appendix 10: Carbon amounts stored in plant biomass of DDF between 2010 and 2017

Plot no.	Dominant species	Carbon in plant biomass (mg/ha)								
		Bark			Stem			Branch		
		2010	2017	net	2010	2017	net	2010	2017	net
1	*S. obtusa*	2.94	3.11	+0.17	29.43	31.15	+1.72	8.28	9.34	+1.06
2	*S. obtusa*	2.67	2.99	+0.32	26.72	29.91	+3.19	7.90	9.49	+1.59
3	*S. obtusa*	2.31	2.63	+0.32	23.12	26.34	+3.22	7.41	8.55	+1.14
4	*S. obtusa*	2.56	3.03	+0.47	25.62	30.22	+4.60	6.74	8.47	+1.72
5	*S. obtusa*	1.70	2.15	+0.44	17.02	21.45	+4.43	4.25	5.60	+1.35
6	*S. obtusa*	2.56	2.79	+0.23	25.60	27.93	+2.33	8.06	8.93	+0.86
7	*S. obtusa*	2.85	3.95	+1.10	28.47	39.42	+10.95	10.96	15.14	+4.18
8	*S. obtusa*	2.01	2.43	+0.43	20.05	24.33	+4.28	5.48	6.74	+1.26
9	*S. obtusa*	2.15	2.29	+0.13	21.53	22.86	+1.34	6.84	7.25	+0.41
10	*S. obtusa*	3.13	3.39	+0.26	31.35	33.92	+2.58	9.59	10.91	+1.32
	Mean	2.49	2.88	+0.39	24.89	28.75	+3.86	7.55	9.04	+1.49
	S.D.	0.42	0.51	0.26	4.23	5.14	2.59	1.82	2.49	0.96

Appendix 10: Carbon amounts stored in plant biomass of DDF between 2010 and 2017 (cont.)

Plot no.	Dominant species	Carbon in plant biomass (mg/ha)								
		Bark			Stem			Branch		
		2010	2017	net	2010	2017	net	2010	2017	net
11	*S.siamensis*	2.83	3.66	+0.84	28.27	36.56	+8.29	7.55	10.46	+2.90
12	*S.siamensis*	2.41	2.68	+0.27	24.11	26.83	+2.72	8.29	9.41	+1.12
	Mean	2.62	3.17	+0.55	26.19	31.70	+5.51	7.92	9.93	+2.01
	Mean	2.51	2.93	+0.42	25.11	29.24	+4.14	7.61	9.19	+1.58
	S.D.	0.40	0.52	+0.27	3.99	5.21	2.70	1.67	2.30	+0.97

Appendix 11: (continued from Appendix 10)

Plot no.	Dominant species	Carbon in plant biomass (mg/ha)								
		Leaf			Root			Total		
		2010	2017	net	2010	2017	net	2010	2017	net
1	*S. obtusa*	1.42	1.32	-0.10	5.96	6.01	+0.05	48.04	50.93	+2.90
2	*S. obtusa*	1.22	1.18	+0.05	5.33	5.65	+0.32	43.84	49.21	+5.38
3	*S. obtusa*	0.94	1.03	+0.08	4.41	4.94	+0.53	38.20	43.50	+5.30
4	*S. obtusa*	1.38	1.46	+0.07	5.42	6.11	+0.69	41.73	49.28	+7.55
5	*S. obtusa*	1.01	1.19	+0.17	3.75	4.59	+0.84	27.74	34.98	+7.24
6	*S. obtusa*	0.97	1.03	+0.05	4.82	5.21	+0.39	42.02	45.89	+3.87
7	*S. obtusa*	0.70	0.96	+0.26	5.79	6.54	+0.75	48.75	66.00	+17.24
8	*S. obtusa*	1.03	1.20	+0.18	4.19	5.00	+0.81	32.76	39.71	+6.95
9	*S. obtusa*	0.88	0.91	+0.04	4.13	4.37	+0.24	35.52	37.68	+2.16
10	*S. obtusa*	1.35	1.33	-0.01	6.13	6.41	+0.28	51.55	55.98	+4.43
	Mean	1.09	1.16	+0.07	4.99	5.48	+0.49	41.01	47.31	+6.30
	S.D.	0.23	0.17	0.10	0.80	0.73	0.26	7.16	8.74	+4.03
11	*S.siamensis*	1.49	1.68	+0.19	5.89	7.28	+1.39	46.03	59.64	+13.61
12	*S.siamensis*	0.74	0.78	+0.04	4.28	4.71	+0.43	39.83	44.42	+4.59
	Mean	1.12	1.23	+0.12	5.09	5.99	+0.91	42.93	52.03	+9.10
	Mean	1.09	1.17	+0.08	5.01	5.57	+0.56	41.33	48.10	+6.77
	S.D.	0.26	0.24	0.10	0.80	0.87	+0.34	6.69	8.74	+4.25

Restoration of Water Storage Potential in a Degrade Dry Dipterocarp Forest by Enrichment Planting of Pine in Huai Hong Khrai Royal Development Study Center, Northern Thailand

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Abstract

This research aims to assess water storage potential in the dry dipterocarp forest (DDF) with enrichment planting of three needle pine (*Pinus kesiya*), and contribution of 34-year-old planted pine to the water storage. Vegetation survey was carried out in the forest using 10 plots, each of size 40 × 40 m^2. Plant data were obtained by measuring stem girths at 1.3 m above ground and heights of all trees with height over 1.5 m. Plant features, standing biomass, and stored amounts of water were measured. Fresh samples of bark, stem, branch and root of common plant species in the forest were taken on sampling days within three seasons. Three soil pits were made in the three selected plots, and soil samples along 100 cm depth were taken in the same days of collecting the plant samples for studing maximum water holding capacity (MWHC) and water storage. The DDF was divided into three stands based on the most dominant tree species, which were *Shorea obtusa*, *Dipterocarous tuberculatus* and *Dipterocarpus obtusifolius*. The DDF was composed of 86 species (69 genera and 36 families) and had the plant biomass at 101.62±27.58 Mg ha^{-1} which contained the average amount of water at 123.24±11.40 m^3 ha^{-1}. The pine contributed to 36.04% of the total biomass. The water amounts stored in plant biomass varied with season: intermediate (127.01 m^3 ha^{-1}) in winter (November 23, 2017), the lowest (104.91 m^3 ha^{-1}) in summber (April 20, 2018), and the highest (137.80 m^3 ha^{-1}) in rainy season (July 18, 2018). The maximum water holding capacity (MWHC) of 100 cm soil was estimated to be 5,089.50±364.68 m^3 ha^{-1}. The amounts of water in the soil in the winter, summer and rainy season were calculated as 3,656.95±474.72 m^3 ha^{-1} (72.17% of MWHC), 3,682.09±264.01 m^3 ha^{-1} (72.36%), and 4,164.99±437.87 m^3 ha^{-1} (81.73%), respectively. After 34 years of enrichment planting of the pine, this species contributed to 32.65% water storage in the forest, and could increase 48.48% water storage in the DDF (if the pine was not planted).

Keywords: Biomass water/ Dry dipterocarp forest/ Plant biomass/ Soil water/ Water storage

1. Introduction

Forest ecosystem can storage water in various components after rainfall. As the rain falls into the forest, a part is intercepted by forest canopy and later lost into the atmosphere through evaporation, and the remains pass the canopy as throughfall (The incident precipitation which falls through the vegetation canopy) and stem flow (The rainfall which reaches the soil by flowing down the stem) to forest floor. Organic layers on the forest floor can absorb a part of water, and the remained amounts are infiltrated into mineral soil and moved as surface runoff to stream water. The water is also lost from forest floor and soil through evaporation. Some water is retained by soil organic matter and particles particularly silt and clay while the excess amount percolates into underground water table and moved out into the streams. Plants usually absorb a large amount of water as well uptake nutrients from soil solution for their physiological processes, growth throughout the life cycle. The functional role of hydrologic cycle in forest ecosystems is important to maintain all organisms including plants, animals and microbes (Landberg and Gower, 1997; Waring and Running, 1998; Kimmins, 2004; Chang, 2006).

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In Thailand, rainfall is only one source of water supply to watersheds and all types of ecosystem, and the forest ecosystem is considered as the most effective ecosystem in hydrologic cycle through many processes as compare to the others. However, different forests have variable roles on the hydrologic cycle. Typical five forests in northern Thailand are classified: (1) the dry dipterocarp forest, (2) mixed deciduous forest, (2) dry evergreen forest, (4) pine forest (pine-dry dipterocarp forest and pine-lower montane forest) and (5) the montane forest (Smitinand et al., 1980, Santisuk, 1988; Khamyong et al., 2004, 2016). These forests have different ecological natures. Most literature studies focus on inputs of precipitation into the forest ecosystems and movement of water through many processes, particularly interception–evaporation by forest canopy, throughfall, stemflow, plant uptake, transpiration, water flow through vegetation, evaporation from soil, infiltration into soil, drainage and runoff, stream flow, etc. However, very few data are available for the water quantity stored in the plant biomass of forests. No data are available for the dry dipterocarp forest. As for the montane forest, Khamyong et al. (2014a) provided the pioneer work on water storage in plants and soils in two community montane forests of Karen tribe in northern Thailand. Khamyong et al. (2014b) and (Summanochitraporn, 2014) examined and evaluated the role of reforestation in watershed hydrology, the water storage potential of a 22-year-old teak and three needle pine plantations in Chiang Rai province, northern Thailand.

This research paper aims to assess the water storage potential of the plants and soils of the dry dipterocarp forest with enrichment planting of three needle pine (*Pinus kesiya*), and to find out the contribution of planted pine on the water storage. The data provide useful information for forest conservation and watershed management.

2. Methodology

2.1 Study area

This research was conducted in the Huai Hong Khrai Royal Development Study (HHKRDS) Center established in 1982 in the Huai Hong Khrai small watershed, Doi Saket district, Chiang Mai province. It is about 27 km to the north of Chiang Mai city, on the road from Chiang Mai province to Chiang Rai province. The establishment of the Center was initiated by the King (Rama 9), and it was meant to be a place of study for the people in the north about integrated watershed management, forestry at the upstream, fishery at the downstream, and agriculture in the in-between areas. The Center covers an area of about 1,360 ha with an altitude range between 350 m and 591 m m.s.l. Many activities of forest and wildlife management, agriculture, and fishery are demonstrated in this Center for the study of officers and people for the extension. Foreign visitors also come here for learning.

There are three seasons in this area: rainy season (May to September), winter (November to February) and summer (March to April). Meteorological data recorded between the years 1985 and 2011 using instruments in the Center report the following: average annual rainfall, 1,328.9 mm; average maximum and minimum air temperatures, 32.2°C and 18.9°C; and average water evaporation, 1,222.6 mm per year (Khamyong et al., 2016). The two deciduous forests including the dry dipterocarp forest and the mixed deciduous forest are distributed in most of the area of the Center. The DDF on volcanic rock covers the front and eastern part of the Center. Two soils are developed on the volcanic rock, the deep soil of Order Oxisols and the shallow soil of Order Entisols, and this research was carried out in the forest on the Oxisols.

2.2 Plant community study

Census of plant species composition, richness, and diversity in the DDF with enrichment planting of pine was taken. Field vegetation survey in the forest was carried out using the method of plant community analysis. The sampling plot was 40 × 40 m^2 in size, and the numbers of 10 plots used, which were arranged randomly in the forest areas based on different topographic conditions including altitude, slope aspect, position and gradient. Stem girths at breast height (gbh, 1.3 m above ground) and tree heights of all species with height over 1.5 m were measured. All plots were located using the GPS. The field plant data were later calculated for species diversity (Krebs, 1985) and forest condition index (FCI) based on an equation studied by Seeloy-ounkeaw et al. (2014).

$$FCI = \sum n_1.10^{-4} + n_2.10^{-3} + n_3.10^{-2} + n_4.10^{-1} + 1(n_5) + 2(n_6) + \ldots\ldots$$

where n_1 = number of tree individuals having gbh <25 cm
n_2 = number of individuals having gbh 26 to <50 cm
n_3 = number of individuals having gbh 51 to <75 cm
n_4 = number of individuals having gbh 76 to <100 cm
n_5 = number of individuals having gbh 101 to <200 cm
n_6 = number of individuals having gbh 201 to <300 cm

2.3 Standing plant biomass

The standing biomass amounts in the stem, branch, leaf, and root organs were calculated using allometric equations studied in the deciduous forests in Thailand by Ogino et al. (1967).

$$W_S = 189\,(D^2 H)^{0.902}$$
$$W_B = 0.125 Ws^{1.204}$$
$$1/W_L = (11.4/Ws^{0.90}) + 0.172$$

where W_S = stem biomass in kilogram
W_B = branch biomass in kilogram
W_L = leaf biomass in kilogram

The unit of stem diameter (D) and tree height (H) was meter. The root biomass was calculated using an equation given by Ogawa et al. (1965), which is $W_R = 0.026\,(D^2 H)^{0.775}$. The units used for this equation were kilogram for root biomass (W_R), centimeter for stem diameter (D), and meter for tree height (H).

2.4 Water storage in plants and soils

2.4.1 Water storage in plant biomass

Samples of fresh bark, stem, branch, and leaf on the standing trees of seven abundant species (*Pinus kesiya*, *Shorea obtusa*, *Dipterocarp obtusifolius*, *Dipterocarp tuberculatus*, *Aporosa villosa*, *Irvingia malayana* and *Wendlandia tinctoria*) in the DDF were taken three times within three seasons: winter (November 23, 2017), summer (April 20, 2018) and rainy season (July 18, 2018). Four stem-gbh classes of <25 cm, 26-50 cm, 51–75 cm, and >76 cm were divided for big tree species, and applied two or three gbh classes for the medium-sized and small tree/shrub species. Three tree individuals of each species were used as the sample trees for each gbh class. The fresh plant samples of 10 to 30 grams were oven dried at 75°C until constant weights were achieved, and later quantified for their water content. The water quantity in the biomass of each species was calculated by multiplying its biomass with the water content of each stem-gbh class. The average water content values of these species were used for calculating the amounts of water in the plant biomass of other species in the forest.

2.4.2 Water storage in soils

The soil (Order Oxisols) derived from the volcanic rock in the DDF was studied by making three pits, 1.5 m × 1 m × 1 m in size, in selected three plots, and soil samples were collected along the depth using a 100 cm^3 corer in three seasons as the same days of collecting the fresh plant samples from eight soil depths with three replications: 0-5, 5-10, 10-20, 20-30, 30-40, 40-60, 60-80 and 80-100 cm. Some physical properties, organic matter content, maximum water holding capacity (MWHC), and field moisture content, on the sampling days were later analyzed in a laboratory.

The MWHC was determined from the field capacity (FC) (Brady and Weil, 2010). Water was added into the soil sample with the 100 cm^3 corer until the soil sample was completely saturated with water, and the water allowed to drain out of the macro pores. Then, the soil samples were oven dried at 105°C within a few days or until they achieved constant weights, and later, their moisture content values were determined. Also, the soil was sent to be field water capacity, and it was later measured for moisture content by volume. The FC was calculated using the equation FC=Vw/Vt, where Vw is the volume of the water and Vt is the total volume of the soil. Finally, the amount of water storage per unit area in each soil layer was determined and the total amount within the soil depth per unit area was calculated.

3. Results

The results of this study include findings on plant community structures, amounts of standing plant biomass, values of water content, and water storage in plant biomass and soils within three seasons of the DDF with enrichment planting of three needle pine.

3.1 Assessment of plant community structures, diversity, and forest conditions

Based on Smitinand (2014), the woody plants sampled within 10 sampling plots, each of size 40 × 40 m^2 (0.16 ha), were identified to be a total of 83 species, 69 genera and 36 families (Table 1). These included 15 big trees, 24 medium-sized trees, 21 small trees, 16 shrubs, 3 climbers and 3 unknown species. The forest was divided into two stands based on the dominant tree species: seven plots of *Dipterocarpus tuberculatus*, two plots of *Dipterocarpus obtusifolius* and one plot of *Shorea obtusa*. The species richness of these stands varied between 22 and 45 species per plot, 36$\pm$7 on average, and had densities of 1,688 to 3,606 trees ha^{-1}. The pine density contributed to 5.53$\pm$3.61% of the total density.

The quantitative characteristics of plant species in the forest are shown in Table 2. Twelve species had the highest frequency value (100%) including *Pinus kesiya, Dipterocarpus tuberculatus, Shorea obtusa, Aporosa villosa, Wendlandia tinctoria, Pterocarpus macrocarpus, Strychnos nux-vomica, Canarium subulatum, Syzygium cumini, Dalbergia oliverli, Gridelia retusa* and *Quercus kerrii*. The dipterocarps of *Dipterocarpus obtusifolius* and *Shorea siamensis* had the values as 70% and 10%, respectively.

Average density of all species over 1.5 m heights was 2,729 trees ha^{-1}. The species with the highest density was *Dipterocarpus tuberculatus* (473 trees ha^{-1}), followed by *Shorea obtusa* (291), *Aporosa villosa* (266), *Dipterocarpus obtusifolius* (201), *Wendlandia tinctoria* (151), *Pinus kesiya* (146), *Gluta usitata* (143), *Symplocos recemosa* (76), *Strychnos nux-vomica* (71), *Canarium subulatum* (60), *Dalbergia cultrata* (54) and *Pterocarpus macrocarpus* (52). These 12 species accounted for 72.70% of the total density.

The dominance of tree species was calculated from the stem basal area by measurement of stem girths at the breast height (gbh). *Pinus kesiya* had the highest dominance (31.81%), followed by *Dipterocarpus tuberculatus* (16.79), *Shorea obtusa* (12.06), *Dipterocarpus obtusifolius* (11.87), *Gluta usitata* (6.34), *Aporosa villosa* (3.52), *Pterocarpus macrocarpus* (2.03), *Semecarpus ancardium* (1.78), *Dalbergia cultrata* (1.54), *Strychnos nux-vomica* (1.27), and *Wendlandia tinctoria* (1.22). These 11 species accounted for 88.20% of the total dominance.

The importance value index (IVI) combines three factors of the relative frequency, relative density and relative dominance into a measure that can be used to imply the ecological influence of each species in the DDF. The species with the highest IVI was *Pinus kesiya* (13.32% of all species), followed by *Dipterocarpus tuberculatus* (12.31), *Shorea obtusa* (8.51), *Dipterocarpus obtusifolius* (7.07), *Aporosa villosa* (5.36), *Gluta usitata* (4.70), *Wendlandia tinctoria* (3.19), *Pterocarpus macrocarpus* (2.25), *Strychnos nux-vomica* (2.23), *Symplocos recemosa* (2.03), *Dalbergia cultrata* (1.93) and *Canarium subulatum* (1.79). These 12 species accounted for 64.69% of the total IVI value.

As shown in Table 3, the values of the Shannon-Wiener Index (SWI) as indicating plant species diversity were different among plots, 3.49 to 4.31 (3.87$\pm$0.24 on average), while the forest condition index (FCI) values were measured to be a range of 1.03 to 10.98 (6.18$\pm$3.24 on average). If the pine trees were not planted in the forest, the values would decrease to 3.79$\pm$0.25 for the SWI, and 2.70$\pm$2.24 for the FCI.

3.2 Growth and population of pine

Within 10 plots, a total of 233 individuals of pine was found. The biggest pine had the stem-gbh of 129.5 cm with 25.5 m height while the values as 10.9 cm and 4.1 m were belong to the smallest tree. The number of trees in the gbh classes of <25, 26-50, 51-75 and >75 cm was 5, 26, 97 and 105 trees, respectively, whereas the height classes of <5, 6-10, 11-15, 16-20 and >20 m had the number of 2, 19, 96, 89 and 27 trees. The average values of gbh and height were 72$\pm$22.02 cm (30.58% coefficient of variance, C.V.) and 15.1$\pm$3.97 (26.29%), respectively. The seedlings of pine did not observe in the forest. Thus, the annual growth rates of pine varied from 0.5 to 6 mm with the mean value of 3.4 mm. The variable growth might be caused by competition with other tree species and the influences of site factors.

Table 1. A species list of tree species in the DDF with planted pine

Family	Scientific name	Growth form
1. Acanthaceae	1. *Justica modesta* (Bremek.) V.A.W. Graham	shrub
2. Anacardiaceae	2. *Buchanania lanzan* Spreng.	big tree
	3. *Gluta usitata* (Wall.) Ding Hou	big tree
	4. *Lannea coromandelica* (Houtt.) Merr.	big tree
	5. *Semecarpus anacardium* Linn.f.	medium tree
3. Apocynaceae	6.*Aganosma marginata* (Roxb.) G.Don	climber
4. Bignoniaceae	7. *Markhamia stipulata* (Wall.) Seem.	medium tree
	8. *Heteropanax sulfureum* Kurz.	small tree
	9. *Stereospermum cylindricum* Pierre ex Dop.	medium tree
	10. *Stereospermum neuranthum* Kurz	medium tree
5. Burseraceae	11. *Canarium subulatum* Guillaumin	big tree
6. Celastraceae	12. *Celastrus paniculata* Willd.	climber
7. Clusiaceae	13. *Garcinia cowa* Roxb. ex Choisy	small tree
8. Combretaceae	14. *Terminalia alata* Heyne ex Roth	big tree
	15. *Terminalia chebula* Retz. var. *chebula*	medium tree
9. Chrysobalanaceae	16. *Parinari anamensis* Hance	medium tree
10. Dilleniaceae	17. *Dillenia obovata* (Blume) Hoogland	small tree
11. Dipterocarpaceae	18. *Dipterocarpus obtusifolius* Teijsm. ex Miq.	big tree
	19. *Dipterocarpus tuberculatus* Roxb.	big tree
	20. *Shorea siamensis* Miq.	big tree
	21. *Shorea obtusa* Wall. ex Blume	big tree
12. Ebenaceae	22. *Diospiros ehretioides* Wall. ex G. Don	small tree
13. Ericaceae	23. *Craibiodendron stellatum* (Pierre) W. W. Sm.	small tree
14. Fabaceae	24. *Acacia catechu* (L.f.) Willd.	medium tree
	25. *Albizia odoratissima* (L.f.) Benth.	medium tree
	26. *Albizia chinensis* (Osbeak) Merr.	big tree
	27. *Butea superba* Roxb.	climber
	28. *Dalbergia cultrata* Graham ex Benth	big tree
	29. *Dalbergia dongnaiensis* Pierre	medium tree
	30. *Dallbergia oliveri* Gamble	medium tree
	31. *Dallbergia velutina* Benth.	climber
	32. *Indigofera sootepensis* Craib	shrub
	33. *Leucaena leucocephala* (Lam.) de Wit	small tree
	34. *Millettia xylocarpa* Miq.	medium tree
	35. *Millettia extensa* (Benth.) Baker	medium tree
	36. *Peltophorum pterocarpum* (DC.) Backer ex K. 52. 36.Heyne	medium tree
	37. *Pterocarpus macrocarpus* Kurz	big tree
	38. *Spatholobus parviflorus* (DC.) Kuntze	climber
	39. *Xylia xylocarpa* Taub. Var. *kerrii* Nielsen	big tree
15. Fagaceae	40. *Quercus kerrii* Craib.	medium tree
	41. *Lithocarpus elegans* (Blume) Hatus. ex Soepadmo	medium tree
16. Hypericaceae	42. *Cratoxylum formosum* Byer subsp. *pruniflorum* Gogel.	small tree
17. Irvingiaceae	43. *Irvingia malayana* Oliv. ex A. W. Benn.	big tree
18. Lamiaceae	44. *Vitex peduncularis* Wall. ex Schauer	medium tree
19. Lauraceae	45. *Litsea glutinosa* (Lour.) C.B. Rob.	medium tree
	46. *Phoebe lanceolata* (Nees) Nees	medium tree

Table 1. A species list of tree species in the DDF with planted pine (cont.)

Family	Scientific name	Growth form
	47. *Colona flagrocarpa* (C.B. Clarke)	small tree
	48. *Decaschistia siamensis* Craib	shrub
	49. *Sterculia balanghas* L.	shrub
20. Melastomataceae	50. *Memecylon plebejum* Kurz var. *plebejum*	shrub
21. Meliaceae	51. *Chukrasia tabularis* A. Juss.	medium tree
22. Moraceae	52. *Ficus* sp.	medium tree
23. Myrtaceae	53. *Eucalyptus camaldulensis* (Dehnh.	medium tree
	54. *Syzygium albiflorum* (Duthie & Kurz) Bahadur & R.C.	medium tree
	55. *Syzygium cumini* (L.) Skeels	small tree
24. Ochnaceae	56. *Ochna intergerrima* (Lour.) Merr.	small tree
25. Oleaceae	57. *Chionanthus ramiflorus* Roxb.	small tree
	58. *Olea salicifolia* Wall. Ex G. Don	small tree
26. Opiliaceae	59. *Meilentha suavis* Pierre	small tree
27. Pentaphylacaceae	60.*Anneslea fragrans* Wall.	small tree
28. Phyllanthaceae	61. *Antidesma acidum* Retz.	shrub
	62. *Antidesma ghaesembilla* Gaertn.	shrub
	63. *Antidesma sootepene* Craib	shrub
	64. *Aporosa villosa* (Wall. ex Lindl.) Baill.	shrub
	65. *Bridelia retusa* (L.) A. Juss.	medium tree
	66. *Glochidion zeylanicum* (Gaaertn.) A. Juss.	shrub
	67. *Phyllanthus emblica* L.	small tree
29. Pinaceae	68. *Pinus merkusii* Jungh. & de Vriese	big tree
30. Rhamnaceae	69. *Zizyphus rugosa* Ram.	climber
31. Rubiaceae	71. *Catunaregam spathulifolia* Tirveng.	shrub
	72. *Gardenia sootepensis* Hutch	small tree
	73. *Gardenia obtusifolia* Roxb. ex Gordon	shrub
	74. *Haldina cordifolia* Ridsd.	medium tree
	75. *Ixora cibdela* Craib	shrub
	76. *Morinda coreia* Ham.	small tree
	77. *Pavetta indica* RL.	shrub
	78. *Wendlandia tinctoria* (Roxb.) DC.	small tree
	79. *Vangueria pubescens* DC.	shrub
32. Saliciaceae	80. *Casearis gallifera* Tathana	small tree
34. Symplocaceae	81. *Symplocos recemosa* Roxb.	small tree
35. Strychnaceae	82. *Strychnos nux-vomica* L.	shrub
36. Ulmaceae	83.*Ulmus lancaefolia* Roxb. ex Wall.	small tree
Unknown	84. Climber -1	climber
Unknown	85. Climber -2	climber
Unknown	86. Climber -3	climber

Table 2. Quantitative characteristics of tree species in the DDF with planted pine

No.	Plant name	Frequency (%)	Density (trees ha^{-1})	Relative value (%)			IVI	
				Freq.	Dens.	Domi.	(300)	%
1	*P. kesiya*	100	145.63	2.82	5.34	31.81	39.96	13.32
2	*D. tuberculatus*	100	472.50	2.82	17.32	16.79	36.92	12.31
3	*S. obtusa*	100	290.63	2.82	10.65	12.06	25.53	8.51
4	*D. obtusifolius*	70	201.25	1.97	7.38	11.87	21.22	7.07
5	*A. villosa*	100	265.63	2.82	9.73	3.52	16.07	5.36
6	*G. usitata*	90	142.50	2.54	5.22	6.34	14.10	4.70
7	*W. tinctorial*	100	150.63	2.82	5.52	1.22	9.56	3.19
8	*P. macrocarpus*	100	51.88	2.82	1.90	2.03	6.75	2.25
9	*S. nux-vomica*	100	70.63	2.82	2.59	1.27	6.68	2.23
10	*S. recemosa*	90	75.63	2.54	2.77	0.79	6.09	2.03
11	*D. cultrata*	80	54.38	2.25	1.99	1.54	5.78	1.93
12	*C. subulatum*	100	60.00	2.82	2.20	0.34	5.36	1.79
13	*S. cumini*	100	41.88	2.82	1.53	0.82	5.17	1.72
14	*S. nacardium*	70	37.50	1.97	1.37	1.78	5.12	1.71
15	*D. oliverli*	100	23.13	2.82	0.85	0.90	4.56	1.52
16	*B. retusa*	100	33.75	2.82	1.24	0.29	4.34	1.45
17	*S. parviflorus*	90	31.25	2.54	1.15	0.55	4.23	1.41
18	*I. malayana*	40	51.25	1.13	1.88	0.99	4.00	1.33
19	*C. formosum*	80	43.13	2.25	1.58	0.13	3.96	1.32
20	*A. fragrans*	90	30.00	2.54	1.10	0.31	3.94	1.31
21	*Q. kerrii*	100	16.88	2.82	0.62	0.47	3.90	1.30
22	*D. obovata*	60	43.75	1.69	1.60	0.55	3.84	1.28
23	*A. odoratissima*	80	22.50	2.25	0.82	0.42	3.50	1.17
24	*B. lanzan*	90	12.50	2.54	0.46	0.25	3.24	1.08
25	*C. ramiflorus*	70	26.88	1.97	0.98	0.14	3.10	1.03
26	*Z. regosa*	70	18.75	1.97	0.69	0.12	2.78	0.93
27	*M. scutellatum*	40	38.75	1.13	1.42	0.20	2.74	0.91
28	*V. peduncularis*	70	15.63	1.97	0.57	0.04	2.58	0.86
29	*C. apathulofolia*	70	11.88	1.97	0.44	0.07	2.48	0.83
30	*S. siamensis*	10	41.88	0.28	1.53	0.66	2.47	0.82
31	*G. sootepensis*	60	15.63	1.69	0.57	0.16	2.42	0.81
32	*P. emblica*	70	9.38	1.97	0.34	0.05	2.37	0.79
33	*U. lancaefolia*	60	8.75	1.69	0.32	0.03	2.04	0.68
34	*P. pterocarpum*	40	13.13	1.13	0.48	0.16	1.77	0.59
35	*D. ehretioides*	40	6.88	1.13	0.25	0.06	1.44	0.48
36	*X. xylocarpa*	20	14.38	0.56	0.53	0.32	1.41	0.47
37	*P. lanceolata*	30	11.88	0.85	0.44	0.01	1.29	0.43
38	*T. chebula*	40	3.13	1.13	0.11	0.01	1.25	0.42
39	*G. zeylanicum*	30	7.50	0.85	0.27	0.05	1.16	0.39
40	*G. cowa*	20	14.38	0.56	0.53	0.06	1.15	0.38
41	*M. coreia*	30	3.75	0.85	0.14	0.05	1.03	0.34
42	*L. glutinosa*	30	4.38	0.85	0.16	0.02	1.03	0.34
43	*S. neuranthum*	30	4.38	0.85	0.16	0.02	1.02	0.34
44	*A. chinnensis*	30	2.50	0.85	0.09	0.05	0.98	0.33
45	*C. stellatum*	10	15.00	0.28	0.55	0.12	0.95	0.32

Table 2. Quantitative characteristics of tree species in the DDF with planted pine (cont.)

No.	Plant name	Frequency (%)	Density (trees ha^{-1})	Relative value (%)			IVI	
				Freq.	Dens.	Domi.	(300)	%
46	*P. anamensis*	20	6.25	0.56	0.23	0.05	0.84	0.28
47	*O. salicifolia*	20	2.50	0.56	0.09	0.08	0.73	0.24
48	*O. intergerrima*	20	4.38	0.56	0.16	0.00	0.73	0.24
49	*D. dongnaiensis*	20	2.50	0.56	0.09	0.04	0.70	0.23
50	*C. tabularis*	20	2.50	0.56	0.09	0.01	0.66	0.22
51	*G obtusifolia*	20	2.50	0.56	0.09	0.01	0.66	0.22
52	*D. velutina*	20	1.88	0.56	0.07	0.03	0.66	0.22
53	*C. gallifera*	20	1.88	0.56	0.07	0.03	0.66	0.22
54	*C. paniculata*	20	1.88	0.56	0.07	0.01	0.64	0.21
55	*A. marginata*	20	1.88	0.56	0.07	0.01	0.64	0.21
56	*A. ghaesembilla*	20	1.88	0.56	0.07	0.00	0.63	0.21
57	*H. cordifolia*	20	1.25	0.56	0.05	0.02	0.63	0.21
58	*A. catechu*	20	1.25	0.56	0.05	0.01	0.62	0.21
59	*S. albiflora*	20	1.25	0.56	0.05	0.01	0.61	0.20
60	*E. camaldulensis*	10	6.88	0.28	0.25	0.01	0.55	0.18
61	*M. stipulate*	10	4.38	0.28	0.16	0.00	0.45	0.15
62	*H. sulfureum*	10	2.50	0.28	0.09	0.07	0.44	0.15
63	*T. alata*	10	2.50	0.28	0.09	0.03	0.41	0.14
64	*M. extensa*	10	1.25	0.28	0.05	0.05	0.38	0.13
65	*I. cibdela*	10	2.50	0.28	0.09	0.00	0.38	0.13
66	*S. cylindricum*	10	1.88	0.28	0.07	0.01	0.36	0.12
67	*B. superba*	10	0.63	0.28	0.02	0.04	0.35	0.12
68	*V. pubescens*	10	1.25	0.28	0.05	0.01	0.34	0.11
69	*L. coromandelica*	10	1.25	0.28	0.05	0.00	0.33	0.11
70	*S. balanghas*	10	1.25	0.28	0.05	0.00	0.33	0.11
71	*M. suavis*	10	1.25	0.28	0.05	0.00	0.33	0.11
72	*D. siamensis*	10	1.25	0.28	0.05	0.00	0.33	0.11
73	*C. flagrocarpa*	10	0.63	0.28	0.02	0.01	0.31	0.10
74	Climber-1	10	0.63	0.28	0.02	0.01	0.31	0.10
75	*P. indica*	10	0.63	0.28	0.02	0.00	0.31	0.10
76	*L. leucocephala*	10	0.63	0.28	0.02	0.00	0.31	0.10
77	*L. elegans*	10	0.63	0.28	0.02	0.00	0.31	0.10
78	*Ficus sp.*	10	0.63	0.28	0.02	0.00	0.31	0.10
79	*M. xylocarpa*	10	0.63	0.28	0.02	0.00	0.31	0.10
80	Climber-2	10	0.63	0.28	0.02	0.00	0.31	0.10
81	Climber-3	10	0.63	0.28	0.02	0.00	0.31	0.10
82	*A. incidum*	10	0.63	0.28	0.02	0.00	0.31	0.10
83	*B. mollis*	10	0.63	0.28	0.02	0.00	0.31	0.10
84	*I. sootepensis*	10	0.63	0.28	0.02	0.00	0.30	0.10
85	*J. modesta*	10	0.63	0.28	0.02	0.00	0.30	0.10
86	*A. chinensis*	10	0.63	0.28	0.02	0.00	0.30	0.10
	Total	3,550	2,729	100	100	100	300	100

Table 3. Plant communities within 10 sampling plots in the DDF with planted pine

Plot no.	Dominant species	Species richness	Density (ha^{-1})	Pine density (ha^{-1})	Pine density %	SWI A	SWI B	FCI A	FCI B
1	*D. tuberculatus*	45	3,356	175	5.21	4.319	4.239	7.72	3.79
2	*D. tuberculatus*	34	3,606	106	2.94	3.664	3.575	10.98	2.45
3	*D. tuberculatus*	30	1,788	56	3.14	3.902	3.812	6.74	1.52
4	*D. tuberculatus*	22	1,688	69	4.07	3.491	3.381	3.23	0.51
5	*S. obtusa*	40	3,594	219	6.08	4.047	3.957	9.53	6.17
6	*D. obtusifolius*	40	3,231	163	5.02	3.708	3.5982	9.49	6.82
7	*D. tuberculatus*	31	1,856	263	14.14	3.857	3.8147	5.12	2.47
8	*D. obtusifolius*	37	2,813	69	2.44	3.888	3.8178	1.03	0.78
9	*D. tuberculatus*	38	2,817	256	9.09	4.124	4.0574	3.56	0.74
10	*D. tuberculatus*	38	2,538	81	3.20	3.767	3.6775	4.39	1.75
Mean		36	2,729	146	5.53	3.870	3.790	6.18	2.70
S.D.		7	741	80	3.61	0.24	0.254	3.24	2.24
C.V. (%)		19.44	27.17	55.080	65.1680	6.180	6.580	52.40	82.80

Remark: A = DDF with planted pine, B = DDF without planted pine

3.3 Amount of standing plant biomass

Table 4 shows the amounts of standing plant biomass within 10 plots of two stands in the DDF with planted pine. The average amount of biomass in the 10 plots was measured as 101.62±27.58 Mg ha^{-1}, divided into bark, stem, branch, leaf, and root organs at 1.72 (1.69%), 64.07 (63.04%), 17.89 (17.60%), 2.34 (2.30%) and 15.59 (15.37%) Mg ha^{-1}, respectively. The biomass amounts in these stands varied in range of 58.73 to 105.54 Mg ha^{-1}.

Among 86 species (Table 5), the biomass amount of pine was the highest, 36.79 Mg ha^{-1} or 36.20% of the total biomass in the forest. The tree species with the lower amounts were in the following order: *Dipterocarpus tuberculatus* (15.97 Mg ha^{-1}), *Dipterocarpus obtusifolius* (12.47), *Shorea obtusa* (12.19), *Gluta usitata* (6.59), *Ptercarpus macrocarpus* (2.45), *Aporosa villosa* (1.90), *Semecarpus anacardium* (1.51), *Dalbergia cultrata* (1.40), *Irvingia malayana* (1.08), etc. These 10 species accounted to 92.35 Mg ha^{-1} or 90.87% of the total biomass. Therefore, the enrichment planting of this pine could increase a large amount of the plant biomass in the degrade DDF.

Table 4. Amount of plant biomass within 10 sampling plots in the DDF with planted pine

No.	Biomass ($kg\ ha^{-1}$) Bark	Stem	Branch	Leaf	Root	Total
1	1,949.54	74,214.80	20,944.33	2,666.43	17,998.62	117,773.71
2	1,645.19	64,094.77	16,877.81	2,576.49	16,326.56	101,520.82
3	1,583.64	58,994.80	19,871.46	1,931.10	13,105.68	95,486.67
4	1,049.69	37,329.26	9,221.41	1,588.28	9,541.67	58,730.31
5	2,835.99	92,935.35	27,796.45	3,071.92	21,787.17	148,426.88
6	1,879.68	86,803.07	26,453.03	2,698.91	19,853.49	137,688.18
7	1,527.66	67,398.09	16,308.99	2,582.23	17,725.98	105,542.94
8	1,306.50	45,122.01	12,323.41	1,809.38	10,702.61	71,263.90
9	1,574.72	57,910.82	13,570.53	2,374.81	15,446.83	90,877.71
10	1,857.48	55,898.94	15,567.37	2,137.10	13,455.26	88,916.14
Mean	1,721.01	64,070.19	17,893.48	2,343.66	15,594.38	101,622.73
S.D.	476.69	17,219.33	5,948.17	464.49	3,925.79	27,575.27
C.V.	27.70	26.88	33.24	19.82	25.17	27.13

Table 5. Amounts of standing plant biomass of tree species in the DDF with planted pine

No.	Name	Biomass (kg ha^{-1})					
		Bark	Stem	Branch	Leaf	Root	Total
1	*P. kesiya*	241.37	23,895.62	4,502.90	947.83	7,199.13	36,786.85
2	*D. tuberculatus*	368.41	9,832.66	3,398.81	341.96	2,030.21	15,972.05
3	*D. obtusifolius*	183.35	7,851.06	2,688.82	226.33	1,524.52	12,474.08
4	*S. obtusa*	402.75	7,424.84	2,600.52	240.62	1,525.98	12,194.71
5	*G. usitata*	109.51	4,082.36	1,541.85	97.55	759.19	6,590.47
6	*P. macroocarpus*	42.45	1,509.70	579.10	36.21	278.45	2,445.90
7	*A. villosa*	29.09	1,202.46	272.04	69.63	316.51	1,889.74
8	*S. anacardium*	32.01	941.09	298.34	34.88	199.55	1,505.87
9	*D. cultrata*	30.62	874.22	266.58	33.91	190.29	1,395.63
10	*I. malayana*	19.85	671.03	233.39	19.18	132.62	1,076.07
11	*S. nux-vomica*	27.68	597.35	144.45	33.28	152.74	955.50
12	*D. oliverli*	28.92	530.17	163.24	20.47	115.93	858.73
13	*W. tinctoria*	21.44	488.28	95.91	33.70	143.43	782.77
14	*S. parviflorus*	21.51	487.72	136.56	21.73	111.86	779.37
15	*S. cumini*	18.02	439.09	122.12	19.91	102.18	701.32
16	*D. obovata*	11.28	360.33	129.59	10.58	72.83	584.61
17	*S. recemosa*	16.45	347.16	78.59	21.00	93.72	556.93
18	S. siamensis	14.45	328.26	83.48	17.09	81.50	524.77
19	*Q. kerrii*	10.05	259.83	73.36	11.23	58.61	413.08
20	*A. odoratissima*	8.18	254.46	79.70	9.09	54.11	405.54
21	*A. fragrans*	6.78	157.57	42.93	7.50	38.27	253.06
22	*X. xylocarpa*	9.00	156.20	37.34	9.01	40.59	252.14
23	*B. lanzan*	4.42	152.43	48.42	5.24	31.95	242.46
24	*C. subulatum*	6.89	145.54	33.25	8.76	39.90	234.35
25	*B. pierrei*	6.32	124.19	27.28	7.81	34.17	199.77
26	*M. scutellatum*	-	79.36	12.26	6.08	25.32	123.02
27	*P. pterocarpum*	3.47	75.09	17.81	4.28	19.44	120.09
28	*G. sootepensis*	3.52	62.95	12.81	4.30	18.26	101.84
29	*C. ramiflorus*	2.95	58.90	12.49	3.85	16.80	94.99
30	*C. formosum*	2.76	54.08	11.51	3.55	15.94	87.84
31	*C. stellatum*	2.73	50.55	11.14	3.19	14.09	81.69
32	*Z. rugosa*	2.65	49.20	10.13	3.32	14.29	79.60
33	*M. extensa*	5.14	46.25	14.10	2.07	10.87	78.44
34	*O. salicifolia*	1.52	47.87	14.74	1.67	9.92	75.73
35	*B. superba*	4.88	43.92	14.84	1.57	9.57	74.79
36	*D. serrulata*	1.57	35.57	9.90	1.59	8.16	56.78
37	*C. spathulifolia*	1.48	32.20	8.12	1.70	8.21	51.71
38	*D. velutina*	1.13	26.98	7.54	1.18	6.05	42.89
39	*A. lebbeks*	1.10	26.36	7.38	1.15	5.89	41.89
40	*D. ehretioides*	1.25	24.37	4.75	1.72	7.21	39.29
41	*P. emblica*	1.23	21.96	4.27	1.56	6.59	35.61
42	*G. cowa*	1.34	20.93	3.50	1.69	7.04	34.50
43	*M. coreia*	1.12	21.26	4.51	1.38	5.91	34.18
44	*D. dongnaiensis*	0.87	20.16	5.13	1.04	4.89	32.09
45	*G. zeylanicum*	0.92	18.05	3.62	1.24	5.32	29.15

Table 5. Amounts of standing plant biomass of tree species in the DDF with planted pine (cont.)

No.	Name	Biomass (kg ha^{-1})					
		Bark	Stem	Branch	Leaf	Root	Total
46	*P. anamensis*	0.94	17.25	3.17	1.28	5.35	28.00
47	*V. peduncularis*	0.85	13.30	2.10	1.12	4.70	22.07
48	*T. alata*	0.75	11.72	2.24	0.85	3.54	19.09
49	*C. gallifera*	0.51	11.82	2.69	0.70	3.10	18.82
50	*U. lancaefolia*	0.70	10.98	1.69	0.93	3.86	18.16
51	M. brunosis	0.64	10.09	2.08	0.69	2.87	16.37
52	*L. gutinosa*	0.51	8.07	1.35	0.65	2.68	13.26
53	*S. neuranthum*	0.41	6.36	1.01	0.53	2.20	10.51
54	*A. catechu*	0.34	5.27	1.03	0.38	1.57	8.58
55	*C. paniculata*	0.32	5.09	0.93	0.38	1.59	8.32
56	*E. camaldulensis*	0.28	4.40	0.60	0.40	1.70	7.38
57	*T. chebula*	0.25	3.93	0.64	0.32	1.35	6.49
58	*C. flagrocarpa*	0.24	3.72	0.72	0.27	1.11	6.05
59	*C. tabularis*	0.19	3.00	0.49	0.25	1.02	4.95
60	Climber-1	0.19	2.90	0.53	0.22	0.89	4.73
61	*V. pubescens*	0.17	2.71	0.47	0.21	0.87	4.44
62	*G. obtusifolia*	0.15	2.33	0.34	0.20	0.86	3.88
63	*A. marginata*	0.14	2.19	0.33	0.19	0.78	3.63
64	*S. albiflora*	0.12	1.83	0.27	0.16	0.65	3.03
65	*S. cylindricum*	0.11	1.73	0.23	0.16	0.66	2.89
66	*P. lanceolata*	0.10	1.54	0.14	0.17	0.76	2.72
67	*I. cibdela*	0.09	1.44	0.20	0.13	0.55	2.42
68	*M. stipulata*	0.09	1.38	0.15	0.14	0.61	2.37
69	*O. intergerrima*	0.06	0.92	0.09	0.10	0.43	1.60
70	*P. indica*	0.06	0.94	0.14	0.08	0.34	1.55
71	*A. ghaessembilla*	0.05	0.77	0.09	0.08	0.33	1.31
72	*L. leucocephala*	0.05	0.76	0.11	0.07	0.28	1.26
73	*L.coromandelica*	0.04	0.60	0.07	0.06	0.25	1.02
74	*Q. elegans*	0.04	0.60	0.08	0.05	0.23	1.00
75	*Ficus* sp.	0.03	0.53	0.07	0.05	0.21	0.89
76	*D. cana*	0.03	0.46	0.06	0.04	0.18	0.78
77	Climber-2	0.03	0.45	0.06	0.04	0.18	0.76
78	Climber-3	0.03	0.45	0.06	0.04	0.18	0.76
79	*A. acidum*	0.02	0.25	0.03	0.03	0.11	0.43
80	*S. balanghas*	0.01	0.17	0.02	0.02	0.08	0.30
81	*M. suavis*	0.01	0.17	0.01	0.02	0.08	0.29
82	*D. siamensis*	0.01	0.14	0.01	0.02	0.07	0.26
83	*B. mollis*	0.01	0.14	0.01	0.01	0.06	0.24
84	*I. sootepensis*	0.00	0.06	0.01	0.01	0.03	0.11
85	*A. acidum*	0.00	0.04	0.00	0.01	0.02	0.08
86	*J. modesta*	0.00	0.04	0.00	0.01	0.02	0.08
Total		1,721	64,070	17,894	2,344	15,594	101,623

3.4 Water storage in plants and soils of DDF with planted pine

3.4.1 Amount of water stored in plant biomass

Data regarding the water contents (% by fresh weight) in the different organs of the eight dominant tree species in the DDF were given in Table 6 (Figure 1 and 2). The water content in the root used the average values of the water content in the stem and the branch because these organs are composed of woody tissues as roots. The water content by fresh weight of these species was varied among species, sampling times and the stem-gbh classes.

The sample trees of pine, *Pinus kesiya*, were divided into two gbh classes, 50-75 cm and 76-100 cm. The average values of water content in bark, stem, branch, leaf and root of trees with the gbh class of 50-75 cm class were 54.81%, 38.17%, 57.33%, 61.74% and 42.74%, respectively. The higher contents were found in those trees with the gbh class of 76-100 cm. The leaf and bark had the highest contents, intermediate for branch and root, and they were the lowest for stem.

Three gbh classes of *Shorea obtusa* were used. The water contents in bark were lower than pine, varied from 47.45% to 49.28%. In stem, they were lower than other organs, and decreased from small trees to bigger trees, 39.05% to 43.29%. The highest values were observed for leaf with decreasing from the small to biger trees (54.91% to 60.64%), and intermediate for branch and root.

There were four gbh classes for *Dipterocarpus obtusifolius*. Their bark contained the variable water contents, 50.18% to 57.36%. In stem, the values were the lowest, 35.70% to 49.34%, whereas the branch and leaf contained the highest contents. The water contents in bark, stem and branch were decreased from the small to bigger trees. They were intermediate for the root.

Dipterocarpus tuberculatus had four gbh classes. The bark contained the contents in a range of 56.37% to 61.96%. In stem, the values were the lowest and decreased from the small to bigger trees, 37.24% to 45.39%, while the leaf contained the highest contents, 65.26% to 67.02%. They were intermediate for branch and root.

Aporosa villosa is a shrub, and two gbh classes were used. The water contents in bark were relatively low, 40.0% and 42.02%. In stem, the values were higher than bark, 50.62% and 51.37%. The highest values were observed in leaf, and lower in branch and root.

The four gbh classes were used for a big tree, *Irvingia malayana*. The bark contained the contents in a range of 49.65% to 61.22%. In stem, the values were the lowest with no clear different among the gbh classes, 35.4% to 39.89%, while the leaf contained the highest values. *Wendlandia tinctoria* is also a shrub, and two gbh classes were used. The water contents in bark were 50.42% and 50.67%. In stem, the values were 46.78% and 50.14%. The highest values were observed in leaf, and lower in branch and root.

In Table 7, the data regarding the water content (% by dry weight) in these eight abundant species are shown. These data were used for the calculation of the amount of water in the standing plant biomass of the dipterocarps. The mean values of the water content in the bark, stem, branch, leaf, and root were used for the estimation of the amount of water in the plant biomass of the other species.

Table 6. Water content (% fresh weight) in organs of dominant species in different stem-gbh classes in the DDF with planted pine

Plant species	Stem-gbh class	Sampling time	Water content (% by fresh weight)				
			Bark	Stem	Branch	Leaf	Root
1. *P. kesiya*		November 2017	50.38	35.77	55.70	59.69	45.73
	50-75	April 2018	58.37	36.98	52.82	61.42	44.90
		July 2018	55.67	41.78	63.45	64.09	52.62
		Mean	54.81	38.17	57.33	61.74	47.75
		November	59.21	42.90	59.33	61.89	51.11
	75-100	April	65.85	33.72	55.36	61.20	44.54
		July	64.62	49.30	63.79	64.95	56.54
		Mean	63.23	41.97	59.49	62.68	50.73

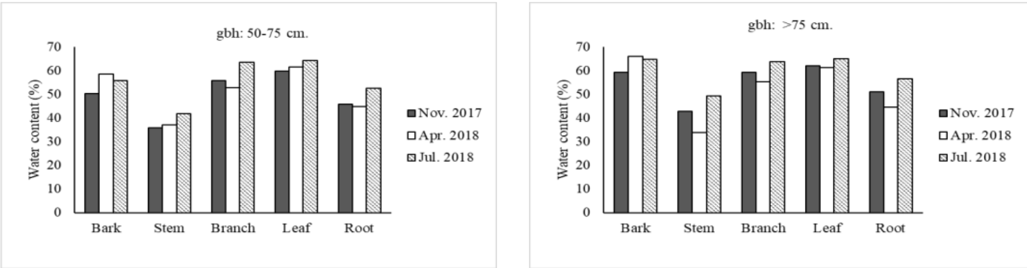
Table 6. Water content (% fresh weight) in organs of dominant species in different stem-gbh classes in the DDF with planted pine (cont.)

Plant species	Stem-gbh class	Sampling time	Water content (% by fresh weight)				
			Bark	Stem	Branch	Leaf	Root
2. *S. obtusa*		November	44.13	32.99	38.13	53.20	35.56
	<25	April	47.86	45.56	52.53	64.33	49.04
		July	50.36	51.31	43.27	64.40	47.29
		Mean	47.45	43.29	44.64	60.64	43.96
		November	49.13	31.25	42.60	51.06	36.93
	25-50	April	46.75	40.20	46.14	61.03	43.17
		July	51.94	45.26	55.72	62.85	50.49
		Mean	49.28	38.90	48.16	58.31	43.53
		November	48.62	29.51	41.81	51.37	35.66
	75-100	April	47.57	47.64	48.91	57.74	48.28
		July	46.49	40.01	53.65	55.63	46.83
		Mean	47.5686	39.05	48.12	54.9193	43.616
3. *D. obtusifolius*		November 2017	64.33	49.22	59.86	59.53	54.54
	<25	April 2018	47.33	45.54	58.01	56.63	51.77
		July 2018	60.42	53.26	67.50	63.62	60.38
		Mean	57.36	49.34	61.79	59.93	55.57
		November	54.49	43.40	60.09	59.24	51.74
	25-50	April	48.55	29.50	60.58	53.29	45.04
		July	58.01	44.60	65.53	63.98	55.06
		Mean	53.68	39.16	62.06	58.84	50.61
		November	48.47	37.67	52.93	57.00	45.30
	50-75	April	49.55	36.92	59.01	58.74	47.96
		July	52.52	44.40	60.42	63.76	52.41
		Mean	50.18	39.66	57.45	59.83	48.56
		November	58.30	28.29	59.26	60.46	43.77
	75-100	April	44.27	35.90	55.11	58.46	45.50
		July	48.31	42.91	60.41	65.25	51.66
		Mean	50.29	35.70	58.26	61.39	46.98
4. *D. tuberculatus*		November	57.17	36.27	35.95	56.22	36.11
	<25	April	52.06	45.77	64.14	69.43	54.95
		July	67.26	54.13	73.58	71.25	63.86
		Mean	58.83	45.39	57.89	65.63	51.64
		November	65.53	36.80	58.80	62.07	47.80
	25-50	April	54.71	41.42	63.09	67.21	52.26
		July	65.65	49.13	68.11	71.77	58.62
		Mean	61.96	42.45	63.33	67.02	52.89
		November	55.58	30.37	53.56	57.58	41.97
	50-75	April	59.92	45.41	65.28	69.14	55.34
		July	66.38	45.32	71.20	69.07	58.26
		Mean	60.62	40.37	63.34	65.26	51.86
		November	58.19	27.89	50.50	55.51	39.20
	75-100	April	52.03	39.87	57.68	68.02	48.77
		July	58.87	44.00	70.13	74.42	57.06

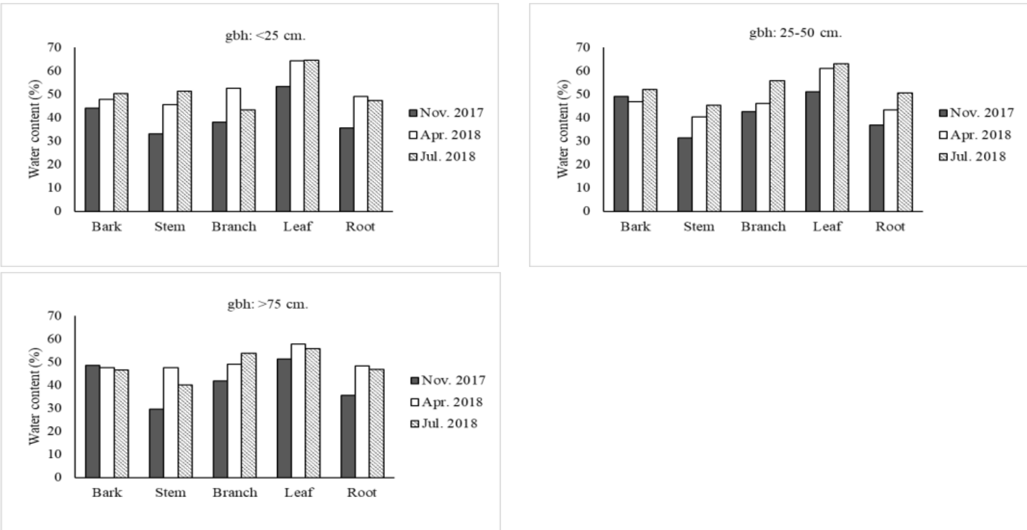
Table 6. Water content (% fresh weight) in organs of dominant species in different stem-gbh classes in the DDF with planted pine (cont.)

Plant species	Stem-gbh class	Sampling time	Water content (% by fresh weight)				
			Bark	Stem	Branch	Leaf	Root
		Mean	56.37	37.25	59.44	65.98	48.34
5. *A. villosa*		November	39.86	49.65	65.96	71.16	57.81
	<25	April	43.73	48.52	59.50	70.20	54.01
		July	42.47	53.69	55.77	85.06	54.73
		Mean	42.02	50.62	60.41	75.47	55.52
		November	41.87	50.10	63.91	72.30	57.01
	25-50	April	39.23	51.85	68.62	75.48	60.24
		July	38.91	52.16	57.30	82.39	54.73
		Mean	40.00	51.37	63.28	76.72	57.32
6. *I. malayana*		November	58.48	36.59	43.50	54.77	40.04
	<25	April	62.12	39.10	51.04	61.99	45.07
		July	63.07	43.98	52.81	63.03	48.39
		Mean	61.22	39.89	49.11	59.93	44.50
		November	52.05	35.72	46.01	47.76	40.87
	25-50	April	50.13	32.80	47.95	57.80	40.38
		July	57.25	39.38	57.28	63.90	48.33
		Mean	53.14	35.97	50.41	56.49	43.19
		November	53.48	40.23	42.43	54.73	41.33
	50-75	April	55.95	28.69	49.47	58.96	39.08
		July	57.78	39.11	52.12	61.52	45.61
		Mean	55.74	36.01	48.01	58.41	42.01
		November	45.27	31.51	38.63	53.41	35.07
	75-100	April	50.05	35.18	47.18	59.77	41.18
		July	53.63	39.55	54.44	62.19	46.99
		Mean	49.65	35.41	46.75	58.46	41.08
7. *W. tinctoria*		November	40.67	48.32	56.26	59.48	52.29
	<25	April	54.14	42.42	53.27	57.91	47.85
		July	62.45	49.58	61.84	67.04	55.71
		Mean	52.42	46.78	57.12	61.47	51.95
		November	45.69	52.00	63.28	67.20	57.64
	25-50	April	49.25	43.33	50.76	50.89	47.05
		July	57.06	54.79	64.20	70.07	59.50
		Mean	50.67	50.04	59.41	62.72	54.73

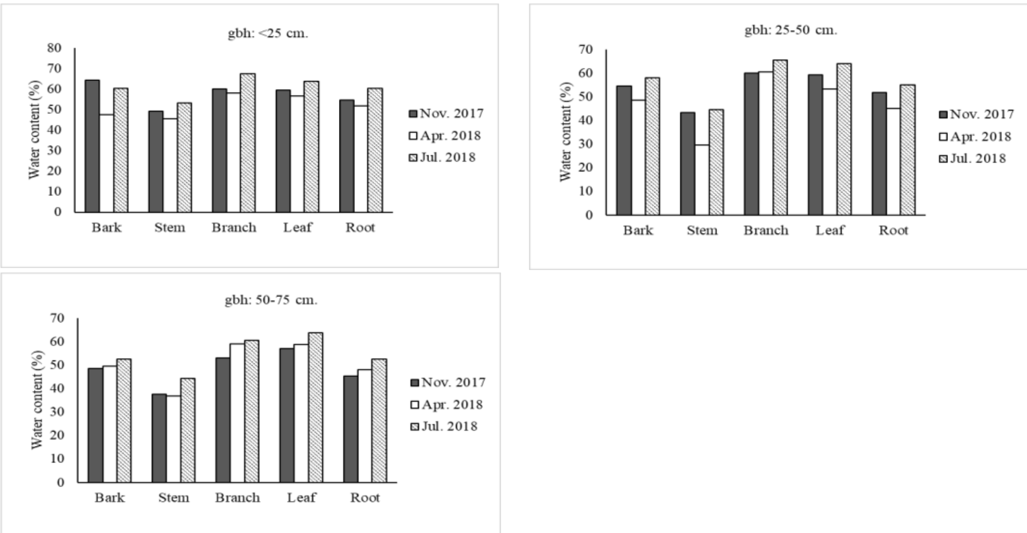
1. ***Pinus kesiya***



2. ***Shorea obtusa***



3. ***Dipterocarpus obtusifolius***



4. ***Aporosa villosa***

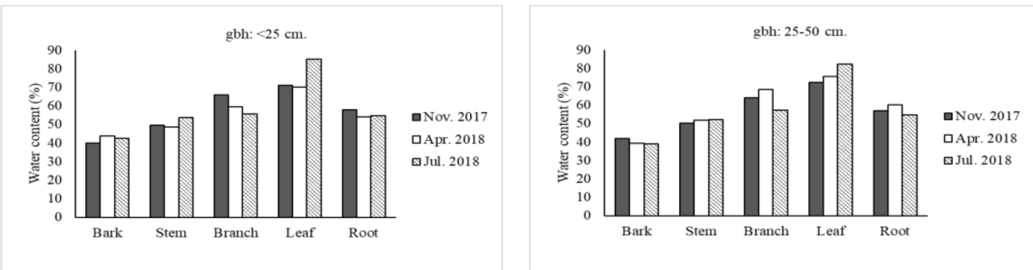
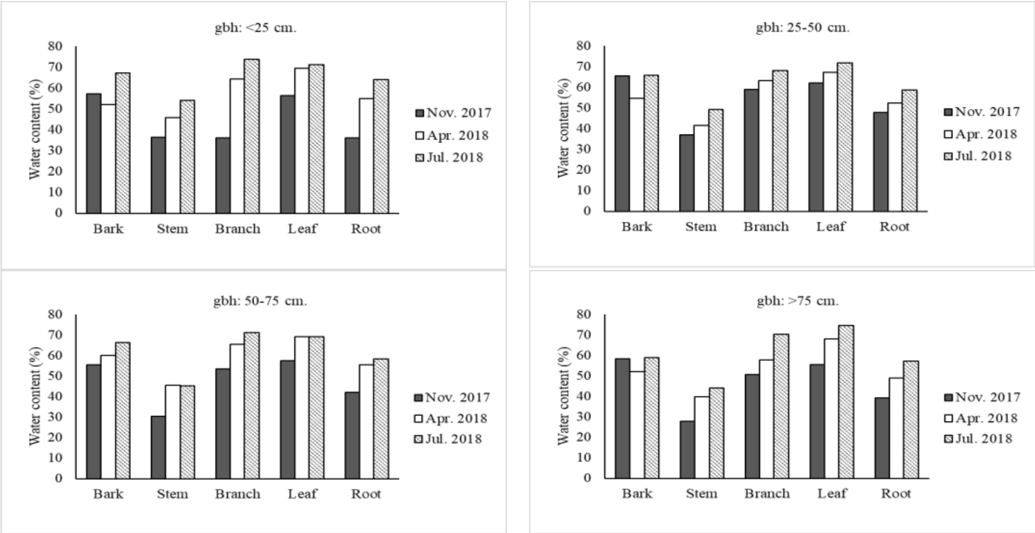
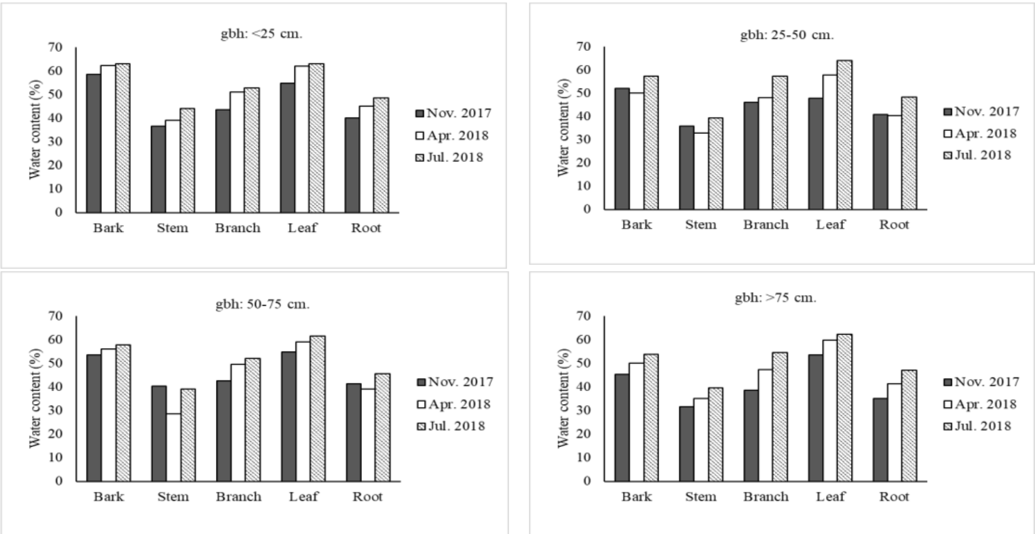


Figure 1. Variation of water content (% fresh weight) in dominant species 1 to 4

5. *Dipterocarpus tuberculatus*



6. *Irvingia malayana*



7. *Wendlandia tinctoria*

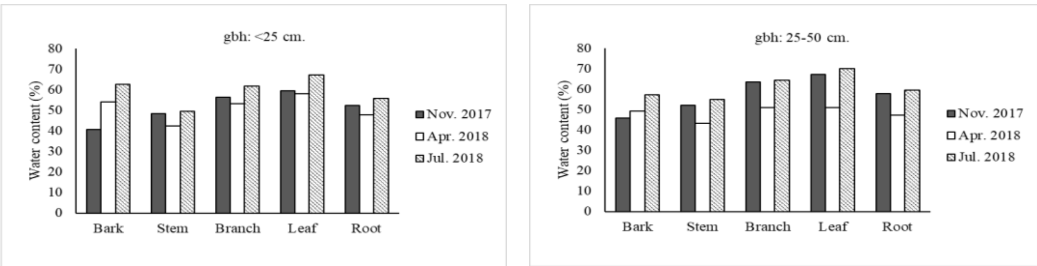


Figure 2. Variation of water content (% fresh weight) of dominant species 5 to 8

Table 7. Water content (% dry weight) in organs of abundant species in different stem-gbh classes in the DDF with planted pine

Plant species	Stem-gbh class	Sampling time	Water content (% by dry weight)				
			Bark	Stem	Branch	Leaf	Root
1. *P. kesiya*		November	125.49	56.11	127.37	148.46	87.32
	50-75	April	146.54	59.08	113.68	159.31	86.38
		July	132.53	73.19	178.58	179.39	125.89
		November	154.83	76.20	146.14	162.50	111.87
	75-100	April	198.06	53.07	124.81	157.89	88.94
		July	205.47	98.41	180.24	186.12	139.33
2. *D. obtusifolius*		November	181.39	98.17	166.32	147.50	143.48
	<25	April	98.95	83.70	139.90	130.65	111.80
		July	153.83	114.12	208.25	175.20	161.19
		November	121.23	77.02	153.04	146.21	112.10
	25-50	April	95.67	45.27	153.88	116.11	99.58
		July	138.82	80.56	195.58	178.11	138.07
		November	94.60	60.47	116.65	132.75	98.14
	50-75	April	98.26	58.66	144.76	142.81	101.71
		July	110.66	79.95	154.30	176.86	117.12
		November	146.43	42.33	147.53	154.00	112.67
	75-100	April	79.64	56.23	127.38	142.27	91.81
		July	94.57	75.41	153.04	187.87	114.23
3. *S. obtusa*		November	79.89	49.61	61.77	114.13	59.69
	<25	April	91.81	85.56	111.11	183.23	98.34
		July	102.17	105.53	89.23	192.78	97.38
		November	97.82	21.01	74.44	104.62	58.22
	25-50	April	88.10	67.34	85.95	156.87	76.64
		July	108.80	84.29	126.75	197.67	105.52
		November	108.50	41.98	72.50	106.98	55.27
	75-100	April	91.21	122.40	97.91	141.65	110.15
		July	88.68	67.01	116.29	132.32	91.65
4. *D. tuberculatus*		November	144.17	59.99	65.91	137.23	64.18
	<25	April	108.92	84.87	190.10	227.50	137.48
		July	212.34	121.58	293.31	248.54	207.45
		November	190.86	58.33	146.94	163.95	130.27
	25-50	April	132.24	70.85	171.81	206.02	121.33
		July	192.95	96.74	220.49	255.29	158.62
		November	125.62	43.95	121.86	135.81	104.52
	50-75	April	150.12	87.44	198.21	225.39	142.83
		July	203.88	83.30	249.11	224.58	166.21
		November	139.50	39.33	18.30	131.24	21.93
	75-100	April	116.85	66.33	137.22	212.80	101.78
		July	143.45	80.14	236.61	320.60	158.38
5. *A. villosa*		November	67.19	99.47	199.39	249.02	148.46
	<25	April	79.54	94.34	148.36	248.64	121.35
		July	75.40	116.06	131.74	569.86	123.90
		November	72.08	100.61	178.80	262.72	144.88
	25-50	April	64.71	107.77	225.90	334.31	166.84

Table 7. Water content (% dry weight) in organs of abundant species in different stem-gbh classes in the DDF with planted pine (cont.)

Plant species	Stem-gbh class	Sampling time	Water content (% by dry weight)				
			Bark	Stem	Branch	Leaf	Root
		July	63.94	109.37	135.93	474.91	122.65
6. *I. malayana*		November	147.26	58.20	81.90	121.22	72.87
	<25	April	171.66	64.33	104.27	166.48	84.30
		July	171.30	78.86	112.16	170.79	95.51
		November	108.53	55.58	85.21	91.43	24.47
	25-50	April	100.55	48.88	92.15	137.81	70.52
		July	138.07	64.98	138.80	177.22	101.89
		November	115.93	67.65	75.93	124.39	70.89
	50-75	April	130.73	45.88	98.04	143.73	71.96
		July	136.83	64.26	108.87	160.41	86.57
		November	84.03	47.13	69.88	115.73	58.30
	75-100	April	102.13	54.89	89.50	148.59	72.19
		July	116.76	65.44	119.55	164.46	92.49
7. *W. tinctoria*		November	68.99	93.71	132.95	148.13	116.95
	<25	April	119.38	73.72	116.54	139.27	95.13
		July	172.32	98.68	162.93	204.91	130.81
		November	85.51	108.39	175.31	207.37	138.47
	25-50	April	97.55	76.76	105.88	108.79	91.32
		July	133.13	121.79	179.55	234.22	150.67

Table 8 shows the amounts of water in the plant biomass within 10 plots of the DDF with planted pine. The average amount of water in the plant biomass of all the stands (10 plots) was calculated as 123.24±11.40 m^3 ha^{-1}. The average values in the 10 plots varied between 70.47 and 179.31 m^3 ha^{-1}. The percentages of the amounts of water in the bark, stem, branch, leaf, and root were in the following order: 1.68%, 44.08%, 19.40%, 3.07%, and 31.74%. The average amounts of water stored in plant biomass on November 23, 2017, April 20, 2018 and July 18, 2018 were 127.01, 104.91 and 137.80 m^3 ha^{-1}, respectively. It was the lowest in summer or dry season, intermediate in winter and the highest in rainy season.

Table 8. Water amounts stored in plant biomass within 10 plots in the DDF with planted pine

Plot	Mount	Water (m^3 ha^{-1})					
		Bark	Stem	Branch	Leaf	Root	Total
1	November 2017	1.95	74.21	20.94	2.67	47.58	147.35
	April 2018	2.44	49.25	28.56	4.87	38.91	124.03
	July 2018	3.02	66.06	39.88	6.02	52.97	167.95
	Mean	2.47	63.17	29.80	4.52	46.49	146.44
2	November	1.65	64.09	16.88	2.58	40.49	125.68
	April	2.09	40.39	22.70	4.51	31.54	101.23
	July	2.63	59.58	29.89	5.56	44.73	142.39
	Mean	2.12	54.69	23.15	4.22	38.92	123.10
3	November	1.58	58.99	19.87	1.93	39.43	121.81
	April	1.89	37.46	27.00	3.37	32.23	101.96
	July	2.37	52.94	37.80	4.16	45.37	142.65

Table 8. Water amounts stored in plant biomass within 10 plots in the DDF with planted pine (cont.)

Plot	Mount	Water (m^3 ha^{-1})					
		Bark	Stem	Branch	Leaf	Root	Total
	Mean	1.95	49.80	28.22	3.15	39.01	122.14
4	November	1.05	37.33	9.22	1.59	23.28	72.46
	April	1.26	23.66	12.52	2.76	18.09	58.29
	July	1.56	34.37	16.07	3.45	25.22	80.67
	Mean	1.29	31.79	12.60	2.60	22.19	70.47
5	November	2.84	92.94	27.80	3.07	60.37	187.01
	April	3.11	69.36	31.97	5.20	50.67	160.30
	July	3.61	78.58	41.96	6.21	60.27	190.63
	Mean	3.18	80.29	33.91	4.82	57.10	179.31
6	November	1.88	86.80	26.45	2.70	56.63	174.46
	April	2.22	51.37	33.12	4.23	42.24	133.19
	July	2.59	73.30	42.40	5.18	57.85	181.33
	Mean	2.23	70.49	33.99	4.04	52.24	162.99
7	November	1.53	67.40	16.31	2.58	41.85	129.67
	April	1.99	42.45	21.94	4.37	32.19	102.94
	July	2.40	58.60	29.37	5.22	43.98	139.57
	Mean	1.97	56.15	22.54	4.06	39.34	124.06
8	November	1.31	45.12	12.32	1.81	28.72	89.28
	April	1.39	28.31	16.51	3.06	22.41	71.68
	July	1.68	38.82	20.17	4.06	29.50	94.23
	Mean	1.46	37.42	16.34	2.97	26.88	85.06
9	November	1.57	57.91	13.57	2.37	35.74	111.17
	April	1.84	40.78	17.39	4.24	29.08	93.34
	July	2.24	50.74	22.87	5.25	36.81	117.91
	Mean	1.89	49.81	17.94	3.96	33.88	107.47
10	November	1.86	55.90	15.57	2.14	35.73	111.19
	April	2.15	43.83	20.25	3.90	32.04	102.16
	July	2.67	49.47	26.03	4.74	37.75	120.66
	Mean	2.23	49.73	20.61	3.59	35.17	111.34
Mean (Nov. 2017)		1.72	64.07	17.89	2.34	40.98	127.01
Mean (Apr. 2018)		2.04	42.69	23.20	4.05	32.94	104.91
Mean (Jul. 2018)		2.48	56.25	30.65	4.99	43.45	137.80
	Mean (all)	2.08	54.33	23.91	3.79	39.12	123.24
S.D.		0.60	0.60	16.83	9.04	1.32	11.40
C.V.		29.03	29.03	30.98	37.80	34.74	29.13

3.4.2 Water storage in soils

The soil derived from volcanic rock in the DDF dominated mainly by *Diterocarpus tuberculatus* was very deep, more than 2.0 meters, and classified into the more developed soil (Order Oxisols) as compared to Order Ultisols. It is the reddish soil containing the high content of iron oxides. In general, the physical properties of the soil, particularly depth, gravel content, bulk density, texture, and organic matter content, have an influence on water movement and retention throughout the soil profile (Brady and Weil, 2010). The data on the physical properties of the soil in the DDF with planted pine are given in Table 10. The bulk density was almost low throughout soil depth, and slightly increased from surface soil to subsoil. The organic matter was high only at the soil surface and decreased to low and very low to subsoil while the gravel content was almost very low. Except for the intermediate content of sand at the soil surface, this soil contained the low content of sand and silt, but the clay content was relatively high throughout the soil profile.

Table 9. Amounts of water stored in biomass of tree species in the DDF with planted pine

Plant species	Water in plant biomass (m^3 ha^{-1})							
	November 2017		April 2018		July 2018		Mean	
	(m^3 ha^{-1})	%	(m^3 ha^{-1})	%	(m^3 ha^{-1})	%	(m^3 ha^{-1})	%
1. *P. kesiya*	43.79	34.48	29.83	28.43	47.11	34.19	40.24	32.65
2. *D. tuberculatus*	20.56	16.19	20.84	19.87	26.52	19.25	22.64	18.37
3. *D. obtusifolius*	16.22	12.77	12.61	12.02	16.49	11.97	15.11	12.26
4. *S. obtusa*	15.68	12.35	14.40	13.73	14.35	10.41	14.81	12.02
5. *G. usitata*	8.64	6.81	7.38	7.03	8.99	6.52	8.34	6.76
6. *P. macrocarpus*	3.21	2.53	2.75	2.63	3.37	2.44	3.11	2.52
7. *A. villosa*	2.31	1.82	2.92	2.79	2.95	2.14	2.73	2.21
8. *S. anacardium*	1.93	1.52	1.67	1.59	2.07	1.50	1.89	1.53
9. *D. cultrata*	1.78	1.40	1.53	1.46	1.89	1.37	1.73	1.41
10. *S. nux-vomica*	1.17	0.92	1.03	0.99	1.32	0.96	1.18	0.95
11. *I. malayana*	1.40	1.10	0.91	0.87	1.14	0.83	1.15	0.93
12. *D. oliverli*	1.09	0.86	0.94	0.90	1.17	0.85	1.07	0.87
13. *W. tinctoria*	0.98	0.77	0.87	0.83	1.10	0.80	0.98	0.80
14. *S. parvifolius*	0.93	0.73	0.78	0.74	1.14	0.83	0.95	0.77
15. *S. cumuni*	0.88	0.69	0.69	0.66	0.85	0.62	0.81	0.66
16. *S. recemosa*	0.68	0.53	0.75	0.71	0.95	0.69	0.79	0.64
17. *D. ovovata*	0.76	0.60	0.65	0.62	0.80	0.58	0.74	0.60
18. *S. siamensis*	0.65	0.51	0.57	0.54	0.72	0.53	0.65	0.53
19. *Q. kerrii*	0.52	0.41	0.45	0.43	0.56	0.41	0.51	0.41
20. *A.odoratisssima*	0.52	0.41	0.44	0.42	0.54	0.39	0.50	0.41
21. *X. xylocarpa*	0.31	0.24	0.27	0.26	0.35	0.26	0.31	0.25
22. *A. fragrans*	0.32	0.25	0.27	0.26	0.34	0.25	0.31	0.25
23. *C. subulatum*	0.31	0.24	0.26	0.25	0.32	0.23	0.30	0.24
24. *B. lanzan*	0.28	0.22	0.25	0.24	0.32	0.23	0.28	0.23
25. *B. pierrei*	0.24	0.19	0.22	0.21	0.28	0.20	0.25	0.20
26. *P. pterocarpum*	0.15	0.12	0.13	0.12	0.17	0.12	0.15	0.12
27. *G. sootepensis*	0.12	0.10	0.11	0.10	0.14	0.10	0.12	0.10
28. *M. scutellatum*	0.14	0.11	0.08	0.07	0.14	0.10	0.12	0.10
29. *O. salicifolia*	0.11	0.09	0.10	0.10	0.13	0.10	0.12	0.09
30. *C. formosum*	0.10	0.08	0.09	0.09	0.12	0.09	0.11	0.09
31-86 Others	1.24	0.97	1.10	1.05	1.42	1.03	1.25	1.02
Total	127.01	100	104.91	100	137.80	100	123.24	100

The soil study on water storage was carried out three times (November 23, 2017, April 20, 2018 and July 18, 2018), as given in the data presented in Table 11. The field capacities of water (% by volume) in three soil pits (pedons) varied with soil depths: 54.62-56.22%, 43.21-52.76% and 44.69-55.58%. The water contents in the three pedons on November 23, 2017 (winter) varied in ranges of 18.96-40.33%, 18.08-37.44% and 33.99-44.37%. They almost increased from surface soil to subsoil resulted from evaporation. The contents in summer or dry season (April 20, 2018) of these pedons were the following order: 30.16-42.49%, 29.77-36.43% and 28.35-39.10%. The higher contents as 35.76-

49.51%, 30.70-40.97% and 33.75-44.27% were observed in rainy season (July 18, 2018).

The values of maximum water holding capacity (MWHC) calculated from the field capacity varied with soil depth of three pedons, and the total amount within 100 cm depth was measured at 5,089.33±364.68 m^3 ha^{-1} on average. This capacity could store the maximum rainfall amount of 508.93 mm. The amounts of water in the winter (November 23, 2017), the summer (April 20, 2018) and the rainy season (July 18, 2018) were determined as 3,656.95±474.72 m^3 ha^{-1} (72.17% of MWHC), 3,682.09±264.01 (72.36%) and 4,164.99±437.87 m^3 ha^{-1} (81.73%), respectively.

Table 10. Physical properties of 100 cm soil (Order Oxisols) under DDF with planted pine

Depth (cm)	Bulk density Mg m^{-3}	Organic matter %	Gravel %	Particle distribution (%)			Texture
				Sand	Silt	Clay	
0-5	1.02	4.34	5.96	68.40	10.00	21.60	sandy clay loam
5-10	1.08	2.06	1.91	49.40	9.00	41.60	sandy clay
10-20	1.09	1.24	1.71	28.40	5.00	66.60	clay
20-30	1.10	0.84	1.82	49.68	10.02	40.30	sandy clay
30-40	1.09	0.61	2.37	34.70	6.30	59.00	clay
40-60	1.13	0.46	1.72	32.68	5.22	62.10	clay
60-80	1.14	0.44	3.12	46.50	23.20	30.30	sandy clay loam
80-100	1.19	0.44	1.58	27.60	6.00	66.40	clay

Table 11. Water contents in 100 cm soil (Order Oxisols) under DDF with planted pine

Soil depth (cm)	Water content (% by volume)				
	Field capacity (%)		November 23, 2017	April 20, 2018	July 18, 2018
	by dry weight	by volume			
			Pedon 1		
0-5	33.80	55.41	29.52	30.16	37.94
5-10	33.15	54.71	32.75	34.19	35.76
10-20	33.14	55.44	18.96	41.46	36.30
20-30	32.40	53.38	28.48	40.88	47.82
30-40	31.93	55.52	28.86	42.49	49.73
40-60	32.33	54.75	40.02	36.70	47.51
60-80	31.50	54.64	39.86	41.77	48.73
80-100	32.96	56.22	40.33	40.80	48.86
			Pedon 2		
0-5	29.48	47.84	18.08	29.77	32.20
5-10	23.99	43.21	31.54	28.36	30.70
10-20	28.48	51.48	31.90	32.41	33.88
20-30	24.53	44.27	31.28	32.29	32.97
30-40	28.31	48.43	25.77	36.22	38.22
40-60	29.61	52.76	37.14	33.98	38.89
60-80	24.06	44.29	33.41	36.02	38.56
80-100	28.02	49.56	37.44	36.43	40.97
			Pedon 3		
0-5	30.17	50.19	33.99	28.35	35.08
5-10	29.51	49.62	35.69	33.52	33.75
10-20	32.86	55.58	39.97	34.42	38.10
20-30	30.43	51.34	39.28	35.37	40.04

Table 11. Water contents in 100 cm soil (Order Oxisols) under DDF with planted pine (cont.)

Soil depth (cm)	Field capacity (%) by dry weight	Field capacity (%) by volume	Water content (% by volume) November 23, 2017	April 20, 2018	July 18, 2018
30-40	29.28	49.08	42.08	36.93	42.34
40-60	28.70	48.26	43.09	36.70	42.00
60-80	26.42	44.69	44.37	38.48	44.27
80-100	31.44	50.72	44.21	39.10	43.89

Table 12. Maximum water holding capacity and water storage with sampling days

Soil depth (cm)	MWHC (m^3 ha^{-1})	Water storage in 100 cm soil (m^3 ha^{-1}) November 2017 m^3 ha^{-1}	November 2017 %	April 2018 m^3 ha^{-1} %	April 2018 %	July 2018 m^3 ha^{-1} %	July 2018 %
Pedon 1							
0-5	277.06	147.62	53.28	150.80	54.43	189.69	68.46
5-10	273.55	163.74	59.86	170.93	62.49	178.82	65.37
10-20	554.39	189.55	34.19	414.63	74.79	363.02	65.48
20-30	533.81	284.81	53.35	408.75	76.57	478.22	89.59
30-40	555.21	288.58	51.98	424.87	76.52	497.30	89.57
40-60	1,095.07	800.34	73.09	734.08	67.03	950.26	86.78
60-80	1,092.76	797.28	72.96	835.31	76.44	974.58	89.19
80-100	1,124.35	806.52	71.73	816.08	72.58	977.29	86.92
Total	5,506.19	3,478.44	63.17	3,955.45	71.84	4609.17	83.71
Pedon 2							
0-5	239.20	90.39	37.79	148.83	62.22	161.01	67.31
5-10	216.05	157.72	73.00	141.82	65.64	153.48	71.04
10-20	514.84	319.01	61.96	324.14	62.96	338.79	65.80
20-30	442.70	312.76	70.65	322.90	72.94	329.68	74.47
30-40	484.31	257.70	53.21	362.17	74.78	382.22	78.92
40-60	1,055.12	742.79	70.40	679.63	64.41	777.89	73.73
60-80	885.88	668.13	75.42	720.47	81.33	771.21	87.06
80-100	991.18	748.84	75.55	728.60	73.51	819.44	82.67
Total	4,829.29	3,297.36	68.28	3,428.54	70.99	3733.71	77.31
Pedon 3							
0-5	250.96	169.95	67.72	141.77	56.49	175.40	69.89
5-10	248.08	178.43	71.92	167.59	67.55	168.73	68.01
10-20	555.75	399.73	71.93	344.19	61.93	381.02	68.56
20-30	513.43	392.83	76.51	353.68	68.89	400.40	77.99
30-40	490.80	420.79	85.74	369.31	75.25	423.41	86.27
40-60	965.23	861.76	89.28	733.99	76.04	839.91	87.02
60-80	893.83	887.30	99.27	769.67	86.11	885.39	99.06
80-100	1,014.42	884.25	87.17	782.06	77.09	877.81	86.53
Total	4,932.50	4,195.04	85.05	3,662.27	74.25	4152.08	84.18
Mean	5,089.33	3,656.95	72.17	3,682.09	72.36	4,164.99	81.73
S.D.	364.68	474.72	11.44	264.01	1.69	437.87	3.83
C.V. (%)	7.17	12.98	15.86	7.17	2.33	10.51	4.69

3.4.3 Water storage in DDF ecosystem (plants and soils) with planted pine

Figure 3 shows the amounts of water stored in the DDF ecosystems (plant biomass and soil system) with planted pine at three sampling days within three seasons. However, the amounts of water in plant biomass and soil could be vary day by day, month by month, and year by year. During a year, the amount of water is usually high in the rainy season and lower in the winter and the summer.

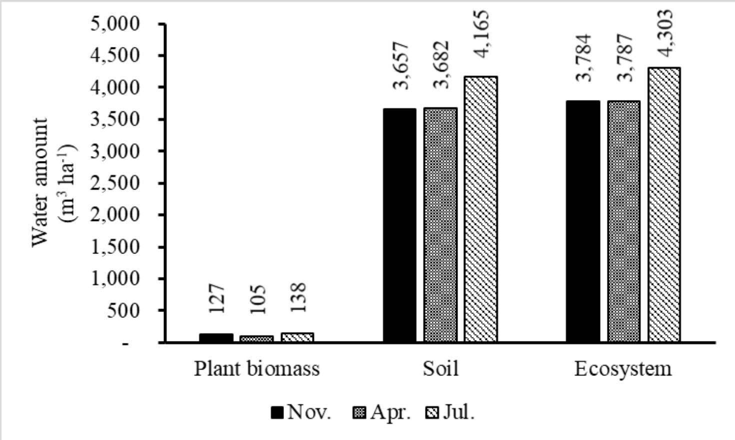


Figure 3. The amounts of water in the plant biomass, soil and ecosystem of DDF

4. Discussion

4.1 Contribution of planted pine to community structures, species diversity and forest condition

The enrichment planting of three needle pine in the degrade DDF could increase species composition, number of species (species richness) as well as species diversity indicated by Shannon-Wiener Index (SWI) of species diversity. The SWI was calculated from the equation derived from species richness and relative population abundance of each species in the plot. One species of pine with the average density of 146$\pm$80 trees ha^{-1} contributed to community structure in DDF, and resulted in the small increase of SWI value from 3.79$\pm$0.25 to be 3.87$\pm$0.24 whereas the forest condition index (FCI) was raised from 2.7$\pm$2.24 to be 6.18$\pm$3.24. These data implied that the enrichment planting could increase species diversity and improve forest condition of the forest after planting for 34 years. This pine occurs naturally in the dry areas with 1,000 to 1,900 m altitude in the lower montane forest of northern Thailand (Seramethakun, 2012; Seeloy-ounkeaw, 2014), and recognized as a fast-growing tree species. Thus, the pine could reduce the time of plant succession and forest development of the degrade DDF. Seeloy-ounkeaw (2014) reported that the utilization community forest (UF) distributed in areas of 1,000 to 1,250 m. Within 50 sampling plots, three needle pine had 98% frequency value with the density of 153 trees ha^{-1}. Its dominance and IVI were measured to be 25.82% and 11.97%, respectively. In the conservation community forest as the watershed covered the areas of 1,100 to 1,800 m altitude, this pine had lower frequency value (58%) with the density of 94 trees ha^{-1}. However, it had the highest values of dominance and IVI among 236 species within 50 plots: 15.77% and 6.51%, respectively.

4.2 Contribution of planted pine to plant biomass and water storage

The enrichment planting of three needle pine in the degrade DDF could increase also plant biomass as well as the water storage potential. The pine produced the amount of plant biomass at 36.79 Mg ha^{-1} (32.29% of the total) within 34 years, and increased the biomass from 65.06 to 99.14 Mg ha^{-1}. This pine species could store the amount of water at 34.08 Mg ha^{-1} (34.04% of the total) and restore the water storage potential in plant biomass in the forest from 65.06 to be 99.14 m^3 ha^{-1}. Therefore, the enrichment planting of this pine could increase 52.38% of the total water storage potential in the DDF (without planted pine). Seramethakun (2012) reported that the natural pine-dry dipterocarp forest dominated by *Shorea obtusa* at Kanlaya Ni Wattana district in Chiang Mai could produce the amount of biomass at 84.96 Mg ha^{-1}, and the pine had the contribution to the biomass at 31.12 Mg ha^{-1} (36.62% of the total). As for the degrade lower montane forest which had the amount of biomass at 79.48 Mg ha^{-1}, the contribution of pine to the biomass was high as 67.94% of the total.

Khamyong et al. (2014a) studied water storage in the lower montane forest in northern Thailand. The community forest of Karen village was divided into the conservation forest (CF) and the utilization forest (UF). Selective tree cutting for house construction and fuelwood was permitted by village regulations only in the UF. The CF was protected for the watershed and become a recovery forest. The amount of standing plant biomass in the CF (252.4$\pm$72.5 Mg ha^{-1}) was higher than that in the UF (139.7$\pm$36.3 Mg ha^{-1}). The amounts of water in the plant biomass varied between seasons. The amounts of water in the CF varied between 208.2$\pm$68.9 and 231.2$\pm$70.7 m3 ha^{-1}, whereas the amounts of water in the UF varied in the range of 107.1$\pm$29.7 to 129.0$\pm$33.3 m^3 ha^{-1}. Thus, the lower montane forest had higher amounts of water stored in plant biomass than the DDF with planted pine.

Different soils have the variable capacity of water storage depending upon soil depth, organic matter, gravel content, bulk density, textures and clay content. The soil in this study was a very deep reddish soil, and classified into Order Oxisols. The bulk density, gravel content and organic matter were almost low throughout the 100 cm depth, but the clay content was high. The soil with the high clay content usually has the high retention of water, but the clay of the Oxisols is aggregated to a strong grade of fine and very fine granular structure which causes it has the rapid permeability after rainfall (Soil Survey Staff, 1999), and the water storage by this soil maybe not high as predicted as other soils with the high clay content. Within 100 cm soil, this soil had the MWHC of 5,089.33$\pm$364.68 m^3 ha^{-1}. This value was nearly the same to the deep soil of Order Ultisols in the lower montane forest, 4,956 m^3 ha^{-1} (Khamyong et al. (2014a). Summanochitraporn (2014) found that the deep soil (the Ultisols) in a 22-year-old teak plantation could store the lower amount of water at 3,617.60 m^3 ha^{-1} while the Ultisols under the 22-year-old three needle pine had the higher value of 5,632.87 m^3 ha^{-1} (Khamyong et. al, 2014b).

5. Conclusions

The enrichment planting of three needle pine (*Pinus kesiya*) in the degrade DDF, covered on the deep soil of Order Oxisols, at the Huai Hong Khrai Royal Development Study Center provided the indirect benefits of ecological influences. This pine species is a fast-growing evergreen tree species, and normally exist in the natural dry dipterocarp forest. The degrade DDF was recovery as this pine increase species richness, diversity and ecological influence as well as the better forest condition by the rapid growth and increasing number (population) of big tree individuals in the forest. The pine species increased the plant biomass and water storage potential in the forest as 56.74% and 52.38%, respectively, of the DDF without planted pine.

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Study of Acid-Catalyzed Esterification Pretreatment of High Free Fatty Acid Crude Rice Bran Oil for Biodiesel Production

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Abstract

This study investigated the acid-catalyzed esterification of two brands of crude rice bran oil (CRBO) with free fatty acids (FFAs) content of about 8 and 10%, respectively. Experimental variables included reaction time, methanol-to-FFA molar ratio and sulfuric acid content with temperature and stirring speed fixed at 60°C and 600 RPM, respectively. A central composite design was used initially for sequential experimentation and followed by a Box-Behnken design to refine the optimum process conditions. Results showed that final FFA in CRBO could be reduced to less than 1% FFA in a single-step. The methanol-to-FFA molar ratio had the maximum influence on the esterification process and was followed by the reaction time and amount of catalyst. The optimum conditions for FFA conversion in CRBO were: reaction time 90 and 48 min, 62:1 and 70:1 methanol-to-FFA molar ratio, 22.5 and 20 % (w/w) sulfuric acid based on FFA for Brand I and II, respectively. Under these pretreatment conditions, initial FFA was reduced to 0.61 and 0.70 %, respectively making the CRBO suitable for biodiesel production.

Keywords: Crude rice bran oil/ Esterification/ Free fatty acid/ Acid-catalyzed methanolysis/ Optimization/ Biodiesel

1. Introduction

The consumption of petroleum based fossil fuels keeps on rising with increasing industrialization all over the world. Concern about the inevitable depletion of fossil fuels has led to an extensive search for alternative fuels that are viable, renewable and environmental friendly energy resources. Biodiesel offers an attractive alternative to petroleum-based fuels and can be produced from a variety of feedstocks such as vegetable oils, animal fats, and waste cooking oil. Biodiesel is biodegradable and non-toxic, and easily blended with petroleum diesel for use in conventional unmodified engines (Mahmudal et al., 2017). Biodiesel is also known for minimizing environmental pollution due to the reduction in exhaust emission and toxic elements (Chen et al., 2018). However, perceived benefits of biodiesel production and use need to be critically analyzed prior to its economic feasibility and commercialization (Amin et al., 2017; Hanif et al., 2018).

The widespread use of biodiesel is mainly limited by its high production cost compared to petroleum-based diesel and current state of the technology to process a wide variety of feedstocks (Baskar et al., 2016; Demirbas et al., 2016; Hanif et al., 2018; Gebremarium and Marchetti, 2017; Aransiola et al., 2014; Tababaei et al., 2015). A reduction in production cost can be achieved through the utilization of inexpensive, low quality feedstocks (Berchmans and Hirata, 2008; Atadashi et al., 2012).

However, the presence of high free fatty acids (FFAs) in low quality feedstocks hinders the transesterification process necessitating a pretreatment step to convert FFA in feedstock to alkyl esters (FAME) by esterification reaction. Therefore, a two-step esterification-transesterification method is often used due to its moderate operating conditions, higher reaction rates and lower cost (Canakci and Van Gerpen, 2001; Ghadge and Raheman, 2005; Wang et al., 2006; Bouaid et al., 2012).

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A distinct advantage of acid-catalyzed esterification step is direct conversion of high FFA content into alkyl esters or biodiesel and thus reducing FFA content to an acceptable level for the subsequent transesterification step (Berchmans and Hirata, 2008; Mohandass et al., 2016; Gunawardena et al., 2017).

The various process variables like temperature, catalyst concentration, amount of methanol, reaction time and agitation are routinely optimized for maximizing FAME yield (Lin et al., 2009; Verma and Sharma, 2016). The application of design of experiment (DOE) and response surface methodology (RSM) for process optimization has been reported in a limited number of studies mainly for the transesterification process (Liao et al., 2011; Kamath et al., 2011). These methods can estimate the effects of individual process variables as well as their interactions leading to the determination of more realistic optimum conditions (Nattapon and Krit, 2015).

Crude rice bran oil (CRBO) is available in large quantities in Asia Pacific region with a huge potential for biodiesel production (Allen Jeffery et al., 2017; Chhabra et al., 2017). However, CRBO remains an underutilized non-edible resource due to rapid spoilage of rice bran by lipase enzymatic action resulting in very high levels of FFAs. Thus, CRBO with high FFA requires acid-catalyzed pretreatment similar to other non-edible oil feedstocks to reduce the final FFA content ≤ 1% suitable for alkali-catalyzed transesterification reaction (Sivakumar et al., 2013; Montefrio et al., 2010). The suitability of enzymatic treatment for the reduction of high FFA in CRBO has been reported in some studies (Wang et al., 2017; Li et al., 2018). However, acid-catalyzed methanolic esterification of CRBO with high FFA has been widely reported in several studies for biodiesel production (Zullaikah et al., 2005; Lin et al., 2009; Amin et al., 2012; Kattimani et al., 2014; Arora et al.,2016).

Many studies have reported optimum conditions for acid-catalyzed esterification for high FFA feedstocks determined by one-factor-at-a-time approach (Hamaze et al., 2015; Farag et at., 2011).However, there are no published studies on the optimization of esterification process of CRBO using DOE and RSM. This study aimed at the determining the optimum process conditions such as reaction time, methanol to FFA molar ratio and amount of catalyst for the esterification of two brands of CRBO with high FFA content. Initially, a central composite design (CCD) was used for sequential experimentation and followed by Box-Behnken design (BBD) to develop models for determining the optimal process conditions for obtaining the final FFA in CRBO less than 1% (w/w).

2. Methodology

2.1 Materials

Two brands of CRBO with FFA were obtained from Kasisuri Co., Ltd. and King Rice bran Oil Company, and designated as Brand I and II, respectively. The reactant solution in esterification process consisted of methanol and sulfuric acid as the catalyst. Potassium hydroxide (laboratory grade, 85%) was used for preparing standard solution to measure the FFA content of oil samples. The CRBO samples used in these experiments were of brownish yellow color in liquid state.

2.2 Esterification

Esterification was carried out in a single-step process with sulfuric acid as homogenous catalyst. The samples weighing 50 g were placed in a conical flask and heated to 60°C by using a hot plate with magnetic stirrer. The mixture of sulfuric acid (based on the weight % of FFA in the oil sample) and methanol (based on the molar ratio of methanol-to-FFA in oil sample) was poured into the heated flask. The agitation speed was fixed at 600 revolutions per minute (RPM) for thorough mixing of methanol and oil. The reaction was proceeded at 60°C for different times based on the experimental design.

Reaction time, molar ratio of methanol-to-FFA and sulfuric acid as catalyst (% w/w FFA) were used as independent variables. Theoretically, one mole of methanol is needed to react with one mole of FFA but excess amount of alcohol is used to prevent the reversible reaction. At the end of reaction, the mixture was allowed to settle down in a separating funnel. The separated sample was washed with distilled water slowly to reduce the pH level to neutral and remove the impurities and dried subsequently in an oven at 100°C to remove the residual water prior to acid value measurement.

2.3 Analysis of FFA

The FFA present in an oil sample was determined as the acid value (AV) by titrating the oil sample into a flask with 10 ml of 2-propanol against standard potassium hydroxide (KOH) solution with phenolphthalein as an indicator. The standard method for FFA titration in oil was followed (AOCS Cd 3d-63).The Acid value (AV) is expressed as (mg KOH/g oil) as shown below

( ) ( ) ( )

( ) ( )

( ) ( )

The strength of KOH solution was standardized using diluted HCL solution.

( )

The correction factor was obtained to adjust the normality of KOH solution in Eq.1.

2.4 Experimental design

The esterification of two brands of CRBO with different initial FFA (%) was carried out using DOE and RSM, respectively. The review of literature indicated that the esterification process of high FFA oils is the most effected by the factors such as the reaction time, methanol-to-FFA ratio and catalysts content whereas the reaction temperature and stirring speed was usually fixed at 60°C and 600 RPM, respectively (Arora et al.,2015; Chai et al.,2014). Therefore, the effects of three independent variables on the conversion of initial FFA in CRBO were investigated. Initially, a five-level-three-factor central composite design (CCD) was used for sequential experimentation and followed by a Box-Behnken Design (BBD) to further improve the models for predicting the final FFA in two brands of CRBO. The independent variables and their levels in coded and actual units are shown in Table 1.

Table 1. Independent variables used for CCD and BBD in esterification experiments.

Variables	CRBO Brand	CCD coded levels				BBD coded levels			
		-1.68	-1	0	1	1.68	-1	0	1
Time, min Z (X_1)	I	40.8	60	120	180	220.8	30	60	90
	II	40.8	60	120	180	220.8	30	60	90
Methanol:FFA (X_2)	I	14.8	25	40	55	65.2	30	60	90
	II	9.8	20	35	50	60.2	30	50	70
Catalysts (% w/w FFA, X_3)	I	11.6	15	20	25	28.4	5	15	25
	II	11.6	15	20	25	28.4	10	15	20

2.5 Statistical analysis

The experimental data obtained for the esterification of the both brands of CRBO were fitted the following second-order polynomial model (Equation 5) using regression analysis in MS Excel.

$$\Sigma \quad \Sigma \quad \Sigma \; \Sigma \qquad ( )$$

Where Y is the response (final FFA conversion esterification were determined by the Excel solver function using the developed models.

3. Results and discussion

3.1 Models based on CCD and BBD

The CCD design had 8 factorial, 6 axial and 6 center point experiments resulting in a total of 20 experimental runs for each brand of CRBO as shown in Tables 2 and 4 based on the coded and actual conditions given in Table 1. A sequential approach was used to analyze the results from the CCD model to evaluate the effects of individual factors and their interactions. Also the direction of steepest gradient for reduction in FFA due to esterification was determined using the Solver function in Excel. Subsequently, the low and high ranges of independent variables in CCD were adjusted for use in the BBD as shown in Table 1. Thus it was possible to obtain the refined optimization conditions from the model based on the BBD. The

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BBD design had a total of 15 experimental runs for each brand of CRBO as shown in Table 3 and 5.

The experimental and predicted values of final FFA in CRBO based on CCD and BBD are shown in Table 2 and 3 for Brand I and in Table 3 and 4 for Brand II, respectively.

Table 2. Final FFA (%) of CRBO Brand I after esterification based on CCD.

Run no;	X1 (Time, min)	X2 (MEOH:FFA)	X3 (% w/w FFA)	Brand I	
				Experimental	Predicted
1	-1 (60)	-1 (25)	-1 (15)	2.78	2.24
2	1 (180)	-1 (25)	-1 (15)	2.66	2.42
3	-1 (60)	1 (55)	-1 (15)	0.92	1.25
4	1 (180)	1 (55)	-1 (15)	1.94	1.36
5	-1 (60)	-1 (25)	1 (25)	1.54	1.84
6	1 (180)	-1 (25)	1 (25)	2.70	2.09
7	-1 (60)	1 (55)	1 (25)	1.10	1.06
8	1 (180)	1 (55)	1 (25)	0.98	1.24
9	-1.68 (40.8)	0 (40)	0 (20)	1.66	1.49
10	1.68 (220.8)	0 (40)	0 (20)	1.24	1.79
11	0 (120)	-1.68 (14.8)	0 (20)	2.06	2.56
12	0 (120)	1.68 (65.2)	0 (20)	1.14	1.02
13	0 (120)	0 (40)	-1.68 (11.6)	1.30	1.77
14	0 (120)	0 (40)	1.68 (28.4)	1.42	1.33
15	0 (120)	0 (40)	0 (20)	1.20	1.17
16	0 (120)	0 (40)	0 (20)	1.26	1.17
17	0 (120)	0 (40)	0 (20)	1.16	1.17
18	0 (120)	0 (40)	0 (20)	1.14	1.17
19	0 (120)	0 (40)	0 (20)	1.06	1.17
20	0 (120)	0 (40)	0 (20)	1.28	1.17

Table 3. Final FFA (%) of CRBO Brand I after esterification based on BBD.

Run no;	X1 (Time, min)	X2 (MEOH:FFA)	X3 (% w/w FFA)	Brand I	
				Experimental	Predicted
1	-1 (30)	-1 (30)	0 (15)	1.70	1.63
2	-1 (30)	1 (90)	0 (15)	1.10	0.97
3	1 (90)	-1 (30)	0 (15)	0.94	1.07
4	1 (90)	1 (90)	0 (15)	0.84	0.91
5	-1 (30)	0 (60)	-1 (5)	1.06	1.28
6	-1 (30)	0 (60)	1 (25)	0.84	0.82
7	1 (90)	0 (60)	-1 (5)	0.86	0.88
8	1 (90)	0 (60)	1 (25)	0.82	0.60
9	0 (60)	-1 (30)	-1 (5)	1.76	1.61
10	0 (60)	-1 (30)	1 (25)	1.06	1.15
11	0 (60)	1 (90)	-1 (5)	1.20	1.11
12	0 (60)	1 (90)	1 (25)	0.70	0.84
13	0 (60)	0 (60)	0 (15)	0.74	0.75
14	0 (60)	0 (60)	0 (15)	0.78	0.75
15	0 (60)	0 (60)	0 (15)	0.74	0.75

Table 4. Final FFA (%) of CRBO Brand II after esterification based on CCD.

Run no;	X1 (Time, min)	X2 (MEOH:FFA)	X3 (% w/w FFA)	Brand II	
				Experimental	Predicted
1	-1 (60)	-1 (20)	-1 (15)	1.68	1.85
2	1 (180)	-1 (20)	-1 (15)	3.06	3.14
3	-1 (60)	1 (50)	-1 (15)	1.02	1.18
4	1 (180)	1 (50)	-1 (15)	1.26	1.46
5	-1 (60)	-1 (20)	1 (25)	1.74	1.93
6	1 (180)	-1 (20)	1 (25)	2.82	3.04
7	-1 (60)	1 (50)	1 (25)	1.32	1.62
8	1 (180)	1 (50)	1 (25)	1.50	1.72
9	-1.68 (40.8)	0 (35)	0 (20)	1.24	0.93
10	1.68 (220.8)	0 (35)	0 (20)	2.34	2.09
11	0 (120)	-1.68 (9.8)	0 (20)	3.18	2.96
12	0 (120)	1.68 (60.2)	0 (20)	1.62	1.28
13	0 (120)	0 (35)	-1.68 (11.6)	2.28	2.10
14	0 (120)	0 (35)	1.68 (28.4)	2.76	2.39
15	0 (120)	0 (35)	0 (20)	1.38	1.42
16	0 (120)	0 (35)	0 (20)	1.44	1.42
17	0 (120)	0 (35)	0 (20)	1.38	1.42
18	0 (120)	0 (35)	0 (20)	1.38	1.42
19	0 (120)	0 (35)	0 (20)	1.44	1.42
20	0 (120)	0 (35)	0 (20)	1.38	1.42

Table 5. Final FFA (%) of CRBO Brand II after esterification based on BBD.

Run no;	X1 (Time, min)	X2 (MEOH:FFA)	X3 (% w/w FFA)	Brand II	
				Experimental	Predicted
1	-1 (30)	-1 (30)	0 (15)	1.44	1.42
2	-1 (30)	1 (70)	0 (15)	0.92	0.97
3	1 (90)	-1 (30)	0 (15)	1.00	0.94
4	1 (90)	1 (70)	0 (15)	0.84	0.85
5	-1 (30)	0 (50)	-1 (10)	1.64	1.54
6	-1 (30)	0 (50)	1 (20)	0.88	0.93
7	1 (90)	0 (50)	-1 (10)	0.92	0.86
8	1 (90)	0 (50)	1 (20)	0.92	1.01
9	0 (60)	-1 (30)	-1 (10)	1.12	1.23
10	0 (60)	-1 (30)	1 (20)	1.00	0.96
11	0 (60)	1 (70)	-1 (10)	0.88	0.92
12	0 (60)	1 (70)	1 (20)	0.84	0.73
13	0 (60)	0 (50)	0 (15)	0.92	0.85
14	0 (60)	0 (50)	0 (15)	0.84	0.85
15	0 (60)	0 (50)	0 (15)	0.80	0.85

3.2 Model evaluation

The coefficients of the developed models based on CCD and BBD using coded independent variables are presented in Table 6 and 7 along with related statistics. The CCD indicated that methanol-to-FFA molar ratio had the maximum effect on FFA conversion in both brands of CRBO as well as its interaction with reaction time in case of brand II. The CCD resulted in model fitting with low R2-value of about 0.669 for brand I with 8% FFA.

However, the model fitting for brand II with 10% FFA was reasonably good with R2- value of about 0.904. A look at the final FFA values for both brands of CRBO revealed that the experimental conditions selected in CCD could not reduce the final FFA below the desired level of 1%.

Accordingly, the experimental conditions were adjusted in the direction of steepest gradient for the BBD as shown in Table 1. The results obtained from 15 experiments runs of BBD in Table 3 clearly indicated the possibility of obtaining the final FFA levels less than 1%. Also there was a marked improvement in the model fitting based on BBD for the both brands of CRBO as shown by higher R2 value and lower SEE in Table 6 and 7. A comparison of experimental and estimated values of final FFA for CRBO based on BBD is presented in Figure 1 for both brands of CRBO. The results for CRBO brand I indicated higher variation compared to Brand II despite the use of higher methanol-to-FFA molar ratio most possibly due to its lower FFA content. Similar findings were reported by Chai et al. (2014) for esterification pretreatment of low FFA feed stocks requiring significantly higher methanol-to-FFA molar ratio. The residual plots for the prediction model developed using BBD clearly indicated random patterns in support of model acceptability for the both brands of CRBO as shown in Figure 2.

Table 6. Coefficients of models based on CCD and BBD for CRBO (Brand I).

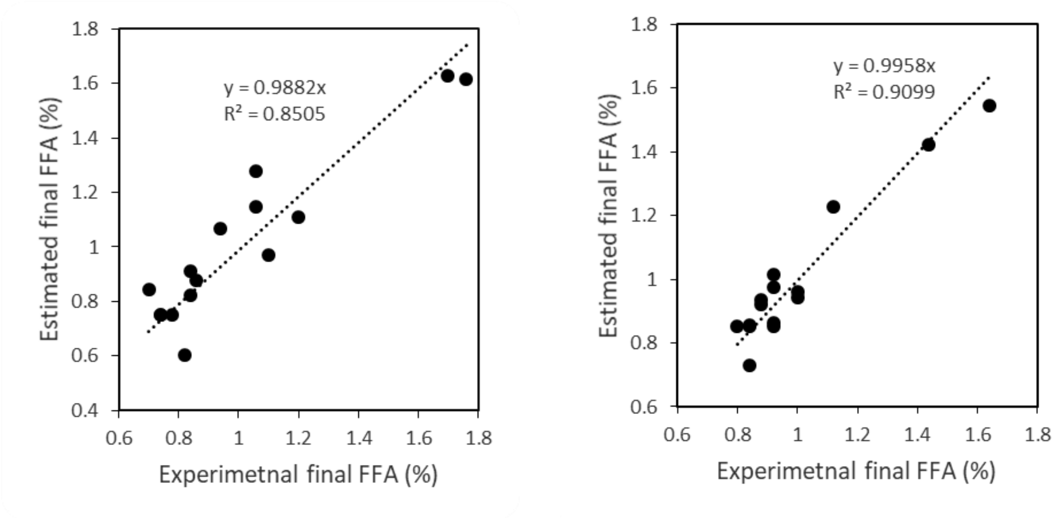
Model coefficients	CCD	p-value	CCD	p-value
β_0	1.17	0.00	0.75	0.00
β_1	0.09	0.49	-0.15	0.08
β_2	-0.46	0.00	-0.20	0.03
β_3	-0.13	0.33	-0.18	0.05
β_{11}	0.17	0.21	0.05	0.63
β_{22}	0.22	0.11	0.34	0.02
β_{33}	0.13	0.30	0.09	0.43
β_{12}	-0.02	0.92	0.12	0.26
β_{13}	0.20	0.92	0.04	0.67
β_{23}	0.05	0.76	0.05	0.64
R^2	0.669		0.869	
R^2_{adj}	0.371		0.632	
SEE	0.467		0.199	
P*value (F>Fcrit)	0.112		0.083	

Table 7. Coefficients of models based on CCD and BBD for CRBO (Brand II).

Model coefficients	CCD	p-value	BBD	p-value
B_0	1.42	0.00	0.85	0.00
β_1	0.35	0.00	-0.15	0.01
β_2	-0.50	0.00	-0.13	0.02
β_3	0.09	0.30	-0.11	0.04
β_{11}	0.03	0.66	0.16	0.04
β_{22}	0.25	0.01	0.03	0.60
β_{33}	0.29	0.00	0.07	0.27
β_{12}	-0.25	0.03	0.09	0.18
β_{13}	-0.04	0.67	0.19	0.02
β_{23}	0.09	0.40	0.02	0.74

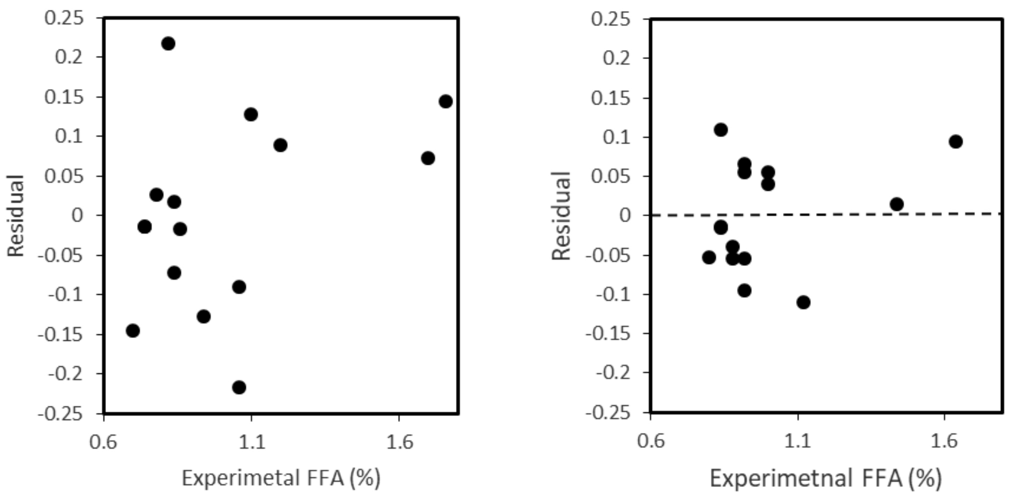
Table 7. Coefficients of models based on CCD and BBD for CRBO (Brand II).(Cont.)

Model coefficients	CCD	p-value	BBD	p-value
R^2		0.904		0.917
R^2_{adj}		0.817		0.768
SEE		0.287		0.114
P*value (F>Fcrit)		0.001		0.030



(a) (b)

Figure 1. Comparison of experimental and estimated final FFA (%) for CRBO based on BBD: (a) Brand I, (b) Brand II.

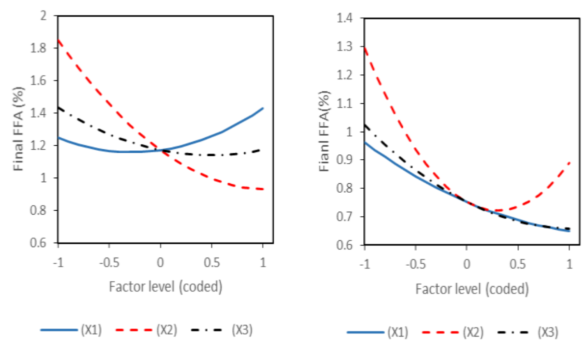


(a) (b)

Figure 2. Residual plot for predicted final FFA (%) based on BBD: (a) Brand I, (b) Brand II.

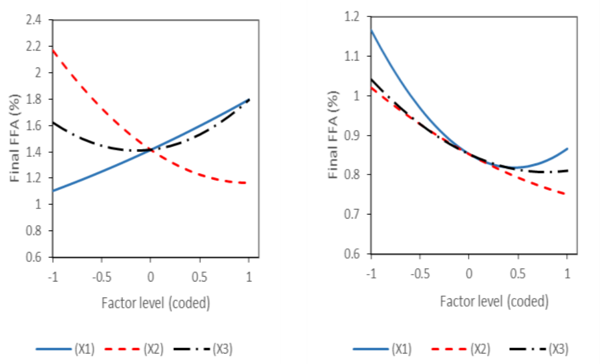
3.3 Sensitivity analysis

The influence of esterification process parameters on the change in final FFA of two brands of CRBO is shown by the perturbation plots in Figure 3 and 4, respectively. These plots show the change in final FFA as each factor moves away from the center point (selected as reference) while holding other variables as the reference value



(a) (b)

Figure 3. Perturbation plots for esterification of CRBO Brand I: (a) CCD, (b) BBD.



(a) (b)

Figure 4. Perturbation plots for esterification of CRBO Brand II: (a) CCD, (b) BBD.

Figure 3 shows that methanol-to-FFA molar ratio was most sensitive to the change in methanol-to-FFA molar ratio for brand I of CRBO with 8 % FFA compared to the reaction time and amount of catalyst. Also the CCD based model required extreme conditions for reducing the final FFA less than 1%. Thus the model based on BBD showed distinct improvement for determining the optimal conditions. Figure 4 presents the results for Brand II of CRBO with 10% FFA. In this case also, the BBD offered clear improvement over the CCD based model. However, the CCD model also depicted the influence of individual factors and their interaction on the esterification due to sequential experimentation.

3.4 Optimization

The models developed using BBD showed a significant improvement following initial experimentation based on CCD. The optimum conditions for the esterification of CRBO are shown in Table 8 based on both CCD and BBD for comparative evaluation. The final FFA levels were predicted in 0.91-1.02 % range by the CCD. However, the models based on BBD predicted significantly lower level of FFA in 0.61-0.70% range for both brands of CRBO. Thus, the reaction time, methanol-to-FFA molar ratio and amount of catalyst could be used in the respective ranges of 48-90 min, 60–70 methanol-to-FFA ratio and 20-23% H2SO4 : FFA (w/w) in general for lowering the high FFA in CRBO to an acceptable level. It should be realized that more precise estimation of process variables is limited by the inherent errors in experimentation, especially when using titration method for FFA determination.

Several studies have reported the optimum conditions for the esterification of high FFA CRBO (Zullaikah et al., 2005; Lin et al., 2009; Amin et al., 2012; Kattimani et al., 2014; Arora et al., 2016). A direct comparison of optimization conditions reported in literature with the results of present study is not possible in an objective way due to very wide variation in the experimental conditions. However, the results reported in previous study as well as the present study were expressed on the comparable basis as shown in Table 9.

Table 8. Optimum conditions for esterification of CRBO.

CRBO Brand	Model	Coded and actual values of independent variables			Initial FFA (%)	Final FFA (%)	FFA conversion (%)
		X_1	X_2	X_3			
I	CCD	-0.2[1] (52.8)*	1.0[2] (90)*	0.3[3] (18.1)*	8	0.91	88.6
	BBD	1.0 (90)	0.1 (61.7)	0.8 (22.6)	8	0.61	92.5

Table 8. Optimum conditions for esterification of CRBO.(Cont.)

CRBO Brand	Model	Coded and actual values of independent variables			Initial FFA (%)	Final FFA (%)	FFA conversion (%)
		X_1	X_2	X_3			
II	CCD	-1 (30)	0.5 (60.8)	-0.3 (13.5)	10	1.02	89.8
	BBD	-0.4 (48.1)	1.0 (70)	1.0 (20)	10	0.70	93.0

Note: 1) time (min), 2) Methanol:FFA molar Ratio, 3) Catalyst:FFA (w/w) % (coded values) (*) Actual values

Table 9. Comparison of optimum process conditions for homogenous acid-catalyzed esterification of high FFA crude rice bran oil**.**

No	Initial FFA(%)	Molar Ratio (MEOH: FFA)	MEOH % (w/w)	H_2SO_4 (% w/w)	Time (min)	Temperature (°C)	Final FFA (%)	FFA conversion (%)	Reference
1	79 (Type I)	10:1	89.7	2	180	60	0.8	99	Amin et al., 2012.
	6 (Type II)	10:1	6.8	2	180	60	3.2	46.7	
2	12	53.9:1	73.4	0.5	90	60	1.8	85	Arora et al., 2016.
3	8 (Step I)	7.05 :1	6.4	0.5	60	55-60	1.8	90	Kattimani et al., 2014.
	1.8 (Step II)	5.7:1	5.2	0.5	60	55-60	0.6	66.7	
4	20 (Step I)	9.7:1	22.0	1	60	60	2.4	88	Lin et al., 2009.
	2.4 (Step II)	94.4:1	25.7	1	60	60	0.5	81.3	
5	49.8	3.2	18.4	2	120	60	3.2	93.6	Zullaikah et al., 2005.
6	8 (Brand I)	61.7:1	56.0	1.8	90	60	0.6	92.5	This study
	10 (Brand II)	70:1	79.4	2	48.1	60	0.7	93	

In view of practical considerations, use of methanol and sulfuric acid as catalyst were also computed based on 100 g of CRBO in % (w/w) irrespective of the initial FFA. The overall optimal conditons for conversion of FFA in various types of CRBO showed methanol use varying from from 5.2 to 89.7% (w/w), 0.5 to 2% of sulfuric acid, and 48.1 to 180 min of reaction time for different initial FFAs and experimental protocol. While the two-step pretreatment can reduce the initial FFA to less than 1% as reported by Kattimani et al.(2014) and Lin et al.(2009), this study showed the reduction in FFA below 1% in a single-step process. The methanol use in present study is comparable to that reported by Arora et al.(2016)even though the final FFA in their study was 1.8%. There is not much variation in sulfuric acid content ranging from 0.5 to 2% (w/w). These results also support the finding that higher methanol-to-FFA molar ratio is required for the conversion of low initial FFA in a variety of oil samples Chai et al. (2014). Overall, the results of this study indicate a significant improvement in determining the optimum conditions for the esterification of CRBO with initial FFA in 8-10%.

4. Conclusion

The DOE and RSM approach was effectively used for the acid-catalyzed esterification of CRBO with high FFA to obtain the final FFA less than 1% (w/w). The CCD could be used initially for investigating the relative effects of process conditions on FFA conversion through sequential experiementation leading to improved process conditions in BBD for subsequent optimization. The developed models accounted for the effects of reaction time, methnaol-to- FFA molar ratio and catalyst concentration on the esterification process of CRBO with initial FFA in 8-10% range. The methnaol-to-FFA molar ratio had the maximum effect on FFA conversion compared to the relatively minor effects of reaction time and catalyst amount. The respective ranges of optimum conditons were 48-90 min of reaction time, 60-70:1 methanol-to-FFA molar ratio, and 20-25.5 % catalyst based on FFA, and for final FFA reduction in approximately 0.6-0.7% range.

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Change of Forest Area and Its Associated CO_2 Emissions at Provincial Level in Southern Part of Thailand

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Abstract

Increasing population and urbanization are factors leading to deforestation that can convert forest area to other types of land uses/covers. Forest controls the balance of atmospheric carbon dioxide (CO_2) due to its significant uptake via photosynthesis process and storage in its biomass production. The objective of this study was to assess the amount of CO_2 emissions resulting from forest area changes at provincial level in the southern part of Thailand. CO_2 emissions from land use changes were estimated using IPCC 2006 Guidelines. From land use classification between reference and study year, 72,078 hectares of forestland were converted to other types of lands: 63,972; 1,735; 1,816; 1,460 and 3,094 hectares, receptively for cropland, settlement, grassland, wetland and others. Remaining forestland was estimated to absorb CO_2 34,219 Gg/year while that for forestland changes estimated CO_2 emission was 38,550 Gg/year. By provincial level out of fourteen provinces, Phang Nga, Surat Thani, and Nakhon Si Thammarat were found to be top three-provinces for CO_2 emission and absorption. To identify a link for future CO_2 management in forest sector, CO_2 emissions were preliminary found to be negatively correlated with provincial population density.

Keywords: Greenhouse gases/ Carbon dioxide/ Land-use change/ Southern Thailand

1. Introduction

For many decades, scientists put a great deal of efforts to quantify influences of carbon dioxide (CO_2) as well as other greenhouse gases on the earth temperature and climate (Meehl and Tebaldi, 2004; IPCC, 2014; Baker et al., 2018). The efforts are not only on assessment how much CO_2 emissions, but also how to control it to be well below the setup target in the atmosphere (450 ppm). This setup value is anticipated that the earth temperature increases will be less than 2°C above preindustrial levels (IPCC, 2014). A more stringent restriction of 1.5°C is remarked in the Copenhagen Accord raised by island states to avoid the threat of sea level rise (Matsuno et al., 2012). Various international organizations, i.e., UNEP, WMO and IPCC also provide the assessment report of causes, effects and ways forward to tackle the earth temperature rising (IPCC, 2006; IPCC, 2014). UNFCCC was later created in 1992 as a framework for international cooperation to combat climate change by limiting global temperature increases and the subsequent impact mitigation and remediation (IPCC, 2014).

Research focus on CO_2 emission reduction is primarily based on energy related sector such as transport, industrial and residential sources as it is a main contributor (IEA, 2009; World Bank, 2014). Recently, land use and land cover changes (LUCC) has also been recognized as an active source and sink for atmospheric CO_2 (Chuai et al., 2015; Zhang et al., 2018) and has been now considered widely as a key driver of global carbon dynamics (Federici et al., 2015; Zhang et al., 2018). Changes of land use, i.e., deforestation are normally functioned of human demand on basic needs and their livelihoods. Understanding the change of land use pattern and its associated CO_2 emissions could help in proper development of measures or policies for future carbon reduction and mitigation of climate impacts.

From land use data, this study found that forest area in southern Thailand was gradually reduced comparing between reference and study

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years in fourteen provinces. Most of the forest was changed mainly to cropland to enhance farming activities. We then investigated above ground CO_2 emissions during the specified timeframe in fourteen provinces of southern Thailand. CO_2 estimates were calculated from conversion of forestland to other types of lands (cropland, settlement, grassland, wetland and others) following the Intergovernmental Panel on Climate Change (IPCC) guidelines. Resulting CO_2 emissions in each province were then linked to basic demographic data using statistical analysis of correlation matrix.

2. Methodology

2.1 Study area

Southern part of Thailand included fourteen provinces as shown in Figure 1 and listed in Table 1 were selected for the assessment of CO_2 emissions. This part is the peninsula between the Andaman Sea and the South China Sea on the western and eastern sides, respectively. Temperatures are generally medium (23-34°C) throughout the year because of the maritime characteristic while abundant of rain naturally occurs (TMD, 2015).

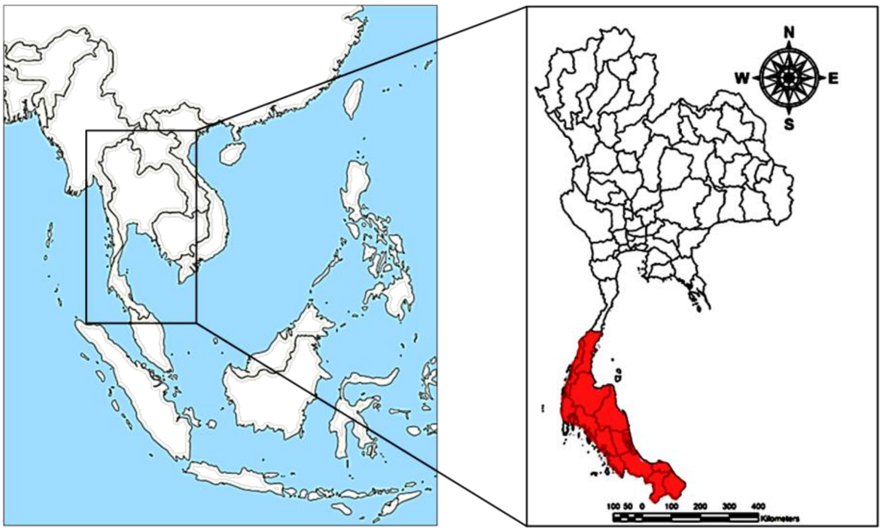


Figure 1. Study area: Southern part of Thailand

2.2 Land use reclassification

Land use data in years between 2007 and 2013 were obtained from the Land Development Department (LDD), Thailand. Reference and study years for CO_2 estimates in different provinces were differed depending on the availability of land use data (Table 1). Year 2007 was selected as the reference year due to available data for fourteen provinces. Land use data were then reclassified following the IPCC 2006 Guidelines for National Greenhouse Gases Inventories into six categories: forestland, cropland, settlement, grassland, wetland and others (IPCC, 2006). Land use changes were identified using overlay technic of GIS application.

Table 1. Land use data of fourteen provinces in Southern Thailand

Province	Ref. year	Study year	Province	Ref. year	Study year
Krabi	2007	2012	Pattalung	2007	2013
Chumphon	2007	2012	Phuket	2007	2013
Trang	2007	2013	Yala	2007	2012
Nakhon Si Thammarat	2007	2011	Ranong	2007	2013
Narathiwat	2007	2009	Songkla	2007	2012
Pattani	2007	2013	Satun	2007	2012
Phang Nga	2007	2013	Surat Thani	2007	2011

2.3 CO_2 emission estimate

In this study, CO_2 emissions for land use change from forestland to other types of land (cropland, settlement, grassland, wetland and others) were estimated following the IPCC 2006 guidelines: Tier 1 (IPCC, 2006). Details of methods and equations used were summarized in Table 2.

Table 2. Equations and default value selection from IPCC: Tier 1 approach

Land conversion	Equations	Parameter	unit	Selected value
Forest remaining forest	$\Delta CB = \Delta CG$	G_w	tonnes d.m. ha^{-1} yr^{-1}	7.0
	$= A * (G_w \times (1+R))$	R	ratio	0.37
	$* CF$	CF	tonnes C (tonnes d.m.)$^{-1}$	0.47
Forest to crop	$\Delta CB = \Delta CG+((0 -$	CF	tonnes d.m. ha^{-1} yr^{-1}	0.47
	$B_{BEFORE}) *$	G_w	ratio	2.6
	$\Delta A_{TO\_OTHER}) * CF$	B_{BEFORE}	tonnes C (tonnes d.m.)$^{-1}$	300
Forest to grass	$\Delta CB = ((B_{AFTER} -$	B_{AFTER}	tonnes d.m. ha^{-1}	16.1
	$B_{BEFORE}) *$	B_{BEFORE}	tonnes d.m. ha^{-1}	300
	$\Delta A_{TO\_OTHER})*CF$	CF	tonnes C (tonnes d.m.)$^{-1}$	0.47
Forest to settlement,	$\Delta CB = ((0 - B_{BEFORE})$	B_{BEFORE}	tonnes d.m. ha^{-1}	300
wetland and others	$*\Delta A_{TO\_OTHER})*CF$	CF	tonnes C (tonnes d.m.)$^{-1}$	0.47

It is to be remarked for parameters indicated in Table 2. ΔCB is annual change in carbon stocks in biomass. ΔCG is annual increase in carbon stocks due to biomass growth. A is area of land remaining in the same land-use category. ΔA_{TO_OTHER} is area of land use converted to another land-use category. G_w is above-ground biomass growth. R is ratio of below-ground biomass to above-ground biomass. CF is carbon fraction of dry matter. B_{AFTER} is biomass stocks on land immediately after the conversion. B_{BEFORE} is biomass stocks on land before the conversion.

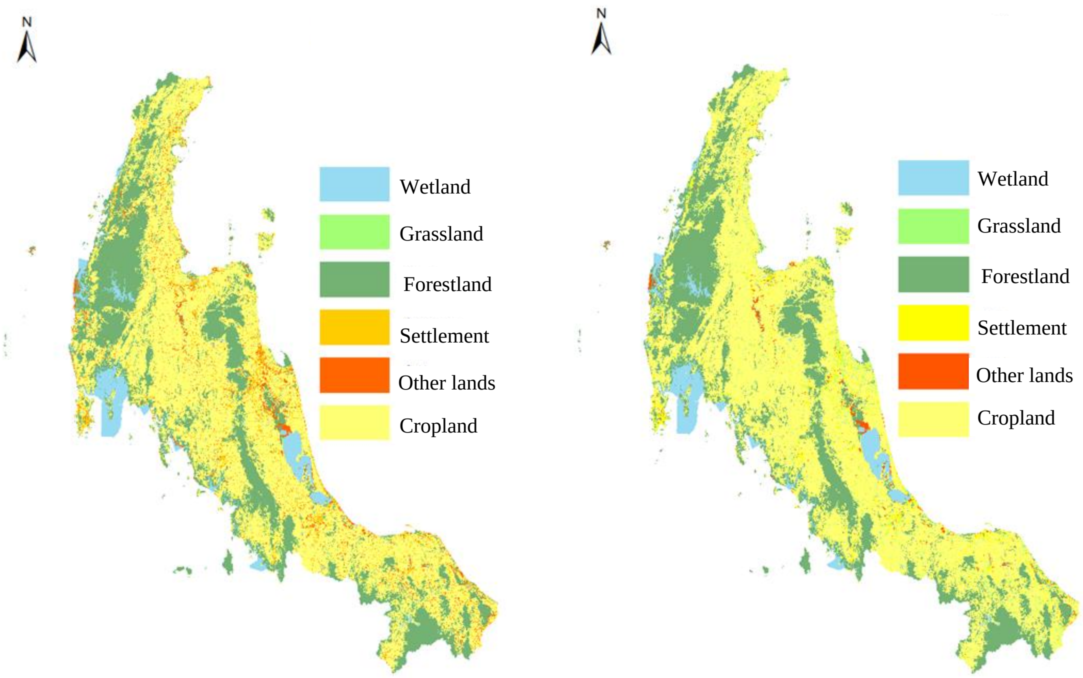
2.4 CO_2 emission and demographic relationships

Statistical analysis using correlation matrix was conducted to identify relationships between estimated CO_2 emissions and absorptions from conversion of forestland to other types of lands and demographic data. Results would be expected to be used for forestland management or land use planning of fourteen provinces in the south of Thailand. Demographic data in the reference year of 2007 such as provincial area and population were collected from the National Statistical Office (NSO) of Thailand (NSO, 2018).

3. Results and discussion

3.1 Land use change

Land use changes between reference and study years were indicated in Figure 2. According to six types of land use reclassification, cropland was found to be the major part of land use in the south of Thailand following by forestland (see also Table 3). It was observed that around 72,078 hectares of forestland was changed to other types of lands: 63,972; 1,735; 1,816; 1,460 and 3,094 hectares, receptively for cropland, settlement, grassland, wetland and others. Particularly, forestland conversion to cropland was around 90% of total change of forestland in the southern part (or around 0.12% to total land area of Thailand). World Bank (2016) also reported the annual deforestation rate of Thailand was around 0.60% per year (from 1973 to 2014). From land use changed in southern Thailand, top three provinces for forestland conversion to cropland were Phang Nga, Surat Thani and Nakhon Si Thammarat, respectively. In Southeast Asia, the demand for crop production has been a major cause of deforestation (FAO, 2018; Imai et al., 2018).



Reference Year

Study Year

Figure 2. Land use reclassification of Southern Thailand

Table 3. Land use changes in Southern Thailand between reference and study years

Land use	Area (ha)		Area change (ha)*
	Reference year	Study year	
Forestland	2,142,603	2,070,525	-72,078
Cropland	4,213,473	4,316,905	103,432
Settlement	310,439	319,489	9,050
Grassland	1,727	2,049	322
Wetland	427,695	429,572	1,877
Others	287,243	244,639	-42,604

Change to	Area (ha)
Cropland	63,972
Settlement	1,735
Grassland	1,816
Wetland	1,460
Others	3,094

Remark: Minus sign (-) is reduction of area

3.2 CO_2 emission

During the emission estimates between the reference and study years in each province, forestland was found to be changed mainly to cropland resulting in CO_2 emissions of around 32,464 Gg/year (Table 4). In contrast, remaining forestland was estimated to absorb CO_2 around 34,219 Gg/year because of its annual increase in carbon stocks due to biomass growth (IPCC, 2006). Other conversions of forestland to other types of land, excluding cropland, were together emitted atmospheric CO_2 around 6,086 Gg/year.

At provincial level for forestland conversion to cropland, Phang Nga (8,470 Gg/year), Surat Thani (7,370 Gg/year) and Nakhon Si Thammarat (4,602 Gg/year), respectively were main contributors for CO_2 emissions in the south of Thailand as the top three-provinces for the highest changes of forestland.

3.3 Relationship between CO_2 emissions and provincial demographic data

Statistical analysis via correlation matrix was used to preliminarily identify a link of CO_2 abortion/emission estimates to basic demographic data (provincial population, area and density). Result was indicated in Table 5. It is interested when we found negative correlation (-0.6092) between CO_2 emissions and population density (significant level at 0.05). Namely, the provinces with lower population density were coincident with more CO_2

emissions due to higher conversion of forestland to cropland. Demographic factors were found to be one of the main cause for lower forest area in Asian countries (Li et al., 2017; Imai et al., 2018) and for global forest changes (Sandel and Svenning, 2013). Particularly for Southeast Asia, population density and potential lowland forest area have been negatively correlated with the remaining forest area (Imai et al., 2018).

Table 4. Change of CO_2 absorption and emission at provincial level in Southern Thailand

Province	CO_2 estimate (Gg/year)						
	Absorption	Emission					
	Forestland	Cropland	Settlement	Grassland	Wetland	Others	Total
Krabi	1,884	1,468	305	63	13	42	1,891
Chumphon	2,750	2,223	26	98	9	141	2,497
Trang	2,448	1,737	25	2,001	6	55	3,824
Nakhon Si Thammarat	3,622	4,085	10	97	28	382	4,602
Narathiwat	2,621	26	56	14	13	38	148
Pattani	230	1,124	62	113	10	45	1,354
Phang Nga	3,042	7,780	86	49	272	282	8,470
Pattalung	1,159	467	11	59	1	24	563
Phuket	185	182	127	24	23	25	381
Yala	3,023	336	10	24	237	65	673
Ranong	3,196	3,351	131	3	83	385	3,953
Songkla	1,706	547	31	15	31	21	643
Satun	1,798	1,941	6	220	9	4	2,180
Surat Thani	6,557	7,197	10	54	19	90	7,370
Total	34,219	32,464	897	2,834	755	1,600	38,550

Table 5. Correlation matrix among CO_2 estimates and related demographic data

	Population	Area	Population density	Absorption	Emission
Population	1.0000	0.7618**	0.6509*	0.2639	-0.0196
Area		1.0000	0.0760	0.7709**	0.4258
Population density			1.0000	-0.4213	-0.6092*
Absorption				1.0000	0.6945**
Emission					1.0000

Remark: ** Significance at 0.01; * Significance at 0.05; n=14

However, in this preliminary analysis, only population, area and density were included as it is represented the basic demographic data. Further analysis to be included other demographic factors, i.e., income, topography (low/high forestlands), occupation and employment rate should help more understanding on the relationship between CO_2 absorptions/emissions and typical demographic pattern in each province.

4. Conclusions

CO_2 emission and absorption from forestland conversion to other types of lands in the south of Thailand were assessed. Forest changed to cropland was found to be the main conversion with associated emission of CO_2 around 32,464 Gg/yr. At provincial level, Phang Nga, Surat Thani and Nakhon Si Thammarat were main driver on CO_2 emissions. Lower population density provinces tended to emit more CO_2.

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Vegetation Survey and Applied Remote Sensing Techniques for Monitoring Carbon Storage in Reclaimed Land of Reforestation at Banpu Lignite Mine, Northern Thailand

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Abstract

Monitoring carbon storage in the above-ground plant biomass on reclaimed land of 18-year-old plantation forest (PF) covered previously by dry dipterocarp forest (DDF) was investigated at Banpu mine, northern Thailand. In 2015, field vegetation survey was done in the PF and adjacent DDF using sampling plots, each of size 40×40 m, 12 plots for the PF and 10 plots in the DDF. Plant data were obtained by measuring stem diameters at breast height (DBH) and tree heights. Amounts of biomass and carbon storage (CS) were calculated using allometric equations. Relationship between the CS and vegetation index of satellite images was taken from 2015 Landsat image for multiple regression analysis to obtain the best correlation. The CS equation used to monitor the CS before and after reforestation included year 1987, 1990, 1995, 2000, 2005, 2010, 2015, 2016 and 2017. The PF and DDF consisted of 47 and 98 species, respectively, whereas the CS amounts were measured at 43.63±8.60 Mg ha^{-1} and 56.26±12.0 Mg ha^{-1}. The best equation for estimating the CS in plots represented correlation of the ratio vegetation index (RVI) and normalized difference vegetation index (NDVI). In the PF, CS = (154NDVI)+(103RVI)-76 (R^2 = 0.96) was used to calculate the CS during and after mining. As for the DDF, CS = (154NDVI)+(103RVI)-76 (R^2 = 0.91) was applied for the CS before mining. The CS amounts in reclaimed land were varied: before mining (1987), 81.74 Mg ha^{-1}, after mining, 0.72 Mg ha^{-1} in 1990 and 10.69 Mg ha^{-1} in 1995, and during stand development (1997, 2000, 2005, 2010, 2015, 2016, 2017) were in the following order; 15.18 Mg ha^{-1}, 24.96 Mg ha^{-1}, 35.12 Mg ha^{-1}, 36.77 Mg ha^{-1}, 41.48 Mg ha^{-1}, 50.08 Mg ha^{-1} and 36.83 Mg ha^{-1}. After 18 years reforestation, the CS in above-ground biomass was increased to 75.55% adjacent natural forest. The decreased CS in 2017 was caused by standing dead trees and illegal tree cutting.

Keywords: Carbon, Monitoring/ Plantation forest/ Remote sensing/ Reclaimed mine land

1. Introduction

Mining is mineral resource extraction for human welfare. In Thailand, mining is one major primary industry which supports infrastructure construction. However, mining industries have perceived negatively due to its impacts on environment and human communities. (Duzgun *et al.*, 2011). The mine companies must follow the laws of reducing environmental and human community impacts and should follow academic principles of mine activities. The Banpu company had engaged in lignite mine by opened pit mining during 1987 and 2014. The original forest on mine land was the dry dipterocarp forest on sandstone. In the first step of mining, the forest was clear cut, surface soil, and materials in deeper layers containing rocks of sandstone, conglomerates and mudstone were moved to the dumping area. Lignite was found at about 50 to 100 m depth in the mudstone layer.

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These rocks containing pyrite and high manganese concentrations, and thus have very strongly acid. In 2004, the company began land reclamation in the area by forest plantation. The main species were teak (Tectona grandis), Acacia mangium, Lagerstroemia tomentosa, Azadirachta indica, Acacia auriculiformis, Senna siamea, Syzygium cumini and Afzelia xylacarpa. The DDF nearby the mine land covers on xeric sites with a poor shallow soil. Dominant species are dipterocarps including Shorea obtusa Shorea siamensis, Dipterocarpus obtusifolius and Dipterocapus tuberculatus. As the same time of clear cutting of the DDF in mine land, the forest outside the mine project was also disturbed by local people through selective tree cutting. Tree cutting in the forest is now prohibited as it is become the community forest. Carbon storage in forest ecosystem is the photosynthetic processes of removing carbon dioxide (CO2) from atmosphere and storage in plant tissues and organs. The CS rates in natural forests depend on plant growths, species diversity and forest conditions. It is recognized that the CS in forest ecosystem can reduce CO2 in atmosphere and mitigate global warming (Landsberg and Gower, 1997; Waring and Running, 1998). However, the CS potentials varied among forest types and subtypes having the poor or good conditions (Seeloy-ounkeaw *et al*., 2014; Phongkhamphunh *et al*., 2015). As for plantation forests, the CS in plant biomass indicates efficacy of forest plantation related to the rates of survival, plant growth and production. The CS is usually increased with plantation ages of variable rates depending upon species and sites. The destruction of planted trees as standing dead trees caused by many natural agents and selectively tree cutting can reduce the production of plantation forest.

Remote sensing and satellite image processing are acceptable detailed information for classification in land cover and monitoring changes of land use (Franklin, 2001). The major utilization of remote sensing in mine monitoring is the inspection of area under planning the satellites and continuous data acquisition, it serves a large collection of historical data (Duzgun *et al*., 2011). The vegetation and soil indices can be used use to monitor the growth of vegetation in reclaimed mine land in comparison with the nearby forest (Suppagarn *et al*., 2016). Pattanakiat *et al*. (2012) used remote sensing technique for estimating the CS in forest plantations of eucalyptus, para rubber, and teak to be the amounts of 25.9, 135.03 and 38.50 Mg ha-1, respectively. The CS rates of plantation forest in coal mine land depended on the plantation ages and increased rapidly at the age of 5 to 6 years after planting (Yusanto, 2016). Boonsang *et al*., (2011) studied the correlation of above-ground carbon sequestration with vegetation by remote sensing techniques of the dry evergreen forest, hill evergreen forest, mixed deciduous forest and the DDF. The coefficient of determination (R^2) of the best-fit model for estimation the CS varied from 0.741 to 0.854. This research aims to monitor change of carbon storage in standing above-ground plant biomass on reclaimed land of Banpu lignite mine, northern Thailand by field vegetation survey and remote sensing (RS) technique. The data are useful information for monitoring the efficacy of reforestation and forest ecosystem recovery in mine land and assess carbon credit for the mining project as well as guidelines for improving forest plantation practices

2. Methodology

This research was conducted at Ban Pu lignite mine, Lamphun province, northern Thailand. It is about 150 km to the south of Chiangmai city. The mine project covers an area of about 184 ha includind 40 ha dumped area with an altitude range from 436 to 507 m above sea level. The original forest in this area is mainly the dry dipterocarp forest covering on sloping areas with the poor forest condition because it is close to the Ban Hong village and the villagers had done the selective cutting of big and intermediate-sized trees since long time ago. It has now become the community forest of the village, and tree cutting is prohibited. The forest along the streams and flat area is the mixed deciduous forest with dominated teak, however, most areas are changed to be paddy fields since the soil is fertile and has the high moistures during the rainy season. The DDF has a poor shallow soil containing many gravels and fragmented rocks of sandstone. At the end of the mining project, the land must be restored by reforestation to be the forest according to the law since it is situated in the national reserved forest, and two deep ponds have remained in the area.

2.1 Vegetation Sampling and Estimation of Biomass-Carbon Amounts

A method of plant community analysis was used for field vegetation survey using sampling plots, each of size 40x40 m, 12 plots in the PF and 10 plots in the DDF. The plots were arranged randomly in these forests. Plant data were obtained by measuring stem girths at breast height (gbh, 1.3 m above ground) and tree heights of all tree species with height over 1.5 m. All plots were located using the GPS. The data were calculated for plant biomass in each plot using allometric equations (1) (Sahunalu, 1995).

$$W = A\,(D^2H)^h \qquad (1)$$

Where, W = biomass of stem, branch, leaf or root (dry weight per area)
D = diameter (cm)
H = tree height (m)
A, h = constant

For the deciduous forest, tree biomass was calculated by Ogino *et al*. (1967).

W_S (stem) $= 189\,(D^2H)^{0.902}$ (kg/tree)
W_B (branch) $= 0.125\,W_S^{1.204}$ (kg/tree)
$: D^2H = m^3$
W_L (leaf) $= 1/(11.4/W_S^{0.90} + 0.172)$ (kg/tree)

Root biomass was calculated by Ogawa *et al*. (1965)
$W_R = 0.026\,(D^2H)^{0.775}$
$: D^2H = (cm^2.m)$

Carbon amounts in biomass of stem, branch, leaf and root were calculated by multiplying biomass amount with the carbon content according to average values investigated by Tsutsumi *et al*. (1983). Average carbon contents in stem, branch, leaf and root are 49.90%, 48.70%, 48.30% and 48.12%, respectively.

2.2 Monitoring Carbon Storage Using Remote Sensing Techniques

The CS in above-ground plant biomass in the PF and the DDF were estimated using LANDSAT-8 image captured in November 2015; Band 2 (Blue: B), Band 3 (Green: G), Band 4 (Red: R) and Band 5 (Near Infrared: NIR). The relationship was taken between the CS (variable independent) with energy reflected from earth's surface, the actual wavelength associated with vegetation including vegetation index, ratio vegetation index (2), normalized difference vegetation index (3), transformed vegetation index (4) and green vegetation index (5).

$$RVI = R/NIR \qquad (2)$$
$$NDVI = (NIR-R)/(NIR+R) \qquad (3)$$
$$TVI = ((NIR-R)/(NIR+R)+0.5)^{0.5} \qquad (4)$$
$$GVI = -0229G-056R+0.6IR+0.49IR \qquad (5)$$

The correlation analysis was used to explore relationship between independent and dependent variables for decision making to choose independent variables in the equation. The stepwise multiple regression analysis was applied as the equation (6). The best equation for estimating the CS was used for calculation of carbon amounts in biomass of the PF and the DDF. The best equation for estimating the CS in the DDF obtained by LANDSAT-5 imageries in 1987, 1990, 1995 before planting in the dumped area. The equation for the PF was applied to calculate the CS after revegetation in reclaimed land by LANDSAT-5 imageries in 1997, 2000, 2005 and 2010, LANDSAT-8 (8 Bit) imageries in 2015, 2016 and 2017.

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_nX_n \quad (6)$$

Where, Y = Carbon Storage from Calculation of Field Data
$X_1, X_2, X_3, \ldots, X_n$ = Satellite Image Data (G, R, IR, IR–R, IR/R, NDVI, TVI and GVI)
a = Y - Intercept
b1, b2, b3, b4,…,bn = Coefficient of Regressi

3. Results

3.1 Species Composition, Richness and Tree Densities in Forest Communities

A total of 47 species (38 genera and 20 families) within 12 plots were existed in the PF. These included 9 big trees, 11 medium trees, 19 small trees, 2 shrubs and 6 climbers. Species richness varied among plots, 5 to 15 species, including planted and successional species. About ten species of native and exotic species were planted. The most abundant species was teak (*Tectona grandis*), followed by *Lagerstroemia tomentosa* and *Azadirachta indica*.

In the DDF, species richness was higher (98 species, 85 genera, 45 families) including 20 big trees, 28 medium trees, 37 small trees, 5 shrubs and 8 climbers. *Shorea obtusa* was the most abundant, followed by *Shorea siamensis* and *Dipterocarpus* *obtusifolius*. All tree species had the great variations of stem girths and heights. Teak had the highest gbh among species, 45.77 cm on average. The others had values in a range of 40.53 to 43.43 cm, except for *Senna siamea* which had the lower value, 28.71 cm. Tree heights of most species were nearly the same, 11 to 12 m, except for *Senna siamea* (8.6 m). All species had almost the same crown widths, 5.5 to 5.9 m. Tree densities in the PF varied among plots, 394 to 1,244 trees ha^{-1}, 661$\pm$231 trees ha^{-1} on average. Teak had the highest density, 314 trees ha^{-1} (47.50% of the total), followed by *Lagerstroemia tomentosa*, *Azadirachta indica*, *Acacia mangium*, *Acacia auriculiformis* and *Senna siamea*. In the DDF, the average density was higher (2,775$\pm$583 tree ha^{-1}). Among 98 species, *Shorea obtusa* had the highest density, 688 trees ha^{-1} (24.79% of the total), followed by *Shorea siamensis*, *Dipterocarpus obtusifoliua*, *Gluta usitata*, *Canarium subulatum*, *Aporosa villosa*, *Lannea coromandelica* and *Dalbergia oliverli*. These 8 species accounted for 72.10% of the total.

3.2 Estimation of Plant Biomass and Stored Carbon by Field Vegetation Survey

In Table 1, the total amounts of above-ground plant biomass (Total AB) within 12 plots of the PF varied from 52.53 to 112.55 Mg ha^{-1}, 88.06±17.38 Mg ha^{-1} on average. It excluded the root biomass of 8.66 Mg ha^{-1}. The biomass in stem, branch and leaf were estimated at 71.71%, 23.52% and 4.76%, respectively. Among 47 species, teak had the highest biomass, 59.06 Mg ha^{-1} or 61.32% of all species. The CS in biomass of the PF also varied among 12 plots, 43.63±8.60 Mg ha^{-1} on average. The biomass in the DDF (Table 2) were varied among 10 plots, 113.47±24.22 Mg ha^{-1} on average with excluding root biomass of 16.89 Mg ha^{-1}. The amounts in stem, branch and leaf were calculated in the following order: 74.38%, 23.12% and 2.49%, respectively. Among 98 species, *Shorea obtusa* had the highest biomass, 26.09 Mg ha^{-1} (20.01% of all species). The average CS amount in the DDF was measured at 56.26±12.0 Mg ha^{-1}. The CS in the PF was 77.55% of adjacent DDF.

Table 1. Carbon amounts stored in plant biomass within 12 plots of the PF

Plot No.	Standing plant biomass (Mg ha^{-1})					Carbon (Mg ha^{-1})				
	Stem	*Branch*	*Leaf*	*Root*	*Tot l (AB)*	*Stem*	*Branch*	*Leaf*	*Root*	*Total (AB)*
PF01	65.17	21.43	3.45	10.28	90.05	32.52	10.43	1.67	4.94	44.62
PF02	80.3	24.83	7.42	9.45	112.55	40.07	12.09	3.58	4.55	55.74
PF03	66.39	23.94	2.67	10.46	93.00	33.13	11.66	1.29	5.04	46.08
PF04	76.65	26.04	5.00	10.27	107.69	38.25	12.68	2.41	4.94	53.34
PF05	62.58	20.17	3.95	8.90	86.70	31.23	9.82	1.91	4.28	42.96
PF06	70.00	24.22	3.20	10.64	97.42	34.93	11.79	1.54	5.12	48.26
PF07	65.76	21.18	4.80	8.64	91.74	32.82	10.32	2.32	4.16	45.46
PF08	58.71	19.15	5.42	8.22	83.28	29.30	9.33	2.62	3.96	41.25
PF09	51.76	15.82	4.22	6.08	71.80	25.83	7.70	2.04	2.93	35.57
PF10	74.11	23.06	5.66	8.00	102.83	36.98	11.23	2.74	3.85	50.95
PF11	38.29	12.06	2.18	5.83	52.53	19.11	5.87	1.05	2.80	26.03
PF12	48.10	16.68	2.38	7.18	67.16	24.00	8.12	1.15	3.45	33.27

Table 1. Carbon amounts stored in plant biomass within 12 plots of the PF (Cont.)

Plot No.	Standing plant biomass (Mg ha^{-1})					Carbon (Mg ha^{-1})				
	Stem	*Branch*	*Leaf*	*Root*	*Tot l (AB)*	*Stem*	*Branch*	*Leaf*	*Root*	*Total (AB)*
Mean	**63.15**	**20.72**	**4.20**	**8.66**	**88.06**	**31.51**	**10.09**	**2.03**	**4.17**	**43.63**
S.D.	*12.28*	*4.18*	*1.55*	*1.67*	*17.38*	*6.13*	*2.04*	*0.75*	*0.80*	*8.60*
C.V.	*19.45*	*20.17*	*36.89*	*19.28*	*19.73*	*19.44*	*20.18*	*36.89*	*19.18*	*19.72*

Table 2. Carbon amounts stored in plant biomass within 10 plots of the DDF

Plot No.	Standing plant biomass (Mg ha^{-1})					Carbon (Mg ha^{-1})				
	Stem	*Branch*	*Leaf*	*Root*	*Total (AB)*	*Stem*	*Branch*	*Leaf*	*Root*	*Total (AB)*
DDF01	88.23	28.65	2.82	17.32	119.70	44.02	13.95	1.36	8.33	59.33
DDF02	92.83	28.92	3.02	18.41	124.77	46.32	14.08	1.46	8.86	61.86
DDF03	75.53	24.42	2.25	14.57	102.20	37.69	11.89	1.09	7.01	50.67
DDF04	53.49	14.82	2.17	11.51	70.48	26.69	7.22	1.05	5.54	34.96
DDF05	85.83	24.29	3.38	18.22	113.50	42.83	11.83	1.63	8.77	56.29
DDF06	83.61	24.69	3.04	17.21	111.34	41.72	12.03	1.47	8.28	55.22
DDF07	89.07	26.34	3.21	18.32	118.62	44.45	12.83	1.55	8.82	58.83
DDF08	72.65	22.96	2.33	14.36	97.94	36.25	11.18	1.13	6.91	48.56
DDF09	81.82	24.60	2.85	16.63	109.27	40.83	11.98	1.38	8.00	54.19
DDF10	120.97	42.68	3.19	22.37	166.84	60.36	20.79	1.54	10.76	82.69
Mean	84.4	26.24	2.83	16.89	113.47	42.12	12.78	1.37	8.13	56.26
S.D.	*17.08*	*6.97*	*0.43*	*2.93*	*24.22*	*8.52*	*3.39*	*0.21*	*1.41*	*12*
C.V.	*20.24*	*26.55*	*15.28*	*17.35*	*21.35*	*20.24*	*26.55*	*15.15*	*17.34*	*21.33*

Table 3. Vegetation index and carbon amounts stored using remote sensing techniques in PF and DDF

Plot	Vegetation Index		Carbon (Mg ha^{-1}) : *Total (RS)*	Plot	Vegetation Index		Carbon (Mg ha^{-1}) : *Total (RS)*
	NDVI	*RVI*			*NDVI*	*RVI*	
PF01	0.657	0.207	57.86	DDF01	0.670	0.198	57.86
PF02	0.752	0.141	70.03	DDF02	0.756	0.139	70.03
PF03	0.671	0.197	51.28	DDF03	0.616	0.237	51.28
PF04	0.724	0.16	35.83	DDF04	0.441	0.390	35.83
PF05	0.642	0.221	56.91	DDF05	0.662	0.203	56.91
PF06	0.681	0.191	53.81	DDF06	0.638	0.221	53.81
PF07	0.667	0.200	61.32	DDF07	0.696	0.179	61.32
PF08	0.553	0.288	47.18	DDF08	0.580	0.266	47.18
PF09	0.517	0.323	52.64	DDF09	0.628	0.229	52.64
PF10	0.700	0.176	76.76	DDF10	0.800	0.111	76.76
PF11	0.339	0.496	56.36				
PF12	0.431	0.398	*11.46*				
Mean	0.61	0.25	*20.33*	Mean	0.65	0.22	56.36
S.D.	*0.13*	*0.11*	57.86	*S.D.*	*0.1*	*0.08*	*11.46*
C.V.	*20.59*	*42.79*	70.03	*C.V.*	*15.15*	*35*	*20.33*

3.3 Monitoring Carbon Storage by Remote Sensing

Table 3 and table 4 shows carbon amounts in above-ground biomass on reclaimed mine land before, during and after mining estimated by remote sensing techniques based on 12 plots and total area (39.75 ha) approaches. The LANDSAT image was interpreted as vegetation index. The significant indices for estimating the CS are NDVI and RVI. The NDVI range of the PF was 0.339 to 0.752 and 0.441 to 0.800 for the DDF. The RVI ranges of the PF and the DDF were 0.141-0.496 and 0.111-0.390, respectively. The CS amounts within 12 plots of the PF and 10 plots of the DDF were correlated to the vegetation index, and then the formula for determining the CS using the remote sensing techniques was obtained. The best-fit model was taken for estimation of the above-ground carbon amounts in study plots of the PF and the DDF. The equation for estimating the CS in the PF was CS = (154NDVI)+(103RVI)-76 with a coefficient of determination (R^2) of 0.96 for calculation of

Table 4. Carbon amounts in above-ground plant biomass on reclaimed land of Banpu lignite mine estimate by remote sensing technique

Year	1987	1990	1995	1997	2000	2005	2010	2015	2016	2017
A. Mean value of total land area;39.75 ha (Mg ha^{-1})										
Minimum	73.12	0.00	9.22	9.22	23.22	23.22	23.31	23.23	1.90	0.79
Maximum	104.00	50.79	16.87	31.95	28.03	45.20	45.17	78.00	184.00	166.00
Mean	81.74	0.72	10.69	15.18	24.96	35.12	36.77	41.48	43.52	36.83
S.D.	*7.40*	*13.75*	*1.45*	*5.22*	*1.09*	*5.43*	*4.07*	*10.51*	*24.14*	*33.81*
B. Mean value of 12 Sampling plots (Mg ha^{-1})										
Minimum	74.13	0.00	1.56	9.31	23.41	28.13	32.82	27.26	30.77	1.10
Maximum	90.51	1.14	10.25	12.83	26.21	44.11	45.17	54.40	56.10	49.13
Mean	79.66	0.09	4.70	10.32	24.78	35.63	37.84	43.85	46.11	30.83
S.D.	*4.58*	*0.33*	*2.56*	*1.06*	*1.08*	*5.16*	*3.33*	*8.43*	*8.19*	*11.90*

The CS during and after mining. As for the DDF, CS = (154NDVI)+(103RVI)-76 with R^2 = 0.91 was used to estimate the CS before mining. The CS amounts in reclaimed land were varied: before mining in 1987, 81.74 Mg ha^{-1}, after mining (1990, 1995), 0.72 Mg ha^{-1}and 10.69 Mg ha^{-1}, respectively, and during stand development (1997, 2000, 2005, 2010, 2015, 2016, 2017) were in the following order; 15.18 Mg ha^{-1}, 24.96 Mg ha^{-1}, 35.12 Mg ha^{-1}, 36.77 Mg ha^{-1}, 41.48 Mg ha^{-1}, 50.08 Mg ha^{-1}and 36.83 Mg ha^{-1}.

The CS in the reclaimed land (Figure 1) was decreased suddenly from 81.74 Mg ha^{-1} in 1987 (before mining) to 0.72 Mg ha^{-1} in 1990 (mining operation) at the rate of 27.01 Mg ha^{-1} per year. The CS had the slow increase from 1990 to 1995 (before planting) at the rate of 1.99 Mg ha^{-1} per year which might be caused by the natural revegetation. The CS was increased in the more rapid rate (2.49 Mg ha^{-1} per year) after planting ground-covered species in 1995 to forest plantation in 1997 and to 2005. After mining (2005 to 2016), the CS during stand development was increased at the rate of 1.50 Mg ha^{-1} per year, and it was declined sharply in 2017 (13.25 Mg ha^{-1} per year) caused by many dead big trees and illegal tree cutting. As the tree age of 18 years old especially teak, the stem size was big enough for wood utilization. The roots were also assumed that damage by toxic substances as tree roots penetrated deeply into the deeper soil horizons in the dumped area.

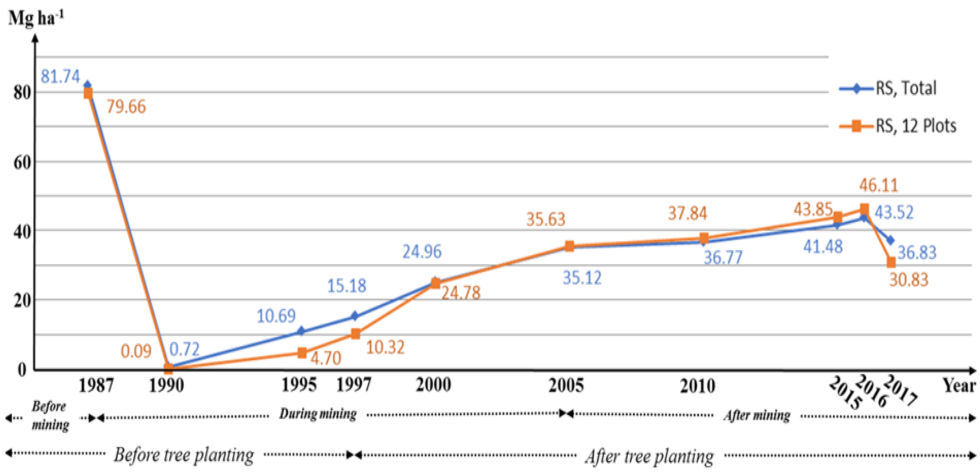

Figure 1. Monitoring change of the CS in reclaimed land of Banpu lignite mine by remote sensing

4. Discussion and Conclusions

The field vegetation survey for estimating carbon storage in the forests provided details of species composition, richness, diversity and quantitative features of plant species. The appropriate plot size and adequate number of sampling plots are required (Greig-Smith, 1983). The biomass amounts in the forests can be estimated using allometric equations, and then measure the CS in the plant biomass by multiplying carbon contents with biomass in different organs including stem, branch, leaf and root. This method has been recognized by most forest ecologists. In this study, the field vegetation study was carried out in 2015 by a method of plant community analysis. The plot size of 40x40 m was used as the minimal area of sampling plot investigated by previous researchers. The number of 12 plots were used for the PF whereas ten plots for the natural forest, the DDF because of more variable plant communities in the PF. The allometric equations of deciduous forest in Thailand studied by Ogino *et al* (1967) and Ogawa *et al* (1965) were used, and the carbon amounts stored plant biomass were calculated. The new allometric equations of the forests in each location cannot obtained at the present because tree cutting in forests is strictly prohibited by the law in Thailand. The contribution of each tree species to the CS in the PF and the DDF was determined. In the PF, variation of plant communities in the dumped area was observed since seedlings of about ten native and exotic species were planted irregularly. As seedlings grew up to be the trees after planting, different plant communities had developed in the area. Within PF 12 plots, seven plots or plant communities were dominated by teak, and the remains were *Azadiracta indica*, *Lagerstroemia tomentosa* and *Acacia auriculiformis*. The different species composition, richness and diversity among plant communities in study plots are thought to have the different percentages of light reflectance according to Kimmins (2004) since they usually have different compensation point, the light intensity which CO_2 uptake is equal to CO_2 release in respiration. The light intensity and reflection from the forest canopy may be different among different sampling plots. In the DDF, the variable plant communities were also caused by dominant species, species composition, tree densities and forest conditions. Within 10 plots, seven plots were dominated by *Shorea obtusa*, and the remains were *Shorea siamensis* and *Dipterocarpus obtusifolius*. The species richness, tree densities and forest condition index varied in the following order: 29 to 50 species, 271 to 608 trees ha^{-1}, and 0.50 to 6.68.

The field study practiced in 2015 provide significant data for the first evaluation of the CS in the PF and the DDF, however, monitoring by remote sensing could provide the estimated data of the CS before, during and after mining. It can save the time consume and reducing cost of the monitoring (Suppagarn *et al*., 2016). The application of remote sensing technology to monitor the reclaimed reforestation area is the good choice but it has some limitations of selecting the satellite images. Field data collection should be taken at the same time as satellite imagery data (Nuanurai, 2005). Boonsang *et al*., (2011) recommended that the moisture or drought at any season have effects on the record of satellite data. In this study, the study area was deciduous forests, therefore, the remote sensing technique could be not taken in May to September (rainy reason) caused by the cloud effect, and from February to April (dry season) because it was the fall season and no leaves on the trees. The period between November and January may be suitable for the investigation and use of satellite images for studying the CS in the forests of northern Thailand.

The CS monitoring in above-ground plant biomass on reclaimed land of Banpu lignite mine could be obtained by remote sensing techniques combined with the field vegetation survey. The techniques can save cost and time, and estimate the CS in the past. Application of the techniques to other reclaimed mine lands of reforestation are useful and will give the comparative results. In this study, the CS was declined after 2016 because some standing dead trees were observed by the impacts of toxic substances in deep layers of the soil in the dumped area, and illegal tree cutting by local people. Improving techniques of forest plantation is required to increase the plant species diversity, biomass and carbon storage.

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Evaluation of Cassava Germplasm for Drought Tolerance Breeding Program in Thailand

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Abstract

Even though cassava can survive in arid agricultural areas, drought can cause cassava root yield tremendous reduction depending on stress severity and affected cassava developmental stage. To be prepared for more severe drought stress in the future due to climate change, cassava germplasm of 255 varieties, which were originated in Thailand and from other countries, were evaluated together with five check varieties in augmented design for experimental trial. Relative leaf size, relative leaf greenness, relative height, and relative leaf retention were calculated from the data collected at 8, 10, and 12 months after planting which were the periods which cassava facing drought until recovery from drought in Huay Bong district, Nakhon Ratchasrima province. The normal distribution of normalized data of relative values indicated that from 8 to 10 months after planting was the period for cassava adaptation to drought and from 10 to 12 months after planting was the period for cassava growth recovery from drought stress. There were positive correlations among these traits except for plant height. K-mean clustering analysis showed cassava varieties grouped upon the different responses to drought adaptation and recovery patterns. Taken from overlapped varieties appearing in all advantage adaptation pattern groups, eight varieties of 255 became promising varieties suggested for drought tolerant breeding program due to the abilities to maintain leaf expansion, leaf greenness, height growth, and leaf retention through drought period.

Keywords: Cassava/ Drought tolerance/ Leaf expansion/ Leaf greenness/ Leaf retention/ Plant height

1. Introduction

Thailand also faces a challenge of global climate change as other countries particularly on agricultural impact. Within this decade, Thai farmers had to confront unpredictable severe drought and waterlogging problems. Even though cassava (*Manihot esculenta* Crantz) can adapted to drought-prone areas better than cereals and legumes (Jarvis *et al*., 2012), a huge reduction in biomass (-87%) and root yield (-95%) also be reported in Aina et al. (2007). Cassava has five distinctive developmental stages (Alves, 2002) which are 1) emergence of sprouting stage, 2) leaf and root system development stage, 3) canopy establishment, 4) carbohydrate accumulation in root stage, and 5) dormancy stage. According to this model, dormancy stage starts at 10 months after planting. However, this model was proposed in the environment having rainfall almost all year round. Therefore, confronting water shortage at different stages can cause different effect to root yield. The most critical stage is in the early stage starting from sprouting until the end of canopy establishment especially in the first five months after planting. Water deficit in the first two months could cause yield loss from 32 to 60% (Alves, 2002). Conner and Cock (1981) also reported that cassava leaf responded quickly to drought via leaf falling. However, recovery is the strategy that cassava can adapt to drought stress. Vandegeer *et al*. (2013) reported that cassava at 4 months after planting treated with water deficit at 25% of field capacity for two weeks had yield loss to 83% with leaf area reduction to 45%. However, after re-watering for

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two weeks, cassava can recover to reach the same level as yield of control. In most region of Thailand, rainy season last only 8 to 9 months which means cassava in Thailand will turn into dormancy right at the end of canopy establishment stage (Onwuem, 1978). The similar problem also occurred in Latin America, (de Oliveira et al., 2017) reported the total annual rainfall in their drought tolerance evaluation field in 2012/2013 for 164 mm which could be distributed into 71, 49, 16, and 27 mm quarterly and in 2013/2014 for 289 mm which could be distributed into 166, 58, 12, and 53 mm quarterly. With this rainfall pattern, plant may encounter water deficit in the age of 6-9 months after planting which is in the period of canopy establishment and carbohydrate accumulation which not cause severe yield reduction as drought effect in the early stages, but it can cause moderate reduction of yield (Alves, 2002). For this reason, cassava production in rain-fed areas in Thailand cannot meet the optimum yield.

Traits involving root yield have been reported profoundly in aspects of agronomic traits such as root yield *per se*, dry matter yield, starch content, starch yield and harvest index. Other traits which can assist cassava to reach optimum yield or maintain yield under constraints in both biotic and abiotic stresses. Drought tolerant traits are one of the important characters for promoting cassava yield under water shortage conditions such as ability to maintain leaf expansion and leaf retention, closing stomata, osmotic adjustment and increase root length (Okogbenin *et al*., 2013; El-Sharkawy, 1993). In a large germplasm evaluation, the effect of genetic variation is the major variance occurring in each parameter variation. To avoid the genetic variance, the relative values should be used to compare among genetype (de Oliveira et al., 2017). However, in previous experiments, the relative values were calculated from agronomic traits such as root yield *per se* by comparing values under well-watered and drought conditions (de Oliveira et al., 2015; de Oliveira et al., 2017). There have never been former studies before to evaluate relative values for drought adapting traits such as leaf expansion, leaf retention, and height. Thus, the objective of this study was to evaluate relative change in leaf size, leaf greenness, plant height, and leaf retention to group varieties from these traits and identify cluster that have positive adaptation toward drought and recovery from drought periods. The impact of this study to cassava breeding program is to identify accessions which have good drought adaptation and drought recovery to be used as parents in breeding program to cross with high yield varieties to produce lines with ability to maintain optimum yield under water deficit conditions.

2. Methodology

2.1 Experimental design

Two hundred and fifty five cassava varieties were evaluate together with five check varieties which were Kasetsart 50 (KU50), Huay Bong 60 (HB60), Huay Bong 80 (HB80), Rayong 72 (R72), and Rayong 5 (R5). Experimental design was augmented design for 21 plots. There were 20 varieties in each plots which were 15 varieties from germplasm collection and 5 check varieties. Plant density was 1x1 m^2. Each variety was grown in line for 10 plants per line. Cassava stakes were planted in May 2017 and harvested in April 2018 at Tapioca Development Institute (TDI), Dan Khun Thot district, Nakhon Ratchasima province. The soil texture in the experimental site was loam with 51% sand, 34% silt, and 15% clay with 3.19% organic matter. Soil contained 0.06% total Nitrogen, 39 mg kg^{-1} Phosphorus, 99 mg kg^{-1} Potassium, 1,723 mg kg^{-1} Calcium, 62 mg kg^{-1} Magnesium with 0.36 dS m^{-1} under pH 7.8. Plants were fertilized with 46.9 kg ha^{-1} of total N, 46.9 kg ha^{-1} of P_2O_5, and 46.9 kg ha^{-1} of K_2O applied at 30 days after planting.

2.2 Data collection and analysis

Data were collected three times which were at 8 (December 2017), 10 (February 2018), and 12 (April 2018) months after planting. For each trait, three varieties were measured. For leaf size, the middle leaf lope of the first fully expanded leaf of each plant was used to measure lope length and lope width (Okogbenin *et al*., 2013). For leaf greenness, leaf chlorophyll meter was used to measure leaf greenness of the same leaf used for leaf size measurement. Plant height and leaf retention were measured from three plants per variety. Leaf retention was calculated from ratio of plant height with leaf divided by a whole plant height. Each trait was calculated as relative value from one period of time to another period of time to avoid genetic effect on values. Values used in this experiment were normalized using values of five check varieties from every plot. Data after normalization were standardized before cluster analysis using K-mean clustering by R program commanded by STAR software (IRRI).

Values mentioned in the result were described as follows:

ll1 = leaf length measured at 8 months / leaf length measured at 10 months,

ll2 = leaf length measured at 10 months / leaf length measured at 12 months,

lw1 = leaf width measured at 8 months / leaf width measured 10 months,

lw2 = leaf width measured at 10 months / leaf width measured 12 months,

sp1 = leaf greenness measured at 8 months / leaf greenness measured 10 months,

sp2 = greenness measured at 10 months / leaf greenness measured 12 months,

lh1 = plant height measured at 10 months - plant height measured 8 months,

lh2 = plant height measured at 12 months - plant height measured 10 months,

z1 = height with leaf retention measured at 8 months / height with leaf retention measured at 10 months,

z2 = height with leaf retention measured at 10 months / height with leaf retention measured at 12 months,

pz8 = percentage of leaf retention height at 8 months; which was calculated from height with leaf retention at 8 months / total height at 8 months *100

pz10 = percentage of leaf retention height at 10 months; which was calculated from height with leaf retention at 10 months / total height at 10 months *100

pz12 = percentage of leaf retention height at 12 months; which was calculated from height with leaf retention at 12 months / total height at 12 months *100

3. Results

3.1 Distribution pattern of values indicate drought adaption and recovery trends

Cassava germplasm collection was maintained at Tapioca Development Institute (TDI), Dan Khun Thot district, Nakhon Ratchasima province. To evaluate the drought adaptation ability of these varieties/accessions, fully expanded leaf lope size, leaf greenness, leaf retention and plant height were measured and calculated for the relative values which indicated change from one period of time to another. At 8 months after planting in December 2017, even though rainfall started to decrease and RF/PE decrease rapidly lower than 1 indicating water shortage (Table 1), it was the first month in dry season. Therefore, plants still gained moisture from soil. But in February 2018, rainfall decreased with RF/PE lower than 0.5 for three months in a row can cause leaf shedding. From Table 2, ll1 is the ratio of leaf length measured at 8 months to 10 months. If the number was low than 1, it meant the length of the first fully expanded leaf in 10 months after planting was smaller than those in 8 months after planting. In the normalized value of this ratio in cassava germplasm, the maximum was 1.66 which meant that some varieties had higher leaf length in low soil moisture condition than that in higher soil moisture condition. The minimum data showed -0.1 due to the normalized value could have value in negative value. The mean of this ratio was 0.87 which meant that the average adaptation pattern of this germplasm tended to have lower leaf length in dry condition. In 12 months after planting (April 2018), rainfall increased again and RF/PE reached 0.8 (Table1). The re-watered soil moisture could cause higher leaf length compared with those in 10 months after planting. For this reason, the mean of ll2 also higher than 1 indicating most of accessions in this germplasm had recovering sign for leaf expansion. For leaf width (lw) and leaf greenness (sp) ratio also showed the same trend as those in change of the leaf length. Therefore, these relative values over 1 could represent accessions' ability of adaptation in dry soil condition and recovery in re-watered soil condition over the mean of this germplasm. The parameters measured in this experiment were similar to conventional traits monthly measured before harvesting usually be started at 3 months after planting until harvesting in breeding program for drought tolerance. These traits were number of primary stems, number of branching levels, length of primary and secondary stems, leaf retention, height of leafless stem, length and width of fully expanded leaf lobe, carbohydrate content of leaves, stems, and petiole, stomatal conductance and ABA content of leaves and stems and pest and disease incidence (Okogbenin, 2013). Although leaf greenness was the only parameter which had never been used in drought screening, it was reported to correlate with chlorophyll content in leaf which can be decreased during leaf senescence. In the case of drought condition, reduction of chlorophyll content in leaf followed by leaf senescence was caused by low nitrogen uptake and distribution due to water deficit effect (Zhang *et al.*, 2010)

However, in this experiment, the relative values were calculated from these parameters to avoid genetic variance effect on selection process. In previous studies about drought tolerance traits, most of traits had genetic variance overpinned the true value. Okogbenin *et al.* (2003) evaluated fresh root yield, fresh shoot yield, cumulative leaves formed, cumulative leaf scars, root dry-matter content, harvest index and root cyanogenic potential variations in nine cassava varieties developed in humid and subhumid areas in three drought-prone area in Savanna zone of Nigeria. These parameters turned to be influenced by genotype more than locations except for cyanogenic potential which tended to increase upon drought severity. de Oliveira *et al.* (2015) screened 47 cassava accessions using five agronomic traits which were root yield, shoot yield, starch content, number of root and root dry matter content in the field conditions with and without water deficit to evaluate heritability and genetic gains of these traits for tolerance to drought. Again in 2017, five agronomic traits, plant height, root yield, shoot yield, harvest index and dry matter content of roots, were measured under both well-watered and drought stress conditions in 49 accessions selected from cassava groups which were reported to high yield under drought stress and high drought tolerance (de Oliveira *et al.*, 2017). Even in physiological traits such as net photosynthetic rate, photosynthetic nitrogen use efficiency, mesophyll conductance and phosphoenolpyruvate carboxylase activity of upper canopy leaves, they were evaluated in the field for 127 cassava accessions in seasonally-dry and hot in southwest Colombia. However, these photosynthetic traits had variation from genotype influences and also reinforce variation in the field (El-Sharkawy *et al.*, 2008).

For plant height (lh), the value is not the ratio, but the difference of height which indicated plant growth (Okogbenin *et al*., 2013). The mean in the increase of height from 8 months to 10 months was lower than those from 10 months to 12 months. Therefore, it also pointed out the water deficit encounter of overall varieties during the period from 8 months to 10 months and indicated recovery phase of overall varieties during the period from 10 months to 12 months. The ratio of plant height in the part containing leaves (z) also implied the water shortage adaptation and recovery phase in z1 and z2 value respectively. Lastly, the percentage of leaf retention height compared to overall height in 8, 10, and 12 months after planting suggested the change from abundant leaf canopy in 8 months to lower amount in 10 months. During prolonged drought, cassava shedded older leaves and adapted to form smaller new leaves. However, cassava can recover when water becomes available by rapidly forming new canopy leaves with much higher photosynthetic rates compared to unstressed crops which can compensate for yield loss during water deficit period (El-Sharkawy, 2007). Then, the percentage of leaf retention came back to the same level as in the 8 months again in 12 months after planting after a large amount of rainfall in April 2018. This indicated the fast recovery time in cassava especially for reforming leaf canopy capacity as discussed in Vandegeer *et al.* (2013). However, this recovery might had a compensation from loss of root yield as a source of energy for new sprouts.

The correlations (Table3) among values indicated the positive trends of these values which support for decision making for selecting values in K-mean clustering. Relative change in leaf length and leaf width had high positive correlation among other values. Therefore, they were grouped for one set of clustering factors. The change in leaf greenness was grouped with the change in leaf retention because it was positively correlated with the change in leaf retention. Change in plant height rarely correlated with other values, but there was positive correlation with some change in leaf retention pattern. Lastly, the percentages of leaf retention in three months of data collecting were grouped as one set of clustering factors. Even though the relative values were calculated as indices from parameters measured from one condition to another condition to avoid variation from genotype, de Oliveira *et al.* (2017) used geometric mean productivity, drought tolerance index, mean productivity, susceptibility, drought susceptibility index, and yield stability index from values of root yield under fully irrigated condition versus drought affected condition and found that different index led to selection of different cassava accessions which mean productivity, geometric mean productivity, and drought tolerance indices were suitable for selecting cassava genotypes with good agronomic traits, whereas, drought susceptibility index, susceptibility, and yield stability index were suitable to identify cassava genotypes with drought tolerant potentials. Therefore, according to K-mean clustering, the different values led to different groups of cassava accessions selected (Figure 1-4). Thus, the overlapped accessions across these selected groups were determined.

3.2 correlation and cluster analysis

According to ratios and differences evaluated in 3.1, Table 3 showed the correlation between these traits. Most of changes had positive correlation, except for plant height. From these correlation, grouping using these factors in K-mean clustering were performed in the set of traits which had positive correlation to one another.

Table 1. Weather conditions from May 2017 to April 2018 at Tapioca Development Institute (TDI), Dan Khun Thot district, Nakhon Ratchasima province

Factor	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18
RF	108	132	75	185	102	104	157	15	68	6	14	170
PE	135	153	164	154	129	121	133	172	139	148	192	162
RF/PE	0.80	0.86	0.46	1.20	0.79	0.86	1.18	0.09	0.49	0.04	0.07	0.80
RH%	83	78	80	80	83	83	74	66	70	69	70	76
Temp	26.9	27.0	26.2	26.8	26.4	25.5	24.5	22.9	24.1	24	26.4	26.4

RF = monthly rainfall (mm), PE = Pan evaporation (mm), RF/PE = ratio of rainfall to pan evaporation, RH% = Averaged percent relative humidity, Temp = air temperature (°c)

Table 2. Maximum, minimum, mean, and values in check varieties of each traits

Traits	Max	Min	Mean	KU50	HB60	HB80	R5	R72
ll1	1.66	-0.10	0.87	0.97	0.81	1.03	0.90	1.09
ll2	2.58	-1.68	1.20	1.31	1.08	1.43	1.15	1.04
lw1	1.74	-0.05	0.88	0.97	0.83	1.00	0.95	1.15
lw2	4.16	-0.57	1.21	1.41	1.16	1.14	1.24	0.81
sp1	2.96	-0.04	0.93	0.94	0.96	0.96	0.99	0.97
sp2	11.66	-0.09	1.00	1.08	1.06	1.01	1.00	0.96
lh1	83.55	-87.70	7.42	15.22	12.06	18.33	6.90	9.96
lh2	105.84	-40.83	23.29	24.06	18.96	26.74	20.23	39.05
z1	2.83	-0.09	0.82	0.93	0.77	0.92	0.76	1.14
z2	17.55	-1.63	1.66	1.43	0.83	1.08	1.15	1.71
pz8	59.16	1.06	24.33	32.34	45.07	30.74	34.72	27.24
pz10	46.48	-0.22	20.69	30.17	34.86	28.35	26.49	27.34
pz12	53.66	0.55	26.17	32.40	28.29	28.99	25.86	35.14

Table 3. Correlation among traits indicating drought adaptation and recovery

	II1	II2	Iw1	Iw2	sp1	sp2	lh1	lh2	z1	z2	pz8	pz10
II2	0.5218**											
Iw1	0.864**	0.5465**										
Iw2	0.5677**	0.7253**	0.5136**									
sp1	0.6491**	0.528**	0.6695**	0.6119**								
sp2	0.4497**	0.2515**	0.4477**	0.2449**	0.2951**							
lh1	0.1277*	0.1724**	0.1228*	0.0926	0.0781	0.1582*						
lh2	0.0598	0.0262	0.0059	-0.0083	-0.0773	-0.0804	-0.4686**					
z1	0.475**	0.3214**	0.3968**	0.3825**	0.3852**	0.3312**	0.319**	-0.1151				
z2	0.3248**	0.2634**	0.3956**	0.2753**	0.1944**	0.542**	0.0796	0.0677	0.0057			
pz8	0.2224**	0.0816	0.2241**	0.1182	0.3573**	0.0842	0.043	0.0216	-0.0585	-0.1739**		
pz10	0.2819**	0.1382*	0.2209**	0.1413*	0.3324**	0.1853**	0.3633**	-0.0596	0.4962**	-0.225**	0.6967**	
pz12	0.3961**	0.1996**	0.3389**	0.3441**	0.3384**	-0.010	0.0181	0.384**	0.2454**	0.2185**	0.3179**	0.3364**

* means significantly correlated at 95% confidence
** means significantly correlated at 99% confidence

The first clustering was grouped by the standardized values of change in leaf length and width (Figure 1). Four groups were generated. The group which had high in both leaf length and width (in triangle shape) was selected as highly adapted group containing 77 varieties.

The second clustering was grouped by the standardized values of change in leaf greenness and the change in leaf retention height (Figure 2). Four groups were generated. The group which had postive in change of leaf greenness and leaf retention (in plus and cross fugure in figure 2) were selected as highly adapted groups (in different pattern; drought adaptation toward positive zone of z1 and recovery toward positive zone of z2) containing 101 varieties and 133 varieties respectively.

The third clustering was grouped by the standardized values of growth regarding on plant height change and leaf retention change (Figure 3). Four groups were generated. The group which had high in both change pattern in postitve value (in triangle shape and cross shape in figure 3) were selected as highly adapted group containing 83 varieties and 106 varieties, respectively.

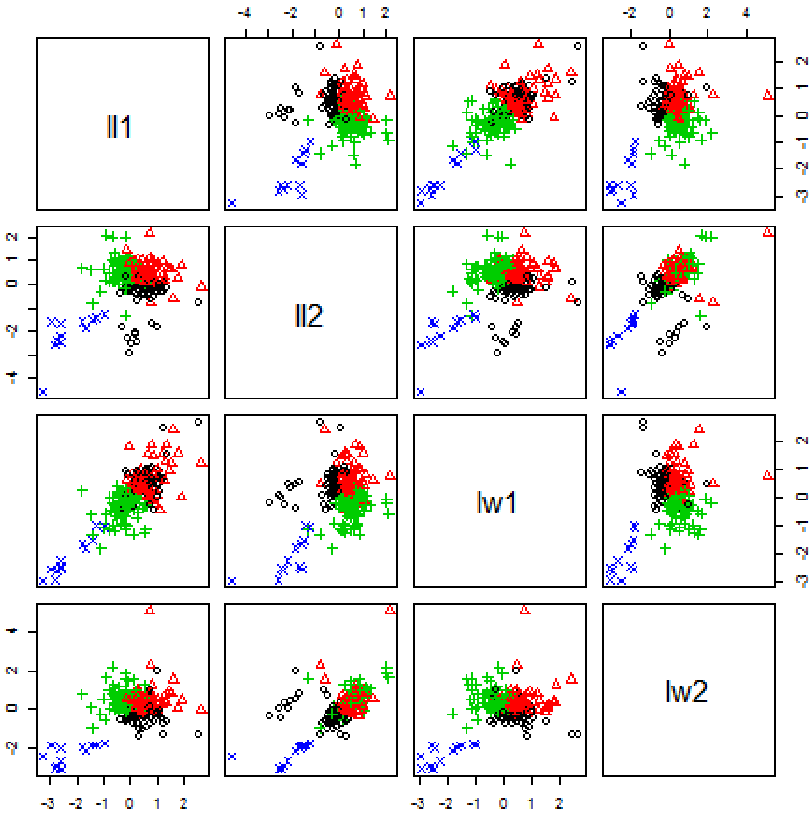

Figure 1. K-mean cluster analysis by value of change in leaf size (length and width)

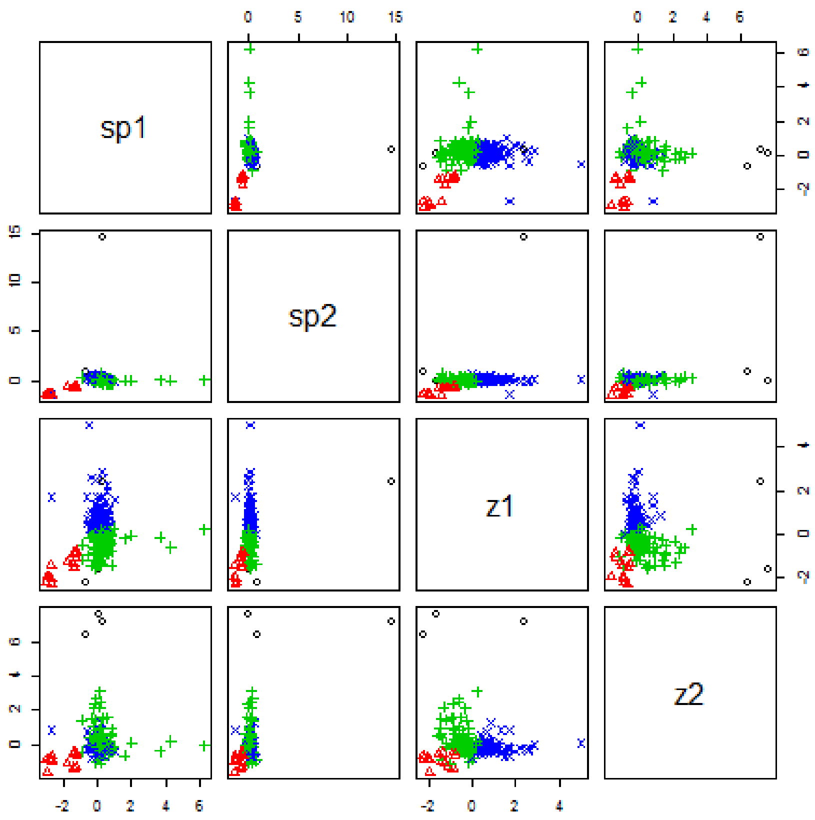

Figure 2. K-mean cluster analysis by value of change in leaf greenness and leaf retention

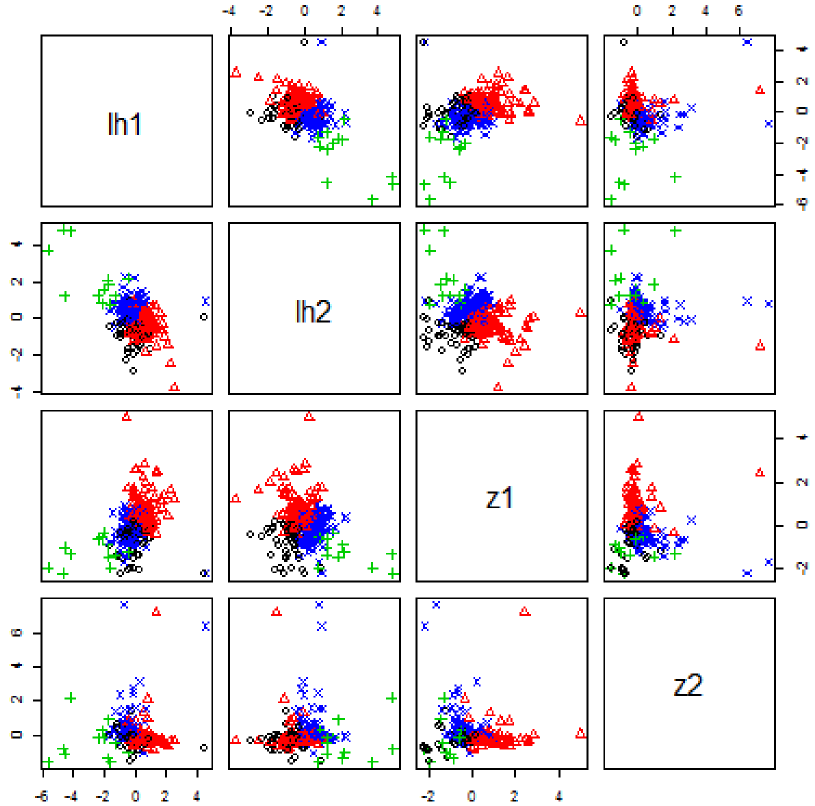

Figure 3. K-mean cluster analysis by value of change in leaf greenness and leaf retention

Lastly, the fourth clustering was grouped by the standardized values of percentage of leaf retention in each month (Figure 4). Four groups were generated. The group which had high leaf retention (in circular shape) was selected as highly adapted group containing 60 varieties. Taken all selected groups from four clusterings into accord, there were 8 varieties overlapping in all selected groups which were KM89-5, Mbar383, MGua62, Mind13, Mind56, unknown16, Giant, Nephngona which will be selected as accessions with broad drought adaptive traits which might not have the highest relative values, but were in the highest groups of most traits (Table4). Therefore, considering overall performance of these accessions, they adapted to dry conditions betten than five check varieties which were Thai popular commercial varieties (Table1). These drought tolerant accessions can be used as parents in breeding program together with these commercial varieties which had high yield potential to generate lines with high optimum yield with ability for drought adaptation. Moreover, if the specific traits, not overall performance, is needed for breeding, accessions with maximum values in Table1 can be selected as parents in breeding program for drought tolerance as well

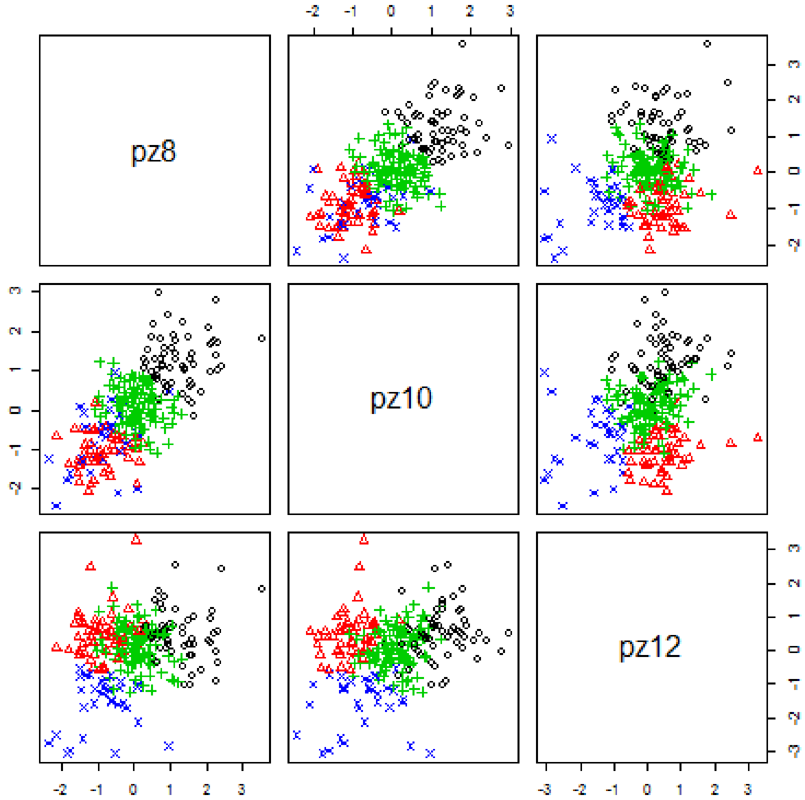

Figure 4. K-mean cluster analysis by value of percentage of leaf retention at 8, 10, and 12 months

Table 4. Relative values in selected accessions of each traits

Traits	KM 89-5	Mbar 383	MGua 62	Mind 13	Mind 56	Unk16	Giant	Neph ngona
ll1	1.08	0.97	0.84	0.95	1.01	0.88	1.20	1.05
ll2	1.04	1.39	1.30	0.74	0.90	1.48	1.10	1.23
lw1	1.21	1.17	0.96	0.91	1.14	1.05	1.04	1.15
lw2	1.31	0.40	1.00	0.81	0.60	1.28	1.15	1.16
sp1	1.09	1.06	0.92	0.91	0.90	0.95	0.96	1.01
sp2	0.79	0.88	1.03	1.00	0.92	1.06	1.12	1.06
lh1	-19.78	7.71	16.81	11.07	3.54	13.13	10.21	10.63
lh2	30.00	38.73	24.17	32.92	32.08	27.50	47.50	41.25

Table 4. Relative values in selected accessions of each traits (cont.)

Traits	KM 89-5	Mbar 383	MGua 62	Mind 13	Mind 56	Unk16	Giant	Neph ngona
z1	0.53	1.12	0.58	0.67	0.84	0.87	0.72	0.78
z2	1.59	1.68	3.33	1.16	1.43	1.25	1.04	0.88
pz8	34.66	20.26	31.33	59.16	32.93	30.68	45.74	46.01
pz10	21.18	21.67	22.73	36.19	26.65	27.50	35.34	35.87
pz12	32.32	34.37	40.99	41.42	31.24	30.55	36.04	30.28

4. Conclusions

The normal distribution of normalized data of relative values indicated that from 8 to 10 months after planting was the period for cassava adaptation to drought and from 10 to 12 months after planting was the period for cassava growth recovery from drought stress. There were positive correlations among these traits except for plant height. K-mean clustering analysis showed cassava varieties grouped upon the different responses to drought adaptation and recovery patterns. Taken from overlapped varieties appearing in overall advantage performance of drought adaptative pattern groups, eight varieties of 255, namely KM89-5, Mbar383, MGua62, Mind13, Mind56, unknown16, Giant, Nephngona, became promising varieties suggested for drought tolerant breeding program due to the abilities to maintain leaf expansion, leaf greenness, height growth, and leaf retention through drought period. These varieties can be used in breeding program for drought tolerance in the future.

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Alternative Energy in Household Using Used Vegetable Oil

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Abstract

Vegetable oil is very important and useful for every household for cooking. However, the used vegetable oil affects the environment and human health a lot when disposed improperly and/or reused. The used vegetable oil can be converted to biofuel energy and used in the household to reduce negative environmental and health effects. This research has used wick combustion process to turn used vegetable oil to heat power in a modified cooking stove. The efficiency of this modified cooking stove was adjusted and compared to commercial LPG stoves. The invented stove works by adding sufficient air for combustion and uses the wick to absorb and transfer material of used vegetable oil. This material allows to continue combustion process on any replacement unlike the conventional wick combustion. The efficiency was tested and compared to commercial 4 LPG stove in terms of time and temperature for heated water to 90°C at high, medium, and low heat power level. The results show that, average time of LPG stove is 3.54, 5.46, and 9.14 minutes, the average temperature in stove is 590.28, 416.81 and 263.34°C, respectively. Invented stove increases air at the airflow rate of 80, 60 and 45 liters per minute to adjust heat power level in high, medium, and low. Then efficiency test of invented stove, the average time is 3.39, 5.27 and 8.67 minutes, the average temperature in stove is 601.40, 381.07 and 261.53°C respectively. According to T-test, there is no difference between both types of stoves. It can be used as a substitute. Thermal efficiency value test of invented stove by Water Boiling Test (WBT) is 48.66 %. Invented stove has break-even point within 8 months, so this invented stove that uses fuel from used vegetable oil is one of the interesting alternative choices. It also helps to solve the environmental problem.

Keywords: Wick combustion / Water Boiling Test (WBT) / Invented stove / Thermal efficiency

1. Introduction

1.1 Statement of Problems

Vegetable oil is a necessary thing as every household to be undeniable. By The data from the Energy for Environment Foundation (2007) indicate that the Thailand has the remaining oil to use. A total of 74.5 million liters per year. By coming from a group of households, reaching 47.2 million liters. As a result, the problem of using vegetable oil repeat. Through repeated food oil for too long, it will have reduced nutritional value. Eating with the use of frying oil repeated. Or in frying oil used with amounts Polar compounds exceeding 25% will cause toxicity in the body. Such as, cause heart disease, cause lung cancer, Liver cancer and bladder cancer, etc. (Food Safety Operation Center Ministry of public health, 2011) In addition, the problem of removal of vegetable oil discarded incorrectly methods also cause environmental problems followed. Vegetable oil is used, then this is another interesting one. To be recycled into renewable energy. Because, as the rest of the household or the store at cooking oil amounting to many, including some industries. This research is a study of the process of creating a stove that uses vegetable oil used to come as fuel instead of gas by using the principle of burning the Wick combustion. This principle, which is different from other research that has ever been about. Creating a stove used vegetable oil into fuel. This principle has the advantage is to reduce clogging of the burners on the stove. This is the main problem that found in vegetable oil stove in general. And the process of stove, not complex, can produce it in every household. Introduced as a

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fuel for cooking In order to reduce the fuel consumption of fried food, fried repeat consumer. And the current era of living costs, increases every day. So the researchers, therefore, there is a concept that will help reduce the cost of using the resource and cost savings in some down. In order to save energy, another way. It is an important alternative to oil as a fuel in addition to direct sales to make biodiesel. And counted as troubleshooting resource. With the energy potential is recycled as a renewable energy change the used vegetable oil as heat energy benefits, Better to neglect and causing other problems followed as mentioned above.

1.2 Objectives of the study

1.2.1 To design and build used vegetable oil fuel stove.

1.2.2 The invented stove efficiency was equal to the regular LPG stove in household scale.

2. Methodology

The research process is divided into 2 phases: 1. the design and build of the used vegetable oil fuel stove. 2. The test of invented stove efficiency.

2.1 The process of designing and building a used vegetable oil stove

The invented stove designing and building were based on oil lamp system which modified the number of burner and aeration into each burner. It will increase the efficiency of combustion and pressure on fuel oil which the fuel will smoothly run into the combustion system. As well as, the smoke was decreased when it was in the complete combustion process. Finally, the flame control was designed to adjusting the heat level as regular LPG stove in household scale.

2.1.1 The study on the oil lamps mechanism and other materials. Normally, the oil lamp is often to see in our daily life which it can ignite through the cotton wick as oil absorber as the lamp fuel. Since the oil lamp mechanism was observed, it was suitable to be a role model of the invented stove. Furthermore, this study was finding for an appropriate oil absorber in the invented stove which absorbs well but heats and melts resistant were greater than cotton rope.

2.1.2 The studied factors that affect the occurrence of flame. The affecting factors in the flame are air and fuel. The flame in the combustion zone is moving toward to the mixture of fuel and air. Hence, the flame occurrence is depending on the ratio of fuel and air as well as it cannot ignite by only fuel or air. Thus, the appropriate ratio to ignite is the right proportion of air and fuel. The mixing is an important part of the combustion process when the appropriate fuel mixed will reduce soot and smoke as well as lead to the complete combustion. Therefore, it will increase the thermal efficiency and flame stability during the combustion (Bapheng, 2004).

2.1.3 The design of material and stove can use vegetable oil as fuel. The design was support to using used vegetable oil as fuel by wick combustion which adds the pressure and air system into the burner. The design was considered with an involved theory and the materials have to have high heat resistant that leads to the durability of the invented stove.

2.2 The efficiency tests of the invented stove

2.2.1 The efficiency testing on fuel absorber materials

- Materials and equipment
  - Materials 3 types
  - Used vegetable oil
  - The lighter
  - Weighing machine
  - Multi-meter model Custom Ahlt-100
  - Container
  - Measuring tape
  - Timer

- Experimental method

(1) The absorbent material cut to three types of length 10 cm.

(2) Pour used vegetable oil 50 gram into a container.

(3) Set the material into oil container and lit up for 10 mins. Then, observe and note the characteristics of flame, soot, flame temperature, the amount of fuel used, and measure the length of each oil absorber material.

(4) The most effective fuel absorber materials will select to use in the invented stove.

2.2.2 The thermal efficiency test of the invented stove which compared to LPG stove.

- Materials and equipment

- Multi-meter model Custom Ahlt-100
- Air Flow Meter
- Aluminum Pot
- Rubber tube
- Weighing machine
- Air Compressor
- Timer
- Thermometer
- The most effective absorbent materials from 2.2.1 test.

- Experimental method

Experiment 1 The thermal efficiency test of the LPG stove from the boiling water test (High heat level).

(1) Pour water volume 600 ml into the aluminum pot and measures the temperature of the water at start point by the thermometer.

(2) Turn on the LPG stove then adjust the heat level to the maximum level.

(3) Set aluminum pot on the stove then starts timing until the water was boiled as temperature 90°C.

(4) Record the time of boiling water and temperature of the combustion reaction.

Experiment 2 The air proportion test of air adding into the invented stove system to getting the boiling water temperature close to LPG stove (High heat level)

(1) Pour water volume 600 ml into the aluminum pot and measures the temperature of the water at start point by the thermometer.

(2) Turn on the invented stove system by adjusting the air flow rate for 10 liters per minute.

(3. Set aluminum pot on the stove then start timing and record water temperature at X minutes.

(4) The experiment was repeated by modification of air flow rate to 20, 30, 40, 50, 60, 70, 80, and 90 liters per minute until the boiling water has temperatures close or equal to 90°C within the time X minutes.

(X=The time of the boiling water from Experiment 1)

* Experiment 1 and 2 are repeated two more times in the medium and low heat level.

**The threshold of the LPG stove heat adjusting:

The LPG stove thermal efficiency testing was tested with high, medium, and low heat level. The high heat is the maximum heat level of each stove, the medium heat is the middle or 90 degrees of the heat level control knop, and the low heat is the minimum heat level of each stove or 45 degrees of the heat level control knop from the turn off point.

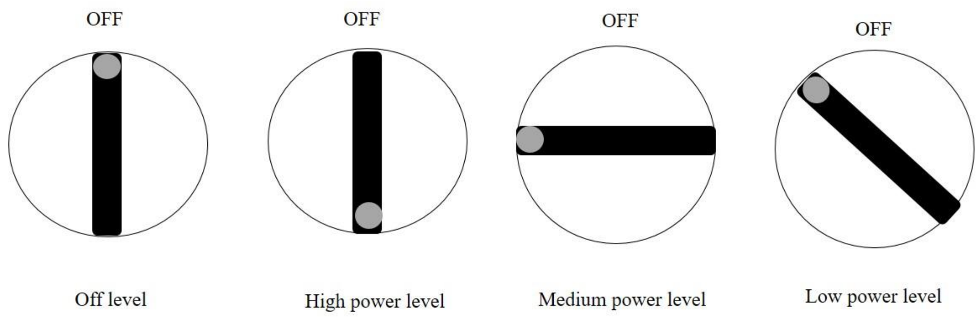

Figure 1. The rotation adjustment strength the LPG stove.

2.2.3 Testing the thermal efficiency of the stove.

- Experimental method

(1) The invented stove equipment and measurement tools preparation.

(2) Pour 1 kg water into the container and record the beginning temperature of water.

(3) Ignite the stove and adjust the air flow rate as high heat level as needed (From experimental 3.3.2.2).

(4) Set the container on the stove then start timing until the water temperature increase to 90°C. As well as, record the amount of water after boiling (Wait until the water cool and measure the amount

of water), and fuel consumption then calculating the thermal efficiency.

This test method is a standard test of Water Boiling Test (WBT) according to equation 1.1–1.3. (Bhattacharya et al., 1998)

$$\eta = \frac{Q_u}{Q_{fuel}} \times 100\% \qquad (1.1)$$

When η The thermal efficiency of the stove, %

Q_{fuel} The heat from the fuel, kJ

Q_u The quantity of heat that use, kJ

The amount of heat that is utilized to find the heat used to warm up and water evaporation. As shown in equation 2.2

$$Q_u = [m_{w,1} C_{p,w} \ (T_{w,b} - T_{w,i})] + [m_{w,2} \ h_{fg}] \quad (1.2)$$

When $m_{w,1}$ Water mass start, kg

$m_{w,2}$ Mass of water that evaporates, kg

$c_{p,w}$ The specific heat of water value 4.186 kJ/kg °C

h_{fg} The latent heat of vaporization of water 2,257 kJ/kg

The amount of heat from the fuel. Find by the equation 2.3

$$Q_{fuel} = m_{fuel} \times LHV \qquad (1.3)$$

When m_{fuel} Total fuel consumption, kg

LHV Lower heating value of the fuel (used palm oil 36,355 kJ/kg) (Bapheng, 2004)

3. Results

3.1 The design and build of cooking stove by fuel used vegetable oil

3.1.1 The design of cooking stove by fuel used vegetable oil

The cooking stove structure was show as figure 2 which designed to support the wick combustion by used cooking vegetable oil fuel. The stove system was increasing the pressure and air flow system into the burner that the design was related to appropriate theories for creating cooking stove as the study objective.

Table 1. Description of invented stove

Description of stove	Size	unit
Height of stove	40	centimeter
Diameter of stove	22.86	centimeter
Diameter of Air tank	15.24	centimeter
Diameter of Burner	1.64	centimeter
Diameter of Air tube	0.7	centimeter
Volume of Fuel Tank	2.076	litter

3.2 The used vegetable oil cooking stove efficiency test result

3.2.1 The efficiency test material of fuel adsorbed

The result of fuel adsorbed material test found material 2 was lack of vegetable oil adsorption ability which effected to the burner igniting. Whereas, material 1 and material 3 were adsorbed better than material 2 which the temperature of the combustion was 110.80 and 113.079°C, respectively. As well as, the fuel use rate of material 1 and material 3 were 5.20 and 5.25 gram, respectively. The combustion of these material were cause of a smoke which material 1 and material 3 were no significantly different in combustion and smoke. Moreover, the 10 minutes combustion test was found material 3 not decreased but material 1 was decreasing to 5.50 centimeters. The combustion test shown material 3 was more efficiency use than material 1 when considered by long term of the efficiency use, time, and price.

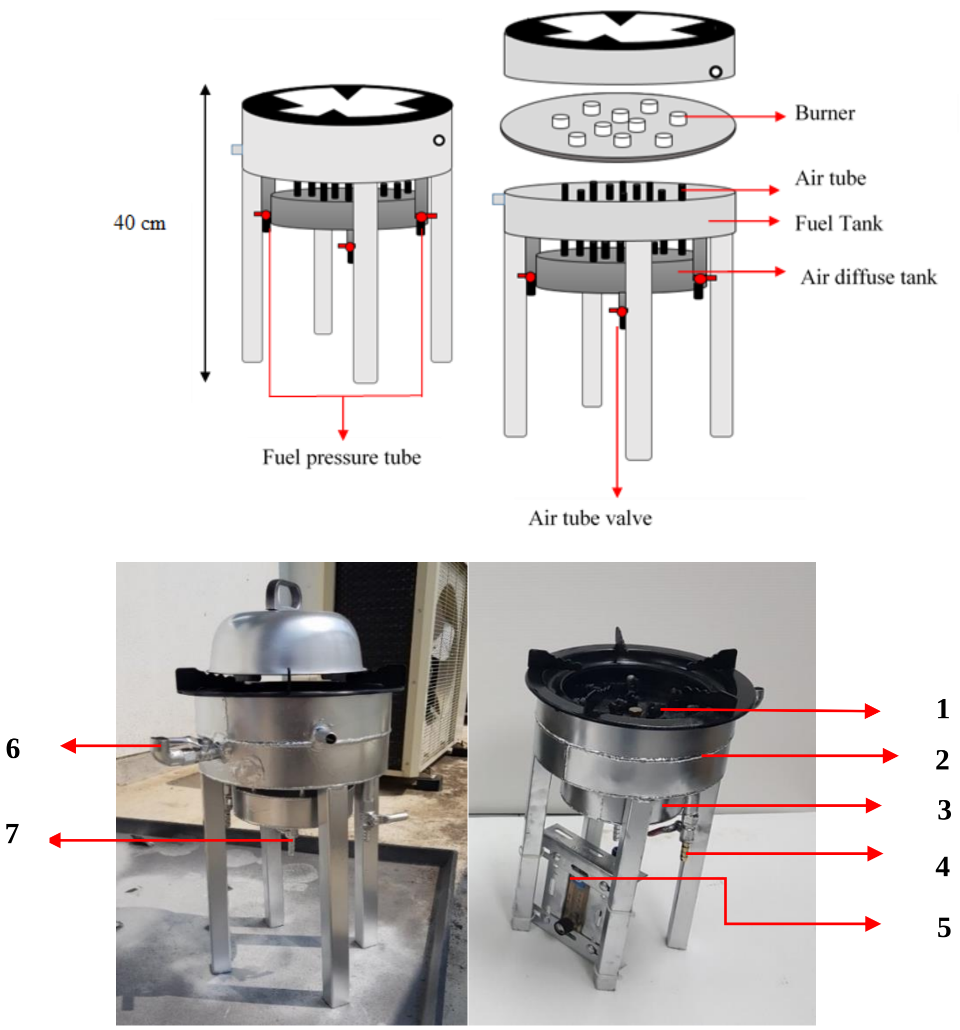

Figure 2. The stove structure

1 Burner
2 Fuel Tank
3 Air diffuse tank
4 Fuel pressure tube
5 Flow Meter
6 Filling pipe fuel
7 Air tube valve

Table 2. The efficiency test material adsorbed vegetable oil combustion

Type of material	Length before burning (cm)	Length after burning (cm)	Soot from burning	Temperature (°C)	Fuel consumption (g)
Material 1	10.00	5.50	smoky	110.80	5.20
Material 2	10.00	*	*	*	*
Material 3	10.00	10.00	smoky	113.07	5.25

* No data, Because the material fire not stuck.

3.2.2 The result of LPG cooking stove testing

The efficiency testing result was show in Table 4.3; the house No.1 was tested at 18.25 which the kitchen is cement room with the ventilator. The stove was using 3-4 times a week for 8 years with good condition but it lightly covered by smut. The house No.2 was tested at 20.45; the kitchen is open air area beside the house with ventilated condition.

The stove label is CUCINA was using every day for 11 years which old condition and covered by smut and cooking oil at the burner. Furthermore, the ventilated condition was a cause of unstable flame. In addition, the house No. 3 was tested at 11.26; the kitchen is cement room with ventilator and windows. The stove label is MEX SCHOTT which using every day cooking for 11 years. The stove has a good condition with light smut. As well as, the house No.4 was tested at 19.05; the kitchen is cement room with windows. The stove label is Rinnai that using 3-4 times a week for 10 years as well as its condition is good without smut.

For the standard testing, this study was testing the household LPG compared to the used vegetable oil cooking stove which test in 4 household. The value will calculated to the average value of heat value and boiler time to compare with the designed stove. The comparing is reference to improving the designed stove which the testing result the temperature of the combustion with various heat levels as high, medium and low were 590.28, 416.81, and 263.34°C, respectively. In the same time, the boiler time as 90°C as high, medium and low heat level were 3.54, 5.46, and 9.14 minutes, respectively.

Table 3. The environmental factors of the LPG cooking stove testing location

Household	Testing date	Start time	Model	Using (year)	Frequency	Kitchen feature
1	18/2/2017	18.25	NA	8	3-4 times/week	Closed mortar room
2	18/7/2017	20.45	CUCINA	11	Everyday	Open air
3	5/11/2017	11.26	MEX SCHOTT	6	Everyday	Mortar room with ventilation
4	16/12/2017	19.05	Rinnai	10	3-4 times/month	Mortar room with ventilation

Figure 3. The LPG cooking stove of thermal performance testing

The testing at household scale in several location found the heat value and boiler time of the LPG cooking stove testing were different. There are variously factors (shown as Table 3) effected to the stove efficiency. These result showing the actual household LPG stove was different both physical structure and potential including to environmental factor is also a control factor. The stove using potential is depending on the air ratio that regular LPG stove has air adjusting which air ratio affect to the heat efficiency. Thus, the LPG stove using without air ratio adjustment is a cause of low heat efficiency. Hence, this study was tested on the household scale to finding the average temperature of the combustion in each level that using the baseline for comparing with the designed stove.

3.2.3 The result of stove uses fuel form used vegetable oil stove testing

The air adding ratio into used vegetable oil stove system for the heat value and boiler time. The stove testing value was similar to the LPG cooking stove value. Furthermore, the result show the air volume was lead to the differ of heat level which the total air volume for heat levels as high, medium, and low were 80, 60, and 45 L/min, respectively. Nevertheless, for the stable fuel pressure, it had to adding air to the system 40 L/min which it will less smoke to no smoke as well as the boiler time as 90°C was similar to LPG cooking stove. This stove system can increase and decrease the heat level by the air addition volume adjusting.

The boiler time testing the high heat was need air flow rate 80 L/min for boiled as 90.83°C at 3.54 minutes and the medium heat was need air flow rate 60 L/min for boiled as 90.17°C at 5.46 minutes. When, compared to the high heat level with air flow rate 45 L/min, the water will boiled as 91.83°C at 9.14 minutes. Furthermore, if added air flow 40 L/min it will boiling to 89.83°C which the most closely to 90°C but it cause of smoke from incomplete combustion process. Therefore, the suitable air flow for the high, medium, and low heat level of this stove were 80, 60, and 45 L/min, respectively. Meanwhile, the suitable fuel pressure is 40 L/min which stable flame and less smoke to no smoke occur.

* Used palm oil from fried chicken shop is fuel being tested in this research.

The comparing of the boiled time between used vegetable oil stove and LPG cooking stove which the boiled time of each heat level was similar. Furthermore, Table 5 is the statistical analysis show the boiled time similarity significant was found in the value of high heat of used vegetable oil stove and LPG cooking stove as 3.39±0.11 and 3.54±0.45 minutes (the significant value is 0.797), respectively. While, the medium heat level, the boiled time was 5.27±0.09 and 5.46±0.25 minutes, respectively with significant value 0.533. As well as, the low heat level, the boiled time was 8.67±0.17, and 9.17±0.55 minutes, respectively with significant value 0.512. Furthermore, the result was analyze by statistical analysis with Independent Samples Test at p=0.05. The analysis found the boiled time as 90°C by high, medium, and low heat level between used vegetable oil cooking stove and LPG cooking stove were not different. The similarity was due to the heat value of their combustion process closely thus the boiled time was not different. Therefore, used vegetable oil stove could be replacing LPG cooking stove.

Table 4. The result of boiling water with invented stove in various air flow rate

Level of heat	Air flow (L/min)	Average time to heated water to 90 °C (minute)	Average temperature of combustion (°C)
high	80	3.39	601.40
medium	60	5.27	381.07
low	45	8.67	261.53

A

B

Figure 4. The characteristics flame of the invented stove
A: no aeration B: aeration 50 l / min

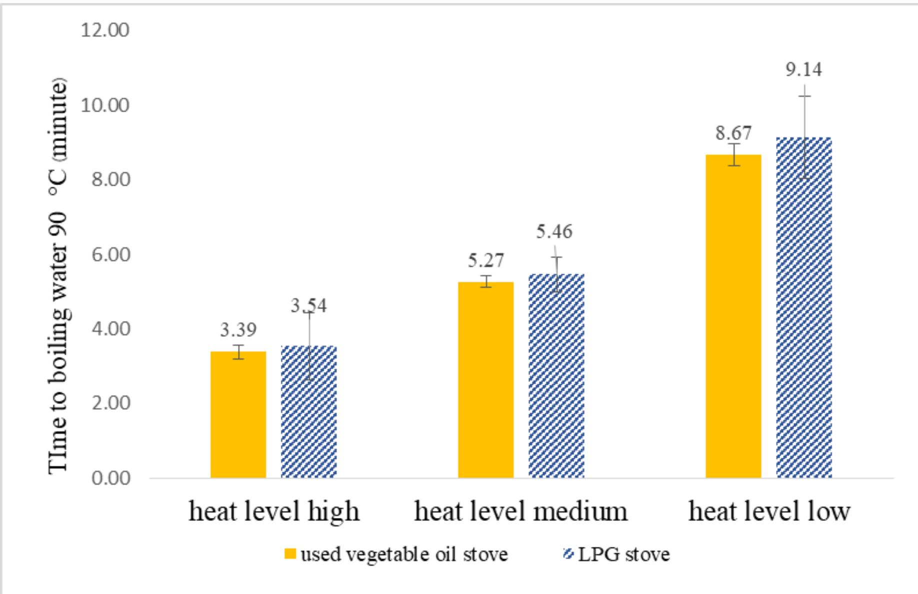

Figure 5. Comparative graph time to boil water of LPG stove and invented stove

Table 5. Independent Samples Test Time to heated water to 90°C of invented stove with LPG stove

Level of heat	N		Mean of Time (minute)		Std. Error Mean		t	Sig. (2-tailed)
	Invented stove	LPG stove	Invented stove	LPG stove	Invented stove	LPG stove		
high	3	4	3.39	3.54	0.11	0.45	-0.27*	0.797
medium	3	4	5.27	5.46	0.09	0.24	-0.67*	0.533
low	3	4	8.67	9.14	0.17	0.55	-0.71*	0.512

*The significant value is p=0.05

3.2.4 The thermal efficiency of invented stove testing result

The air flow rate 50 L/min is the lowest rate due to completely combustion process in used vegetable oil stove. Furthermore, the boiled time test of 1 kg water as 90°C was 6.26 minutes which the average stove heat temperature was 592.73°C. After the boiling, the water weight was decreasing to 0.910 kg, and the fuel using was 34.66 milliliters. When, the air flow rate was adjusted to 100 L/min which the highest air flow rate for completely combustion process in used vegetable oil stove. In this case, the boiled time was 3.14 minutes and the stove heat temperature at boiling point was 637.83°C. Which, the water weight was decreasing to 0.893 kg, and the fuel using was 17.36 milliliters as show as Table 4.15. As well as, the test of the thermal efficiency of used vegetable oil stove with Water Boiling Test (WBT) (Bhattacharya et al., 1998) found the thermal efficiency of air flow rate 50 L/min and 100 L/min were 34.46% and 48.66%, respectively.

Table 5. The thermal efficiency test of used vegetable oil stove

Times	Air flow rate (L/min)	Water volume (kg)	The start temperature	Boiling time as 90°C (min)	The combustion temperature as 90°C (°C)	Water volume After boiled (kg)	Fuel using rate (ml)	η (%)
1	50	1	25	6.48	591.2	0.905	41.53	34.46
2	50	1	24.5	6.29	587.8	0.915	41.53	
3	50	1	24.5	6.01	599.2	0.920	41.53	
Average			24.67	6.26	592.73	0.910	41.53	
1	100	1	25	3.01	612.4	0.889	31.15	48.66
2	100	1	25	3.22	645.8	0.895	31.15	
3	100	1	25	3.18	655.3	0.895	31.15	
Average			25	3.14	637.83	0.893	31.15	

4. Discussion

Chaichana et al. (2013) was study in thermal efficiency of local household cooking stove included by Standard-sized normal stove, Small-sized normal stove, High efficiency stove, Long-lip stove, Middle-sized black stove and Small-sized black stove. This study found high efficiency stove had the highest thermal efficiency as 30.54% followed by Middle-sized black stove, Standard-sized normal stove, Long-lip stove, Middle-sized black stove, and Small-sized black stove which the thermal efficiency 4.29% 21.70% 19.80% 18.46% and 16.66%, respectively. While, Chaiyasomthip et al. (2014) was designed the gasifier stove coupled with biomass stove in household using. The study found the best thermal efficiency was the combination of rice husk and charcoal as 41%. Furthermore, the design used palm oil burner of Ornthong (2009) found the material shape and porosity affected to thermal efficiency as 28%. While, Jenjit (2009) found the highest thermal efficiency of regular gas stove was 29% when the stove covered by porosity material was increasing thermal efficiency to 40%. As well as, K.F.Mustafa et al. (2015) was designed the kerosene mixed with used vegetable fuel stove. This stove had thermal efficiency 31.5%. Therefore, the used vegetable oil stove of this study had thermal performance higher than other studies which it can be alternative choice of household using in the future.

5. Conclusions

The research on the alternative energy in a household using used vegetable oil that design and build used vegetable oil fuel stove. The invented stove efficiency was equal to the regular LPG stove in household scale. The study was included by 2 parts: 1) the design and build the stove 2) the invented stove efficiency test. It can be summarized as follows:

The invented stove was using used vegetable oil as a fuel with wick combustion that adding air and pressure for the steady combustion. Hence, it can adjust the stove heat level as high, medium, and low as LPG stove. Since the material 3 was the most effective fuel adsorption material with long-lasting than others; it is suitable for use in household-scale. The thermal efficiency and water boiling test of LPG stove in household-scale were tested with 4 stoves which the boiling point as 90°C as high, medium and low heat average time were 3.54, 5.46 and 9.14 minutes, respectively. As well as, the combustion temperature was 590.28, 416.81 and 263.34°C, respectively. The appropriate air flows for a high, medium and low heat of the stove were 80 60 and 45 liters per minute, respectively. The water boiling test with these air flow as high, medium and low heat average time were 3.39, 5.27 and 8.67 minutes, respectively while the combustion temperature was 601.40, 381.07 and 261.53°C, respectively. The statistical analysis shown water boiling test of the LPG and invented were not different that the invented stove is replaceable for the LPG stove.

Furthermore, the thermal efficiency test of the invented stove by standard Water Boiling Test (WBT) (Bhattacharya et al., 1998) with air flow rate at 100 L/minute can cause the thermal efficiency for 48.66%. The invented stove breakpoint is 8 months that it is an alternative choice to use used vegetable oil as renewable energy. Therefore, it could reduce the health and environment problem by inappropriate disposal, especially, household and community scale

Table 6. The thermal efficiency of stoves

Reseacher (year)	Stove Type	Thermal efficiency
Chaichana et al. (2013)	Standard-sized normal stove	30.54%
	Middle-sized black stove	4.29%
	Standard-sized normal stove	21.70%
	Long-lip stove	19.80%
	Small-sized black stove	18.46%
	Small-sized normal stove	16.66%
Chaiyasomthip et al. (2014)	The gasifier stove coupled with biomass stove in household using	41%
Ornthong (2009)	The used palm olive oil burner (studied on the effect of material shape and porosity to burner efficiency)	28%
JenJit (2009)	Regular gas stove	29%
	the regular stove covered by porosity material	40%
Mustafa et al. (2015)	the kerosene mixed with used vegetable oil fuel stove	31.5%
Khongaseam (2017)	Alternative energy in household using used vegetable oil	48.66%

6. Acknowledgements

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Sustainable Swine Farming Transformation Case Study of Economic and Environment Evaluations of Turning Ordinary to Smart Green Farming System

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Abstract

This article exhibits about the evaluation of economic and environment aspect in sustainability swine farming from the transformation the ordinary swine farm to smart green farming system. This research used SPM farm, which is located in Ratchaburi province as a study site. The data was collected over a period of 1 year. SPM farm has a solar system covering 7,000 m^2 with 2,985 panels of 300 Wp each, resulting in electricity-generated 1,269.8 MW/year and carbon reduction by 712.36 tCO_2/year in environment point. The other technology is biogas system, the farm was able to produce 1,992 MW/year and carbon reduction by 49.8 tCO_2/year. Moreover, the study has included the possibility of using new ventilation technologies in the farm that would generate 1.63 MW/year and carbon reduction by 0.91 tCO_2/year each fan.

By practicing smart green farming, it does not only elevate the standards of swine farming, but also increases the yield and becomes environmental friendly. Thus, this model encourages investors and farm owners to realize full potential and benefits of smart green farming.

Keywords: Swine farming/ Livestock/ Green energy/ Technology/ Sustainable agriculture

1. Introduction

Agriculture is the main industry in Thailand due to the suitable landscape and climate, especially livestock farming. Thailand is one of the largest agricultural products exporters in the world, and pork almost dominates the margin of Thailand's agricultural export. Swine farming shows the tendency of becoming more industrialized in the near future, as many farms lean towards technology due to the government's "Thailand 4.0" policy that encourages every industry to advance in technology. This research aims to build the framework for smart green farming, by integrated processes of material management in ways best appropriated to recycle the waste from swine farm outputs into a subsidiary energy to be used in farms and make the most efficient use of non-renewable resources.

In the analyzing process of smart green conceptual model, the objective aims to measure the environmental and economical impact evaluation, as to cover every area. For the environmental impact evaluation, CO_2 emission reduction (tCO_2/year) of every approach, it will be derived from the usage of electricity produced in house, as a subsidiary of normal buy-in electrical power, will be taken into the account. As for economical impact evaluation the cost reduction after installation from each approach will be evaluate, and also accounted the payback period of each approach after installation. Moreover, providing investors with a MS Excel program as a tool to help them calculate the appropriate size of each technology.

This sustainable swine farming practice is focus on provides food sustainably based on environmental protection, while enhanced economy viability of farm operation and improves quality of neighboring society and to make long lasting relationship with the society. Follow up papers will provide more details about sustainability on swine farming transformation as a farm sharing-model.

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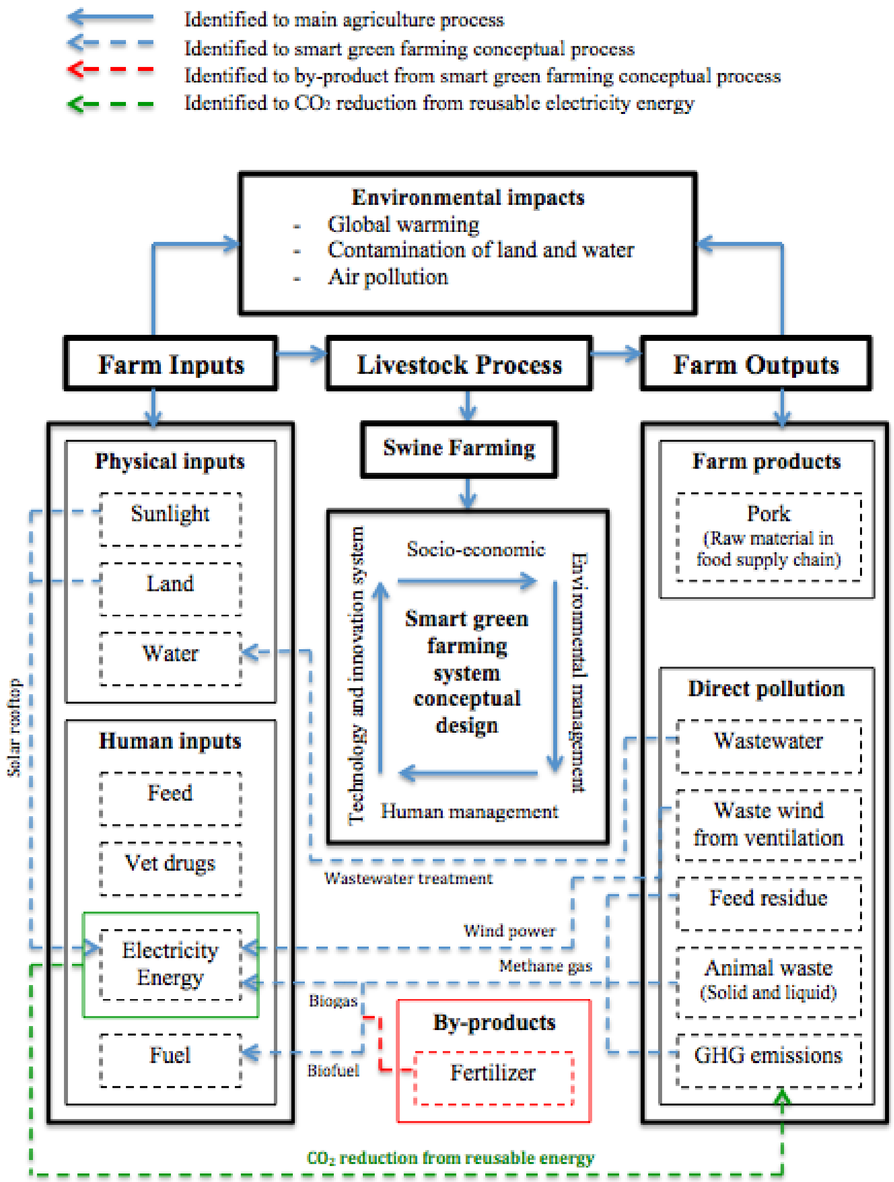

Figure 1. Conceptual framework

2. Methodology

2.1 Research design process: This research methodology used consisted of primary data and secondary data. The secondary data are mainly collected by mean of a literature review. The information used to design the conceptual framework that performs the smart green farming conceptual model, also to be a guideline for the interview questions. For Primary data was collected in the study site survey, by using initial interview method. Mainly in form of semi-structured interviews for qualitative data and collect quantitative data. The gathering of the data was collect depend on approach of installation in farm.

2.2 Fieldwork process: The study site is select from characteristic and type of farming, also operation in farm systems that are properly use to test the select group of innovations as follow.

- Overview of The study site: SPM farm is the successful large-scale swine farm, which has number of swine population more than 600 units.
  - Located at: 125 Moo 8 Donsai, Pakthor, Ratchaburi province 70140, Thailand.
- Technologies for installation in SPM farm

(a) Biogas system enables biogas recovery and organic fertilizer to treatment organic solid waste from biogas process.

(b) The solar rooftop system for energy generation used on-farm

Figure 2. Technologies for installation in SPM farm

2.3 Data Analysis process: The data analysis will be divided into two parts which both are examined by analysis environmental and economic impact evaluation as follow:

2.3.1 The study of technologies applied in farm

2.3.1.1 Conversion of bio-waste into organic fertilizer:

- Environmental impact evaluation: By collecting bio-waste residue from the biogas process to be process in organic fertilizer process to reduce air pollution, the biggest concern of swine farm to the neighboring social gathering.

- Economic impact evaluation: Organic fertilizer is considered as a by-product that can be sell with the market price of 2 to 3 baht per kilogram.

2.3.1.2 Electricity Generating approach:

- Environmental impact evaluation: In part of approach that can generate electricity energy such as conversion of waste wind from ventilation fan into wind power energy (the counter rotate dual fan wind power unit) and rooftop solar plant for energy use on-farm, to be consider as recycling energy to optimize the usage. Generated electricity can be use as a substitute energy in the farm and the differences can be use to calculate the decrease of CO_2 emission from the power plant, CO_2 emission factor of generating electricity has the value of 0.561 $kgCO_2/kWh$ based on TGO, the calculation is as follow:

$$CO_{2\ eq\ reduction\ (B\ or\ C)} = CO_2 \text{ emission factor} \times \text{Electricity output}$$

Where;

- $CO_{2\ eq}$ reduction of each approach in 1 day (tCO_2/day) (B=the counter rotate dual fan wind power unit; C=rooftop solar plant)
- Electricity output of each approach (MWh) = electricity generated (kWh)/1000
- CO_2 emission factor for electricity output 1 MWh = 0.561 tCO_2/MWh

Electricity that is generated from biogas usually comes from combustion of CH_4. To calculate the decrease of CO_2 emission from conversion of bio-waste into bioenergy and bio-fuel approach (biogas), CH_4 emission factor of generating electricity has the value of 0.001 tCH_4 /kWh, and to convert into tCO_2, GWP_{CH4} is to be account in the formula based on Thailand greenhouse gas management organization (TGO), the calculation is as follow:

$$CO_{2\ eq}\ reduction_A = CH_4 \text{ emission factor} \times \text{Electricity output} \times GWP_{CH4}$$

Where;

- $CO_{2\ eq}$ reduction of biogas (A) approach in 1 day (tCO_2/day)
- Electricity output of biogas approach (MWh) = electricity generated (kWh)/1000
- CH_4 emission factor for electricity output 1 MWh = 0.001 tCH_4/MWh
- GWP_{CH4} = Global warming potential of CH_4 = Default 25 tCO_{2e}/tCH_4

Total $CO_{2\ eq}$ reduciton = CO_2 reduction$_A$ + CO_2 reduction$_B$ + CO_2 reduction$_C$

Where;
- CO_2 reduction$_A$ reduction from biogas tank
- CO_2 reduction$_B$ reduction from the counter rotate dual fan wind power unit
- CO_2 reduction$_C$ reduction from rooftop solar plant

- Economic impact evaluation: Energy production from farm's waste by conversion of bio-waste into bioenergy (biogas), conversion of waste wind from ventilation fan into wind power energy, and rooftop solar plant system into the analysis. The electricity production was efficiently subsidized some of the farm's facility, resulting in reduction in the electricity cost. Results were astonishing, it shows the reduction in the farm's electricity cost since the first month and waste from the farm was into some uses. This calculation method will be used if the farm invested in the technologies and uses the produced electricity as a subsidiary of buying from the government electricity plant, then we can find the ratio of the saved electricity cost of each and every technology installed in the farm. Starting from finding the different in the electricity cost before and after the installation of every selected approach as follow:

Different in electricity cost = Electricity cost before installation - Electricity cost after installation

Then, calculated the electricity cost reduction after installation from each approach that can generate electricity by converting the production power into fraction (each approach electricity production divided by total electricity production) and then times the different in the electricity cost to find the effect of each approach per month, the calculation is as follow:

$$\text{Electricity cost reduction}_{(A\text{ or }B\text{ or }C)} = \text{Different in electricity cost} \times \left[\frac{\text{Electricity output}_{(A\text{ or }B\text{ or }C)}}{\text{Total electricity output}}\right]$$

Where;
- Electricity cost reduction after installation of each approach per month (THB)
    - A = biogas process
    - B = the counter rotate dual fan wind power unit
    - C = rooftop solar system
- Different in electricity cost after installed all selected approach per month (THB)
- Electricity output of each approach selected per month (kWh)
- Total electricity output of all selected approach per month (kWh)

Additionally, we accounted the payback period of each approach after installation by formula as follow:

$$\text{Payback period} = \frac{\text{Initial investment}}{\text{Average save cost}}$$

Where;
- Payback period of each approach (Month)
- Initial investment of each approach (THB)
- Average save cost per month (THB)

2.3.2 The study of prototype technologies

Commonly, the Evaporative cooling system (Evap') in the swine farm will result in better quality and quantity comparing to traditional open system. The housing units are usually the size of 12 × 120 m dimension, and there are approximately 10 ventilation fan sizes of 36-50 inches, which produce waste air. We see the opportunities to use the waste air and started to collected various ventilation fans research into this research project. The ventilation fans option are as follow:

3. Results and Discussion

From the interview and observation from SPM farm, it is known that raising a batch of swine will take up to 4 months, and 2 weeks to reset the housing environment. There can be up to 3 batch of swine in a year, resulting in total number of 270,000 swine per year within 7 farms located around Paktor, Ratchburi province. SPM farm decided to take the matter into their priority to solve the waste problem. Then the solar energy and biogas energy were install, and currently researching on new ventilation system to make use of the waste air as in the flow illustrated in the Figure 4.

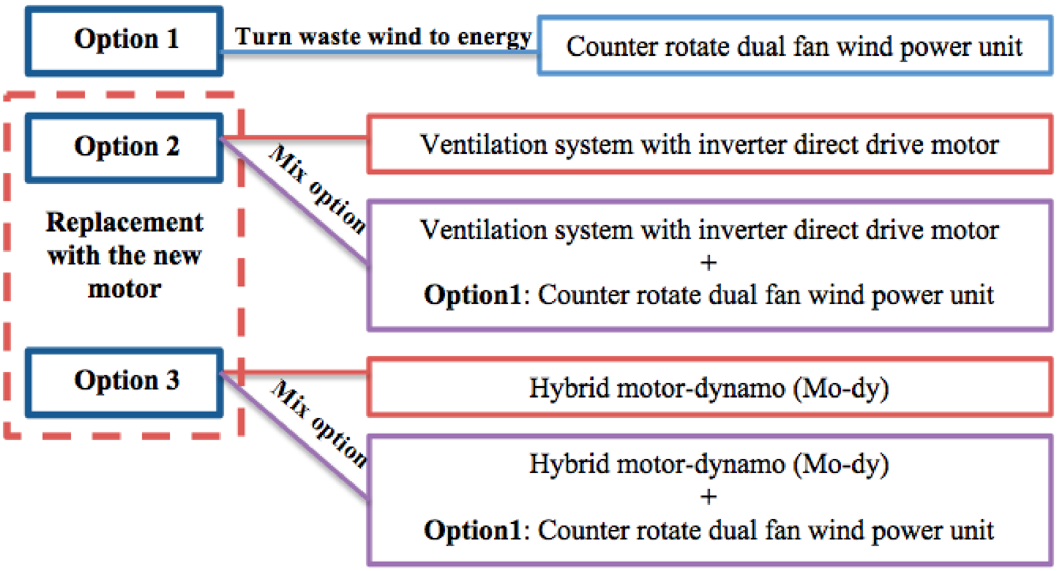

Figure 3. Ventilation fan sustainability frameworks can be divided into 3 options.

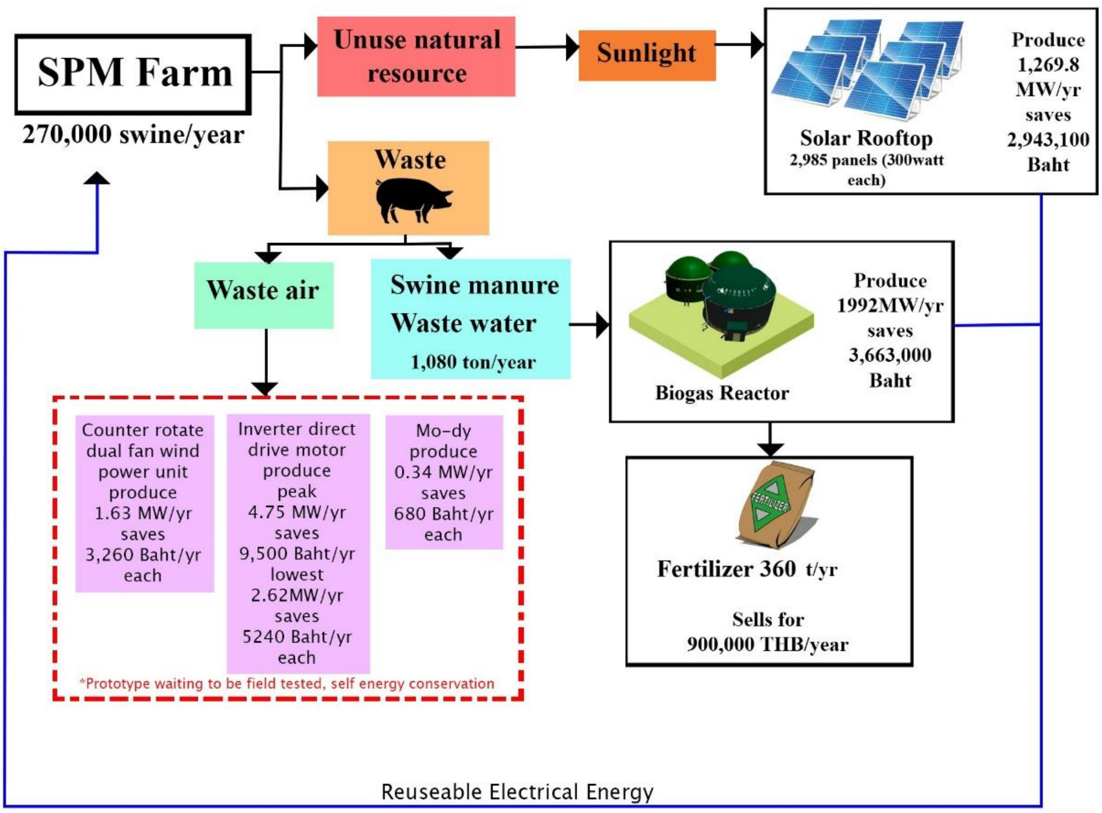

Figure 4. The study flow chart of technologies installed in SPM farm

In each technology applied in the farm system is a way to bring waste into the electricity generating process and helps farm reduce the cost of production as the Table 1 below:

Table 1. Environmental and economic impact evaluation of the solar system, biogas system and ventilation fan prototypes technologies applied to SPM farm.

Technology	Electricity Generation (MW)	Environmental Impact Evaluations (tCO_2/yr)	Economic Impact Evaluations (Baht)	Payback Period (year)
The technologies applied in farm system				
Solar rooftop system	1,269.8	712.36	2,943,100* 457,000	8/4* -
Biogas system	1,992	49.8	3,663,000	
- Fertilizer	-	-	900,000	9/3**
Total	3,261.8	762.16	5,020,000	-
The prototype technologies				
Ventilation fan				
- Option 1	1.63	0.91	3,260	3.07
- Option 2	-	-	-	
○ 50%	2.62	2.66	9,500	0.67-0.37
○ 75%	4.75	1.47	5,240	
▪ Mix option 2+1	-	-	-	
○ 50%	4.25	3.57	12,760	1.59-1.06
○ 75%	6.38	2.38	8,500	
- Option 3	0.34	0.2	680	1.47
▪ Mix option 3+1	1.97	1.11	3,940	2.79

* The electricity cost saves and payback period is based on assumption that if SPM farm invest in the rooftop solar and used electricity by themselves.
** Payback period of biogas system should include the sales of fertilizer, as it is a by-product of biogas system

3.1 Solar rooftop system: Most of electricity-generated use as a supplementary energy in the farm, especially in the swine housing. Moreover EGAT does not buy back the electricity production due to swine farm uses huge amount of electricity, if EGAT buy in from the farm, EGAT will lose a lot of profit. The other condition is the systems have to be near the hub of EGAT in order for EGAT to have lower lost in transfer of electricity (PEA, 2016).

As the ideal efficiency is from the expectation of the solar panel working at 100%, but in fact it couldn't achieve that effectiveness in working condition due to external environmental factors such as, cloudy day, rainy day, the consistency of sunlight strength, and how often the panel were clean so it has nothing to block the sunlight thus result in the fluctuate in payback period of different installation. The system maintenance follows these steps:

- Clean the solar panel with clean wet cloth or sprinkle water on the panel's front and mop it off, this process doesn't involve with any chemical.
- Check the wire and the connector for faulty wire/connection. This is to prevent any accident and to allow good electricity to flow.

3.2 Biogas system: SPM farm used H-UASB system due to it capability of treating waste with high percentage of suspended solid. By store their waste in a wastewater pond that circulates roughly 1,080 ton of swine manure per year. And send into a buffer tank to be ready to put in H-UASB system before converting it into electrical energy.

The biogas system in SPM farm is a 20 year old system which is much more expensive than those newly build as the technology at that time was not as cheap as today. When comparing the investment of covered lagoon biogas system of SPM farm to a modern investment price, it shows that if the system is to be build in with newer technology it would cost 1,480.89 baht per 1 LU and able to generate 226.91 kW/yr per 1 LU. The new system will be able to generate 4 times higher output than the 20 years old system in SPM farm with the similar investment (Greenthaibiogas, 2012).

When comparing electricity cost, the actual cost usage might vary due to energy loss during the energy conversion, storage, and transportation. The leftover wastes will dried up into fertilizer as a by-product that has the selling value of 3 baht per kg.

Which make profit for the farm up to 900,000 baht per year.

From the research of various technology and invention, and field testing at SPM farm as a model of farm-sharing, results were collected and analyzed to be compile in a programmed MS excel data sheet where the investors can use it as a guidance in economic and environment impact factor according to their needs. The data sheet shown the estimated results consist of cost of installation of the technology, minimum and maximum electricity generated, payback period (breakeven point), and carbon emission reduction. There are three main category of programmed data sheet provided, which users can put in their farm specification/need are shown in Figures 5 below:

SOLAR ROOFTOP

Please fill in the information		
The rooftop shade-free spaces	3000	sq.m
The space of solar panel (per 1 panel)	2	sq.m
Spec of solar panel	300	Wp
Cost of solar panel (per 1 panel)	THB 8,500.00	Baht

Results: in case of the space of shade-free rooftop area for installation		
Amount of solar panel installation	1,500.00	Panels
Investment cost of installation (Minimum)	THB 12,750,000.00	Baht

(*The installation fee is not included)

Electricity Generation	Maximum	657.00	MW/yr
	Minimum	164.25	MW/yr
Electricity Cost Saving	Maximum	THB 1,314,000.00	Baht/yr
	Minimum	THB 328,500.00	Baht/yr
Payback Period	Fastest	9.70	Year
	Slowest	38.81	Year
Carbon Emission Reduction	Maximum	368.58	tCO2/yr
	Minimum	92.14	tCO2/yr

Please fill in the information		
Electricity requirement in swine farm (Load require)	164.25	MW/yr
Spec of solar panel	300	Wp
The space of solar panel (per 1 panel)	2	sq.m
Cost of solar panel (per 1 panel)	THB 8,500.00	Baht

Results: in case of the electricity load needed for supplied in farm operation		
Investment cost of installation (Minimum)	THB 12,750,000.00	Baht
Amount of solar panel installation (Minimum)	1,500.00	Panels
The rooftop shade-free spaces for installation	3,000.00	sq.m

Electricity Generation	Maximum	657.00	MW/yr
	Minimum	164.25	MW/yr
Electricity Cost Saving	Maximum	THB 1,314,000.00	Baht/yr
	Minimum	THB 328,500.00	Baht/yr
Payback Period	Fastest	9.70	Year
	Slowest	38.81	Year
Carbon Emission Reduction	Maximum	368.58	tCO2/yr
	Minimum	92.14	tCO2/yr

*Note: The electricity generation and payback period are depend on the climate and spec of solar panel efficiency.

(a) Solar rooftop data sheet: by fill in the rooftop shade-free spaces in the left table or the electricity load needed for supplied in farm operation in the right table to calculated in data sheet, and also the spec of solar panel that choosing installed in system.

BIOGAS

Please fill in the information		
Number of Nursery Swine Population	108000	Head/yr
Number of Fattening Swine Population	108000	Head/yr
Number of Breeder Swine Population	54000	Head/yr

Results			
Amount of Swine Manure		1350	tons/yr
Electricity Generation	Maximum	7695	MW/yr
	Minimum	4860	MW/yr
Investment Cost	Biogas Generator	THB 16,200,000.00	Baht
	Construction Land + PE	THB 34,020,000.00	Baht
Total investor		THB 50,220,000.00	Baht
Electricity Cost Saving	Maximum	THB 20,007,000.00	Baht/yr
	Minimum	THB 12,636,000.00	Baht/yr
Payback Period	Fastest	2.39	Year
	Slowest	3.77	Year
Carbon Emission Reduction	Maximum	192.38	tCO2/yr
	Minimum	121.50	tCO2/yr

Livestock Unit (1 LU = 500kg)	33,912.00	LU
Investment Cost per 1 LU	1,480.89	Baht

(*The investment cost is estimated, please contact the contractor for the actual investment cost.)

Fertilizer from Biogas System	337.5	tons/yr	Maximum Selling Price	1,012,500.00	Baht
			Minimum Selling Price	675,000.00	Baht

* Note: The volume of swine manure depends on the health of swine, quantity and quality of food feeding, which resulting in different volume swine manure in each farm.

(b) Biogas data sheet: by fill in the number of swine population in each range.

Ventilation Fans

Please fill in the number of ventilation fan in each type of installation			
Option 1: Counter rotate dual fan wind power unit		1	Unit
Option 2: Ventilation system with inverter directs drive motor		1	Unit
Mix option 1+2	Option 1: Counter rotate dual fan wind power unit	1	Unit
Option 3:Option 3: Hybrid motor-dynamo (Mo-dy)		1	Unit
Mix option 1+3	Option 1: Counter rotate dual fan wind power unit	1	Unit

	Result					
	OPTION		Maximum Electricity Generation (MW/yr)	Maximum Electricity Cost Saving (Baht/yr)		Carbon Emission Reduction (tCO2/yr)
	Option 1: Counter rotate dual fan wind power unit		1.63	THB	3,260.00	0.91443
Replacement with new motor	Option 2: Ventilation system with inverter directs drive motor					
		Blades rotational speed 50%	2.62	THB	5,240.00	1.46982
		Blades rotational speed 75%	4.75	THB	9,500.00	2.66475
	Mix option	Option 2 (Blades rotational speed 50%) + Option 1	4.25	THB	8,500.00	2.38425
		Option 2 (Blades rotational speed 75%) + Option 1	6.38	THB	12,760.00	3.57918
	Option 3: Hybrid motor-dynamo (Mo-dy)		0.34	THB	680.00	0.19074
	Mix option	Option 3 + Option 1	1.97	THB	3,940.00	1.10517

	Investment Cost *The cost of ventilation fanis not included in calculation.					
	OPTION		Investment Cost (Baht)		Payback Period (year)	
					Slowest	Fastest
Replacement with the new motor	Option 1: Counter rotate dual fan wind power unit		THB	10,000.00	-	3.07
	Option 2: Ventilation system with inverter directs drive motor		THB	3,500.00	0.67	0.37
	Mix option	Option 2 + Option 1	THB	13,500.00	1.59	1.06
	Option 3: Hybrid motor-dynamo (Mo-dy)		THB	1,000.00	-	1.47
	Mix option	Option 3 + Option 1	THB	11,000.00	-	2.79

(c) Ventilation fan prototype data sheet: by fill in the number of ventilation fan option that choosing installed in the farm.

Figure 5. Programmed MS excel data sheet for investor to use as guidance. Consist of (a) Solar rooftop, (b) Biogas, (c) Ventilation fan.

4. Conclusions

This research project aims to be the aid in decision making for those entrepreneurs who are interested in setting up new smart green framing. By leaning toward technology, the production cost will go down drastically resulting in higher income. From the study shows that the solar rooftop system and biogas system of SPM farm can generate total electricity of 3,261.8 MW/year. SPM farm was able to reduce the electricity cost by 4.12 million baht/year and profited 900,000 baht/year from the fertilizer (by-product of biogas system), total in 5.02 million baht benefited in economic aspect. SPM farm have total number of livestock unit (LU) of 33,912 LU, which 1 LU can save the cost by 4,563,000 baht/33,912 LU=134.55 baht/1 LU (*4,563,000 come from electricity cost saved 3,663,000 baht + fertilizer 900,000 baht). These cost saving doesn't include the installation of the prototype ventilation fans that can further reduce CO_2 and save more electricity cost in the rearing operation in swine farm. In order for biogas system to breakeven faster the number of LU should be as close to the max capacity of each generator as possible, by not carefully calculate the ratio of LU to the number of generator might resulting in longer breakeven period.

In the environmental aspect, the electricity production that used as a reusable energy is resulting in CO_2 reduction of 762.16 tCO_2/year, which is the main cause of global warming. Additionally, biogas system plays an important role in reducing air pollution that effect the neighboring residential area as shown in the monitoring result of the questionnaire. By these technologies installation, it helps to improving quality of live for people living nearby and able to coexistence with SPM farm happily in long term. Moreover the farm helps create numerous jobs for the local to have a stable income in socio-economic aspect. But most importantly, strategy for attaining sustainable swine farming transformation should be improving in all the three relationship aspects among economic, environmental, and society, will lead into much more sustainable smart green farming.

However, transforming farm into a smart green farming require a lot of capital investment. So, the investor needs not to install every technology available. This research project is just a guideline to help in decision making by choosing the technology according to the farm needs and adapt it to the waste production from their farm.

5. Acknowledgements

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People's Perception and Adaptive Behaviours for Mitigating Climate Change

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Abstract

The world's population is playing their role in greenhouse gas emissions to the atmosphere due to their activities in daily life such as energy use. Consequently, the global society has been facing with climate change resulting in severe potential impacts. This motivates people in reducing energy consumption and changing behaviours in response to climate change. This study was conducted to investigate individuals' perception on climate change and to investigate their behaviours in response to climate change mitigation. According to the result, it was shown that people well perceived climate change and also were aware of the issue because most people felt interested in climate change and desired to make the difference. For behaviours in response to climate change, most individuals always save energy use such as turning off lights and recycling respectively.

Keywords: Climate change/ Greenhouse gas emissions/ Perception/ Impact/ Mitigation

1. Introduction

Knowledge related to global climate change has widely increased in various environmental principles and dimensions; nevertheless, it is evident that research papers and relevant programmes about this phenomenon have been discussed and operated in many cities (Monsalves-Gavilán et al, 2013). The city is considered to be a more significant place in response to climate change because there is the existence of discussion that cities are the main sources of greenhouse gases emissions (GHG) (Broto and Bulkeley, 2013). In particular, an anthropogenic factor is the main origin to release greenhouse gas (GHG) (Utaraskul, 2015). Climate change is one of the important environmental phenomena of the current society, with global situations that display a big challenge for both developed and developing countries (Monsalves-Gavilán et al, 2013). There are the questions that what and who is most fragile to the impacts of climate change (Broto and Bulkeley, 2013). It is continuously estimated to bring about various big damages to affect people's life, livelihood and properties. Also, natural hazards cannot be independently considered from the change of climatic conditions and its variability (Uy et al., 2015). This is because there is hazardous complexity that can affect the global societies (Linden, 2015). For example, it has effects on changing ranges of wildlife and its habitats, a rise of sea levels and erosion of coastal areas, an increase of intensity and frequency of storms and other situations (Urioste-Stone et al., 2015). More importantly, it has reflected the survival amongst climate change (Sakurai et al., 2011) or uncertainty. Eventually, people should begin with learning in mitigation and adaptation to changes in climatic conditions. Thus, this comes up with the study to investigate the perception of individuals on climate change and to investigate human behaviours in response to climate change.

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2. Methodology

2.1 Participants

This study aims to explore a sample of 146 people, including university students and staff by questionnaire surveys in and around the University of Sunderland, Tyne and Wear, UK. The demographics were divided into three parts. The first is gender (male and female) (Taddicken, 2013). The second is age group (from 14-19 years to 60 years and older) (Taddicken, 2013) and the third is educational levels (A levels, BSc/BA, MSc/MA and PhD). The survey also depended on the major characteristics of background knowledge about climate change such as its causes and potential impacts. Therefore, respondents were also sampled to reveal how they perceive and react to climate change in order to ensure that they have perceived about climate change. According to demographic information such as sex, age groups and study levels, they are control variables at the people's level that these factors might be found to affect perceptions of climate change (Marquart-Pyatt et al., 2014). Thus, these were elicited and this can help to receive the data in depth to compare and assess their perception. Finally, understanding about reported behaviours can lead to beneficial advice to information providers in conserving and decreasing the use of resources such as energy and water (Hayles and Deanb, 2015).

2.2 Design and materials

For the perception of climatic change occurrence, it was examined belief of people in relation to the occurring of climate change that the answers could be set as already occurring, not occurring today but will occur in the future, not occur at all, don't know respectively (Dai et al., 2015). The answering choices of this question were measured by adapting score from Marquart-Pyatt er al. (2014) that showed that the impacts of global warming ‘‘have already started to happen" = 1. Thus, this study were similarly scored from climate change is already taking place = 1, "not occurring today but will occur in the future" = 2, "not occur at all" = 3 and don't know = 4.

As for causes of climate change, human activities seem to be a major cause because human actions have been playing a crucial role in recent changing climate (Smith Jr et al., 2014), and the question was measured as (1 = strongly disagree, 2 = disagree, 3 = not decided, 4 = agree, to 5 = strongly agree) (Smith Jr et al., 2014).

In terms of climate change impacts, the material used in this question was adapted from Wang and Cao's (2015) study that questions contained knowledge about increasing temperatures, glaciers retreating and increasing precipitation. Moreover, there are lots of rain in the UK resulting in the possibility of flooding increased as stated in Taylor et al.'s (2014) study; thus, the aspect of climate change impacts would be asked about temperatures increased, glaciers retreating, precipitation increased and increased occurrence of flooding that all were measured by using Likert scale1–5 score (1 = strongly disagree to 5 = strongly agree) (Wang and Cao, 2015). As for the potential impacts by sectors being adapted from Smith Jr. et al.'s (2014) study for investigating people's perception on potential climate change impacts. From Smith Jr. et al. (2014), these alternative activities were measured by adapting score from not at all affected = 1 to very affected =4.

As for the section of climate change mitigation, prior to individuals' behaviours in mitigating changing climate being elicited, their reactions to climatic change would be examined because of reaction possibly leading to responding behaviours. In terms of reaction, there were 5 choices for answering when respondents feel about climate change such as "Sceptical, Angry, Helpless, Interested and Desire to make difference" and each option could be scored by ranking from 1 = no reaction to 5 = strong reaction (Hayles and Dean, 2015). In terms of people's behaviours in mitigating climate change, activities in reducing energy were adapted from Whitmarsh et al. (2011) and they were measured by scoring from Never = 1 to always = 4.

2.3 Data analysis

Numerical study is conducted by using SPSS version 23.0 statistical software. The data about impacts and human behaviours in mitigation of climate change was entered into the SPSS programme and subjected to descriptive analysis. The data was analysed by descriptive of frequencies to investigate the mean and standard deviation (SD) of data to examine the number and percentage of data.

3. Results

3.1 Demographics

Table 1 lists that the percentage of females participated in this study was higher than males by almost half. For age groups, a group of respondents who were in a range of 20 - 29 years old was the highest percentage, whereas a group of participants who were aged between 50–59 years and 60 years and older was the least percentage. In regard to the group of educational levels, the figure for A level was similar to the figure for BSc/BA level as the most respondents in this study, while the percentage of participants who has already had a PhD qualification were the lowest in this study. However, there was the difference between the proportion of male and female students at the University of Sunderland in 2015 that the percentage of women students was higher than men students and also the high difference of number of educational levels that the figure for undergraduate respondents were more than postgraduate respondents by about Eight times in the same year; hence, this research mainly focuses on A level and Degrees (BSc/BA and MSc/MA).

Table 1. Demographics of this study

Demographics	Number	Percentage
Gender		
Males	53	36 %
Females	93	64 %
Age Groups		
14–19 years	23	15.8 %
20–29 years	96	65.8 %
30–39 years	16	11 %
40–49 years	5	3.4 %
50–59 years	3	2.1 %
60 years and older	3	2.1 %
Education levels		
A level	63	43.2 %
BSc/BA	62	42.5 %
MSc/MA	14	9.6 %
PhD	7	4.8 %

3.2 Perception of climate change and its causes

Table 2 lists information of participants responding to the survey questionnaire. It was evident that the majority of people thinks that climate change has already taken place. Furthermore, the most respondents strongly agreed that human actions have been playing a significant role in climatic change as seen in Table 3.

Table 2. Number of individuals in responses to climate change perception

To what extent do you think climate change is taking place?	Total
1. Already occurring	135
2. Not occurring today but will occur in the future	2
3. Not occur at all	3
4. Don't know	6

Table 3. Number of individuals in perceiving human activities as climate change causes

Do you think that human activities have been playing a crucial role in climate change?	Total
1. Strongly agree	65
2. Agree	59
3. Not decided	16
4. Strongly disagree	3
5. Disagree	3

3.3 Perception of Climate change impacts

Table 4 shows that the highest percentage of individuals agreed with air temperatures significantly increasing resulting from climate change. Moreover, the same percentage of people agreed that climate change caused an increase of precipitation and occurrence of flooding (47%). Likewise, 43% of participants agreed that climate change led to glacier treating. However, when focusing on the figure of people about being more strong perspective, most individuals strongly agreed that flood situations are possibly getting more increased. When looking at the figure for the mean of gender, the average of females in knowing about all impacts from climate change was higher than males as seen in Figure 1. As for educational level, the average of the perception on climate change impacts of individuals who have already had Degrees was higher than individuals who have held A level. Nevertheless, the mean of people who have held A level in perceiving an increase of flooding was higher than those who have had Degrees as can be seen in Figure 2.

Table 4. The perspective of people on the impacts of climate change

The perspective of people on impacts of climate change	Mean	SD	Strongly agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly disagree (%)
1. Air temperatures significantly increasing	3.98	.812	25.9	51.1	18.7	3.6	7
2. Glaciers retreating	4.05	.911	34.5	43.2	17.3	2.9	2.2
3. Precipitation increased	3.74	.887	18	47.5	27.3	5	2.2
4. Increased occurrence of flooding	4.14	.848	36.7	47.5	10.1	5	.7

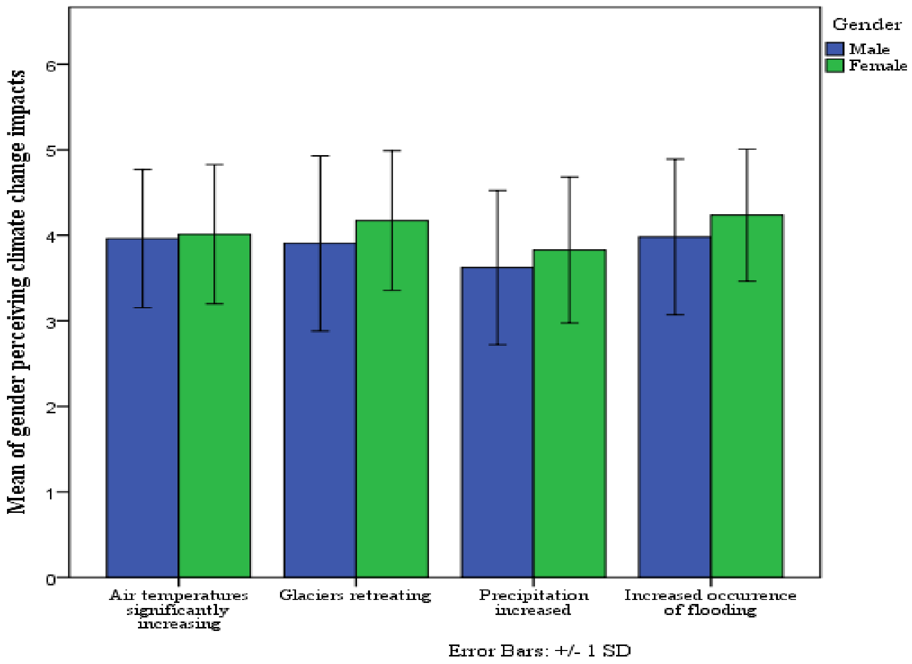

Figure 1. Mean of gender in perceiving climate change impacts

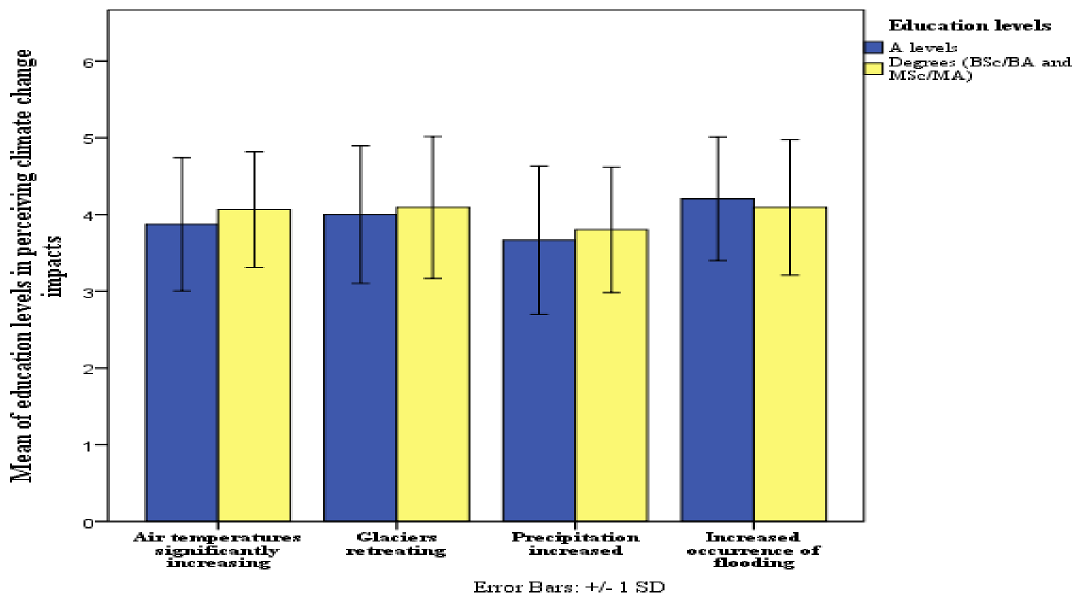

Figure 2. Mean of educational levels in perceiving climate change impacts

In terms of examining the perception on sectors that may be affected by climate change, Figure 3 indicates that the mean of individuals' perception on extreme weather events was the highest, closely followed by the average of perception on ecosystem degradation, farming livelihood respectively. On the contrary, when looking at the small business sector that might be affected by climate change, the average of perception was the lowest in comparison to all sectors. As for considering gender in perceiving sectors that may be affected by changing climate, the average of females in perceiving climate change having influence on most sectors was higher than males as shown in Figure 4. However, when focusing on economic, and resort and outdoor recreational sectors, the average of the perception of males on these two sectors in being affected by climate change was higher than females.

With respect to levels of education, Figure 5 shows that the mean of respondents who [2]have had Degrees in perceiving sectors being affected by changing climate was higher than A level. Furthermore, the average of sampled participants who have held Degrees in perceiving ecosystem degradation and resort sectors was nearly the mean of the perception of people who have had A level certificates. Overall, most individuals from two educational levels perceived that extreme weather events might be very affected by climate change, whereas the mean of the perception of those people about small businesses which might be affected by climate change was the lowest as demonstrated in Figure 5

3.4 Climate change mitigation

Prior to demonstrating the outcome of people in adopting their behaviours in response to climate change, the result of reaction was firstly illustrated as followed. From Figure 6, the high mean of individuals who were interested in climate change was similar to the mean of people who desired to make the difference, but the figure for the average of individuals who wanted to make the difference was a bit higher.

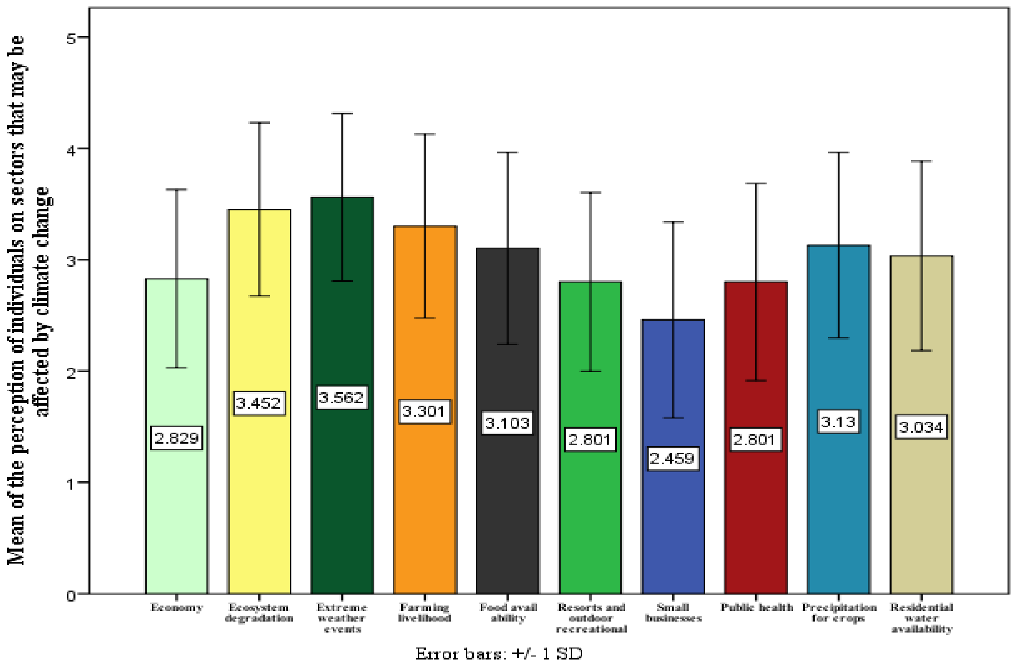

Figure 3. Mean of the perception of people on sectors that may be affected by climate change

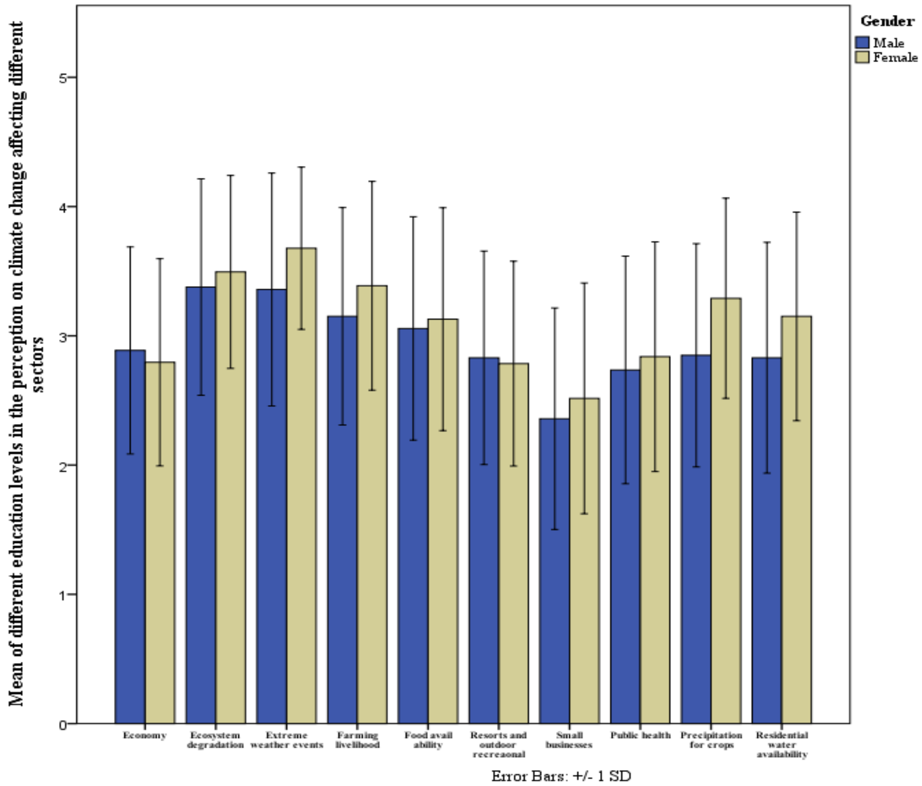

Figure 4. Mean of the perception of gender on sectors that may be affected by climate change

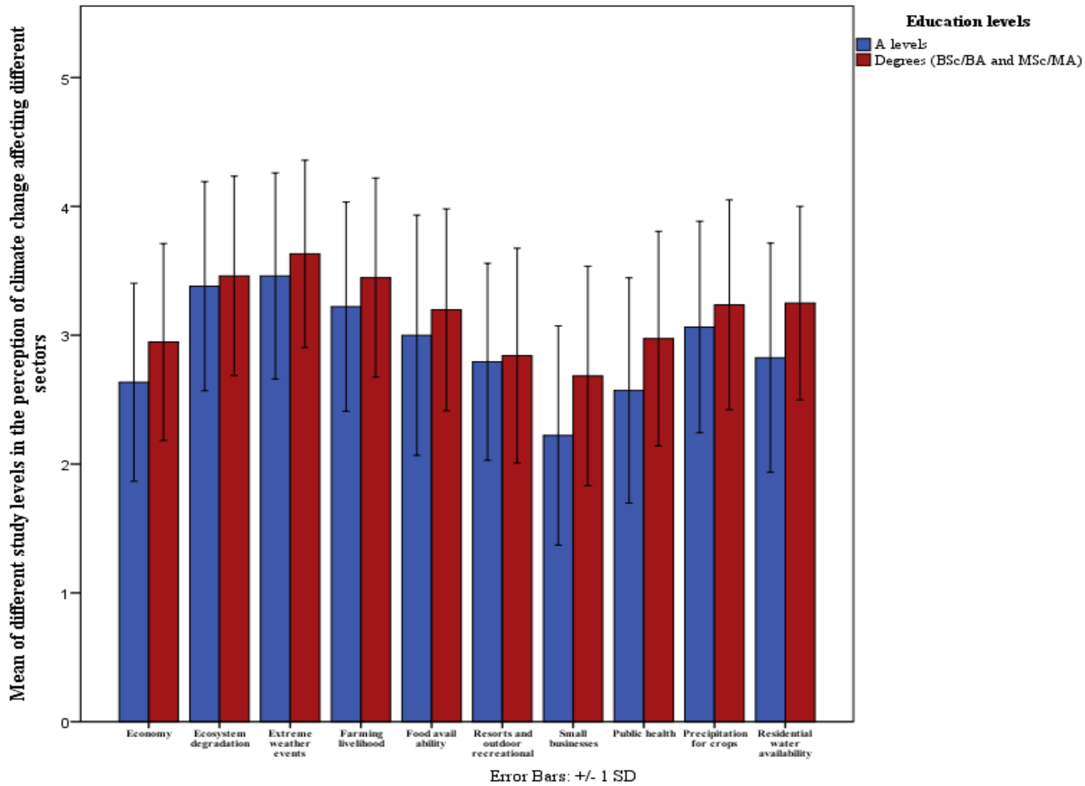

Figure 5. Mean of different education levels on sectors that may be affected by climate change

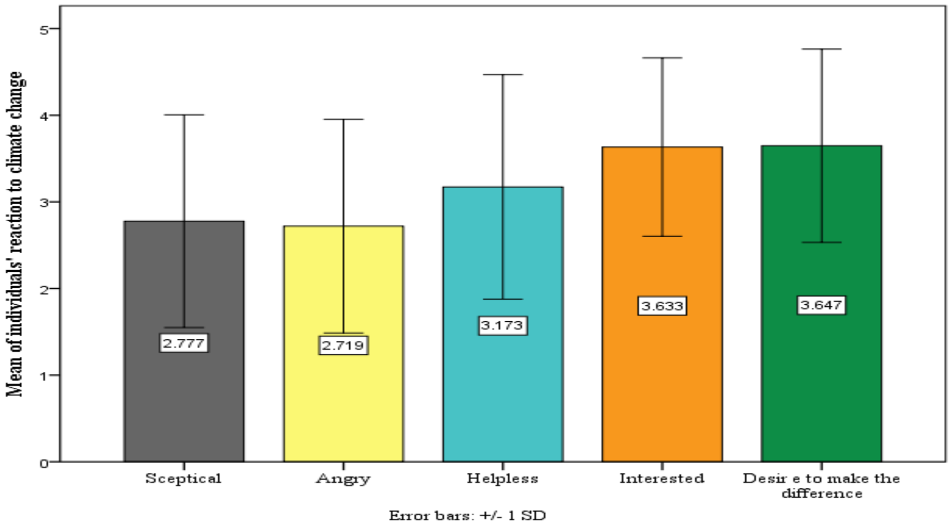

Figure 6. Mean of people's reaction to climate change

Table 5 clearly demonstrates that the highest percentage of people always turn off the lights when they did not use, closely followed by recycling that those individuals always adopt in mitigating to climate change as shown at 56.2 %. In addition, almost 50 % of individuals always turn off the tap during brushing their teeth. Furthermore, the percentage of individuals in walking was at 31.7%. Nevertheless, the percentage of people in reducing buying products with less packaging was lower than other behaviours and the highest percentage of people never cycle as presented at 56.8 %.

When investigating the average and SD of gender in adopting their behaviours in response to climate change, Table 20 shows that the average of females in turning off lights was the higher than males as shown at 3.49 (SD=.86) and 3.19 (SD=1.06) respectively. As for recycling, the average of females in adopting their behaviours in response to climate change was the higher than males at as illustrated 3.37 (SD=.92) and 3.25 (SD=.80) in order. In terms of cycling, the mean of males and females was the lowest at 1.92 (SD=1.07) and 1.68 (SD=.98) respectively; however, the mean of males in cycling was higher than females as shown in Table 6.

According to educational levels, Figure 7 demonstrates that the highest mean of individuals who have held Degree studies was recycling, closely followed by the average of those who turn off the lights when they are not used. Additionally, the same mean of people who have had Degree studies walked and turned off the tap when they brushed their teeth. On the other hand, the average of cycling of people who have held A level study and Degree studies were the lowest in comparison to other behaviours. Nevertheless, the average of buying goods with less packaging of respondents who have had A level study was higher than those who had had Degree studies.

Table 5. Percentage of the frequency of people in adopting their behaviours in mitigating climate change

People's behaviours have been adopted in response to climate change	Always (%)	Often (%)	Occasionally (%)	Never (%)
1. Recycling	56.1	25.6	11.5	5.8
2. Turn off lights that they are not used	62.6	21.6	7.2	8.6
3. Turn off the tap during you brushing your teeth	49.6	18.0	14.4	17.3
4. Sharing vehicle travelling with someone else	20.9	32.4	26.6	20.1
5. Taking public transport for a short journey	21.6	26.6	20.1	31.7

Table 5. Percentage of the frequency of people in adopting their behaviours in mitigating climate change (cont.)

People's behaviours have been adopted in response to climate change	Always (%)	Often (%)	Occasionally (%)	Never (%)
6. Walking	31.7	38.1	20.9	9.4
7. Cycling	10.1	5.8	27.3	56.8
8. Reusing or repairing items before throwing away	18.7	37.4	32.4	11.5
9. Purchasing goods with less packaging	11.5	26.6	33.8	28.1
10. Purchasing environmentally friendly goods	17.3	23.7	39.6	19.4

Table 6. Mean, +/- SD of males and females' behaviours in adopting in response to climate change

People's behaviours have been adopted in response to climate change	Overall		Males		Females	
	Mean	SD	Mean	SD	Mean	SD
1. Recycling	3.32	.909	3.25	.804	3.37	.918
2. Turn off lights that they are not used	3.38	.941	3.19	1.057	3.49	.855
3. Turn off the tap during you brushing your teeth	2.99	1.168	2.91	1.131	3.03	1.193
4. Sharing vehicle travelling with someone else	2.53	1.045	2.55	1.084	2.53	1.028
5. Taking public transport for a short journey	2.42	1.185	2.42	1.126	2.42	1.173
6. Walking	2.94	.948	3.02	.951	2.89	.949
7. Cycling	1.77	1.017	1.92	1.071	1.68	.980
8. Reusing or repairing items before throwing away	2.64	.915	2.70	.890	2.61	.933
9. Purchasing goods with less packaging	2.21	.989	2.26	1.095	2.17	.928
10. Purchasing environmentally friendly goods	2.37	.990	2.40	.987	2.35	.996

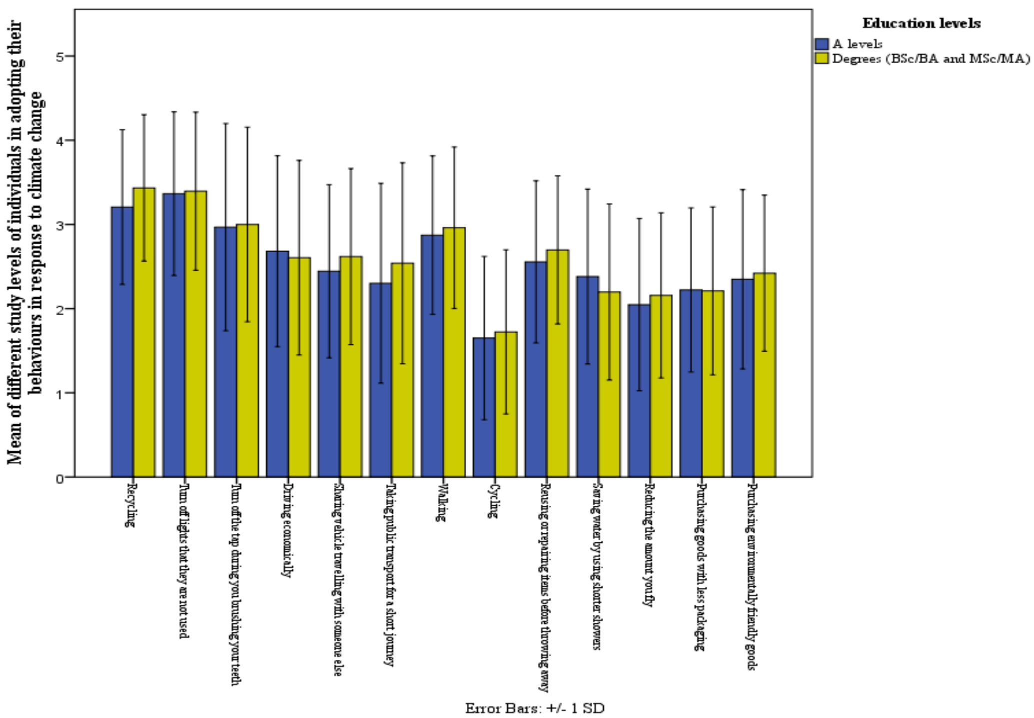

Figure 7. Mean of different educational levels of individuals in adopting their behaviours in response to climate change

4. Discussion

Table 2 indicates that the majority of respondents thought that climate change is already taking place. This outcome conforms to Haylesa and Deanb, (2015) and Dai et al. (2015)'s research that three-fourth of participants believed that climate change is occurring today. This is also similar to Smith Jr. et al. (2014), 100% of participants were also convinced that changes of climate are already happening. However, Finnis et al. (2015) claimed that the belief, which climate change is taking place, was rejected because there is the present variability of the media frequently. For example, USA media builds a ‘‘false balance’’ between believers and sceptics of climatic change.

For changing climate causes, Table 3 shows that the result of the most respondents strongly agreed that human actions have been playing an important role in climatic change. This is because an anthropogenic factor is the main origin to release greenhouse gas (GHG) (Utaraskul, 2015). As a consequence, this rise of the concentration of greenhouse gases have resulted from the emissions from human activities (Gao et al., 2017) such as transport (Sovacool, 2014), industries, deforestations, vehicle emissions and the use of fossil fuels (Whitmarsh et al., 2011). Accordingly, it can be stated that anthropogenic activities now produce major sources of emissions of greenhouse gas to the global atmosphere (Palutikof el al., 2004 and Pidgeon et al., 2008). However, Florides and Christodoulides (2009)'s study explained that natural factors such as changes of the World's orbit, changes of the Sun's intensity, changes of the ocean currents and volcanic emissions contributing to the increasing global temperatures; thus, this might cause changing climatic conditions.

According to climate change impacts, Table 4 shows that the highest percentage of participants agreed that air temperature significantly increasing is from an effect of climate change, followed by flooding increase. This outcome was similar to Wang and Cao, (2015) as stated that the majority of participants had a strong opinion and perceived that there was a steady increase of air temperatures. Moreover, Megersa et al. (2014) also claimed that there was the majority of perception on a temperature rise. This is because an increase of heat wave cause global warming (Marquart-Pyatt et al., 2014). When considering, as a result of human activities as previously stated, emission of greenhouse gases are released to the air leading to global warming (Truelove and Parks, 2012) and also climate change.

As for rising floods, the similar figure of individuals agreed that climate change resulted in an increase of precipitation and flooding occurrence. It is quite reasonable because when there is the increasing rainfall, it may bring about more possible floods. However, when concentrating on the percentage of people about being more strong perspective, most individuals strongly agreed that flood situations are possibly getting more increased. This is because there is more evidence to indicate that personal experiences associated with serious climate situations have an effect on the perception on climate change (Linden, 2015). More importantly, it is claimed that the UK flooding may become significantly severe due to climate condition aspects (Penning-Rowsell et al., 2006) and the population might experience flooding themselves due to several flooding situation in the UK (Taylor et al., 2015).

According to Figure 6, there were the high interesting rate of people and the need to make change. It might be because it is widely recognised about global climate change and the survey indicates that awareness and concern about changes of climate have increased throughout a 20-year period (Whitmarsh et al., 2011). More significantly, this research was undertaken in the UK and it was suggested that the majority of individuals in Europe, which might include the UK, are concerned about changing climatic condition and believe that the global climate is changing (Hayles and Dean, 2015). Additionally, perception on causes of climate change can lead to emission reduction that is a significant factor to affect willingness and behaviours (Howell, 2011). This can lead to reaction and changes of behaviours in response to climate change.

From Table 5, it is clear that there was the percentage of individuals who always turn off lights, which they are not used, was high. This was similar to Whitmarsh et al. (2011) as shown that many people always switch off lights when they are not in use, while Whitmarsh (2009) stated that there was the low number of respondents turning off lights. However, from this study, the percentage of individuals in turning off lights was high because those might want to save money; nevertheless, it might be aware of climate change and energy use in house could be most options of behavioral changes to respond to changing climate (Semenza et al., 2008). According to Howell, (2011)'s study, people are responsible via household energy usage and around 35% of the total greenhouse gas emissions in the UK. Therefore, it could be stated that individuals and offices have searched for alternative solutions to promoting lower carbon lifestyles (Howell, 2011).

In the aspect of recycling, the outcome shows that the percentage of participants who always recycle was the second and this was similar to Truelove and Parks, (2012) as shown that recycling was the second most frequently adopted behaviours, whereas Whitmarsh et al. (2011) indicated that most people always recycle in their study as recycling is very efficient action to mitigate climatic change (Truelove and Parks, 2012) due to greenhouse gas emission reduction from recycling.

5. Conclusions

Most individuals in this research had knowledge background about climate change because the result shows that the majority of participants (135 persons) believed that climate change is taking place and most of them (65 persons) strongly agreed that human activities has been playing a significant role resulting in changing climate. In addition, most individuals (51%) agreed that a significant average increase of air temperatures, closely followed by 47% of individuals agreeing that increasing precipitation and flooding occurrence were the effect of climate change. Turning to reaction and behaviours of people, as for reaction, most people felt interested in climate change and desired to make the difference. Most of them perceived that the origin of climate change causes resulting from human being activities and concern about them, this led to people's behaviours adopting for mitigating climate change. It was clear that most people (63%) always turn off lights when they did not use because people were aware of a climate change issue and it was the easy way in adopting their behaviours for saving energy use at home and also reducing greenhouse gas emissions.

It is expect on the further study that it would be concentrated on spatial studies and the content would be focused on adaptation to climate change.

6. Acknowledgements

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Statistical Downscaling for Regeneration of Historical Daily Rainfall

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Abstract

Historical meteorological record is spatially and temporally limited in developing countries including Thailand. Regarding large uncertainties of future climate change projections, historical record is believed to be more convincible for development of stakeholder's belief and support to government's climate change actions. This study proposes a method to regenerate historical daily rainfall by developing a statistically downscaling relationship between large-scale observed variables and local rainfall station. Khlong U-Taphao Basin in Southern Thailand was selected for demonstration. The area contained 11 daily rainfall stations in which most of them was monitored since 1980. It was found that atmospheric precipitable water, geographic airflow velocity at 850 hPa, vorticities and divergences were a strong rainfall predictor. For model performance, the model well generated rainfall amounts (R^2 in calibration=0.69-0.45, R^2 in verification=0.17-0.86). In contrast, performance of wet day forecasting was not high (*HSS* in range between 0.17 and 0.29). This referred to stochastic components of the downscaling model. The developed model in line with long-term record of large-scale predictors was used to regenerate historical rainfall between 1948 and 2017. It was concluded that the area experienced dramatic increase in wetter conditions including extreme event through 1948 to 2017.

Keywords: Climate change/ Extreme rainfall indices/ Khlong U-Taphao basin/ Statistical downscaling

1. Introduction

Since 1992, after establishment of the United Nations Framework on Climate Change (UNFCCC), anthropogenic climate change issue has been rapidly received attention from national governments, globally. As consequence of the 21st Conference of Party (COP), many countries across the world has legally committed to reduction in national greenhouse gas (GHG) emissions, so called "Nationally Determined Contribution (NDC)". The aim is to control global warming not exceeding 2°C against the pre-industrialized period in order to avoid irreversible impacts. Nevertheless, climate change has gradually unfolded such as increase in global mean temperature, melting of glacier, flood and sea level rise, which has caused immense negative impacts to human beings (IPCC, 2014). By these, simultaneous with GHG emission reduction, adaptation is urgently required for impact prevention and minimization.

Thailand has pledged between 20 and 25 percent GHG emission reduction by 2030 against a Business-As-Usual (BAU) scenario in which the implementation will begin from 2020. Therefore, national governments are currently building preparedness for responding to the commitment through development of strategy, roadmap and action plan. Correspondingly, Thailand's government has enacted the Thailand's Climate Change Master Plan 2015-2050, which acts as a policy framework and guideline for relating agencies to prepare and implement climate change actions consistently across the country (ONEP, 2015). One essential issue for effective implementation of the master plan in a long run is enhancing capacity and awareness of stakeholders to be willing to support government's climate change policies. Yet, stakeholders' awareness on necessity to change their life style to reduce direct and indirect GHG emission and to initiate a systematic adaptation plan, is still limited in a small group such as environmentalist (Hyland et al., 2016). It is led by large uncertainties of impact projections arising from uncertain future GHG emissions as well as parameterization of global climate models, resulting in deterioration of people's belief and support.

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The Intergovernmental Panel on Climate Change (IPCC) summarized a possible future scenario into 4 Representative Pathway Concentrations (RCP) namely, RCP2.6, RCP4.5, RCP6.0 and RCP8.5 in which the global temperature rise in 2100 will be in range of 0.3ºC to 1.7ºC, 1.1ºC to 2.6ºC, 1.4ºC to 3.1ºC and 2.6ºC to 4.8ºC, respectively (IPCC, 2014). Such dispersed future trends cause hardship for governments, non-governmental organizations and academic institutes to inform stakeholders with aim at encouraging them to participate in campaigns for GHG emission reduction and implementation of adaptation measures. In addition, stakeholder who unbelieves that climate change has occurred tends to be relatively inactive to favoring government's action especially for climate change mitigation (Arbuckle et al., 2013). Sufficient long-term meteorological record is believed to be more informative that can lead to development of local people's awareness and willingness to implement climate change actions. However, such record is very limited in terms of temporal and spatial resolutions in developing countries especially for rainfall. For instance, in Thailand, there is approximately 41.6% of total rainfall station (out of 103 stations) monitored by Thai Meteorological Department (TMD) that contains the historical record duration longer than 60 years (as of 2017). It is noteworthy that there is other 1,210 local rainfall stations monitored by local government. Such local rainfall stations are generally initiated their record since 1975 (according to a field survey). Threshold of 60-year record refers to the definition of climate recommended by the World Meteorological Organization (WMO) that 30-year record is minimally needed to represent climate characteristics, thereby 2 periods of 30-year record can be used for investigation change of trend driven by climate change.

This study proposes a method to regenerate historical daily rainfall by developing a statistical relationship between large-scale observed meteorological variables and local rainfall at station scale. The formers are made available globally with long-term record while the latter record duration is often limited. Therefore, the developed relationship can be used to regenerate rainfall data in period before operation of the rainfall station. This application was demonstrated in Khlong U-Taphao Basin in Southern Thailand where it is flood-prone. Consequently, the downscaled daily rainfall was used for assessment of historical extreme rainfall characteristics.

2. Methodology

2.1 Study area

Khlong U-Taphao Basin in Songkhla Province is selected for this study because of economic importance in Southern Thailand, arising from its proximity to Malaysia (Figure 1). The basin area is approximately 2,400 km^2. The region has been statistically experiencing more rainfall extremes (IPCC, 2013; Limsakul and Singhruck, 2016). Annual rainfall amounts in the basin is statistically 1,995 mm and they are governed by 3 tropical monsoons as described below.

- Southeast (S/E) monsoon (February–April), dry season
- Southwest (S/W) monsoon (May–September), moderate wet season
- Northeast (N/E) monsoon (October–January), very wet season

According to a flood record in Khlong U-Taphao basin by the Royal Irrigation Department (RID), the major one entirely occurred in N/E monsoon period. An event included a flood during 21-25 November 1988; 21-25 November 2000; 6-8 November 2009; 20-22 November 2009; 31 October-2 November 2010; and 30 December 2011-1 January 2012, in which most of the mentioned flood event was caused by 3-day accumulative rainfall. Therefore, the studied period in this study was investigated only in N/E monsoon period when it is relevant to highest probability of flood occurrence in the area.

In Khlong U-Taphao Basin, there are currently 11 rainfall stations, as shown in Figure 2, which are monitored by RID and TMD representing meteorological characteristics in the area.

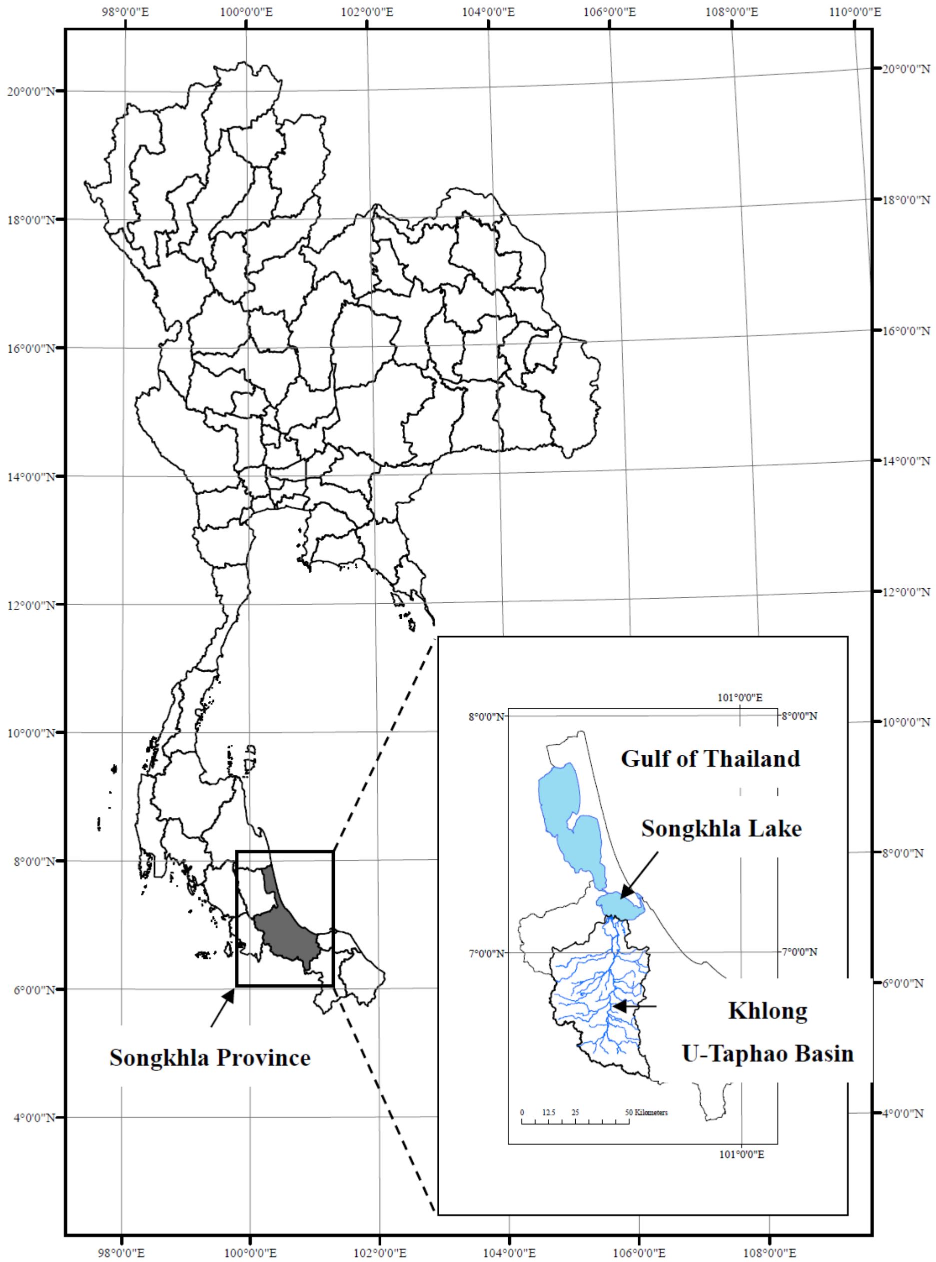

Figure 1. Location of Khlong U-Taphao Basin

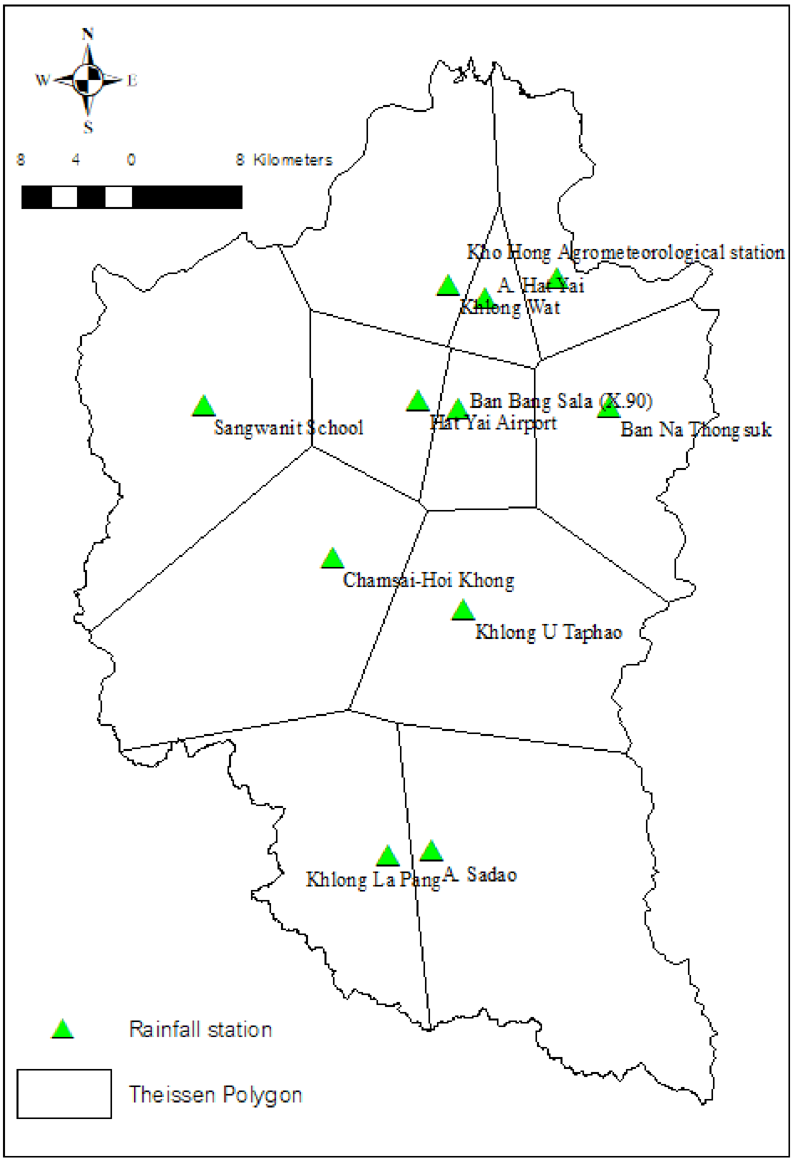

Figure 2. Rainfall stations and their respective Theissen Polygon

2.2 Historical rainfall regeneration

The method of statistical downscaling developed by Wilby and Dawson (2007), so called "SDSM", was adopted in this study for regeneration of historical daily rainfall between 1948 and 2017. SDSM is the combination of multiple regressions and a stochastic weather generator in which the latter one allows the model to simulate multiple daily rainfall series. Thereby, this study generated 100 simulations of daily rainfall to reflect model uncertainties. In order to establish a statistical relationship, a dataset of daily large-scale observed predictors reproduced by the National Centre for Environmental Prediction (NCEP) (Kalnay et al. 1996) with global grid size of 2.5^{o} latitude × 2.5^{o} longitude, as listed in Table 1, was applied. Likewise, daily rainfall record at 11 stations was used as predictands as shown in Figure 2 and summarized in Table 2. It is obvious that 5 rainfall stations have been operated after 1980. Therefore, such observational record may be spatially insufficient to demonstrate impact of climate change on change in rainfall characteristics in the basin.

Table 1. NCEP predictors used for statistical downscaling of rainfall

Code	Description	Code	Description
mslp	Mean sea level pressure	p8_f	Geographic airflow velocity at 850 hPa
p_f	Geographic airflow velocity near the surface	p8_u	Zonal velocity component at 850 hPa
p_u	Zonal velocity component near the surface	p8_v	Meridional velocity component at 850 hPa
p_v	Meridional velocity component near the surface	p8_z	Vorticity at 850 hPa
p_z	Vorticity near the surface	p8zh	Divergence at 850 hPa
p_zh	Divergence near the surface	p500	500 hPa geopotential height
p5_f	Geographic airflow velocity at 500 hPa	p850	850 hPa geopotential height
p5_u	Zonal velocity component at 500 hPa	pr_wtr	Precipitable water
p5_v	Meridional velocity component at 500 hPa	prec	Precipitation total
p5_z	Vorticity at 500 hPa	r500	Relative humidity at 500 hPa
p5zh	Divergence at 500 hPa	r850	Relative humidity at 850 hPa
		rhum	Near surface relative humidity
		shum	Near surface specific humidity

Remark: All variable unit were a dimensionless Z-score with mean zero and standard deviation one, except for prec in which the unit is in mm.

Table 2. Summary of rainfall stations used in this study

No.	Station name	Record period	Missing data (%)	Period for assessment of model performance analysis	
				Calibration	Verification
1	Hat Yai	1969-2017	16.02	1993-2017	1969-1992
2	Kho Hong Agrometeorological station	1969-2017	1.55	1993-2017	1969-1992
3	Hat Yai Airport	1973-2017	1.69	1995-2017	1973-1994
4	Cham Sai Hoi Kong	1988-2008	1.21	1998-2008	1988-1997
5	Sadao	1969-2017	18.92	1993-2017	1969-1992
6	Khlong Wat*	1975-2009	1.46	1975-2009	N/A
7	Sangsawanit School*	2000-2017	1.44	2000-2017	N/A
8	Ban Bang Sala*	2000-2017	1.44	2000-2017	N/A
9	Ban Na Thong Suk*	2000-2017	1.44	2000-2017	N/A
10	Khlong U-Taphao*	1989-2006	5.88	1989-2006	N/A
11	Khlong La Pang*	1980-2017	0.67	1980-2017	N/A

Remark: * N/A denotes the station is *not applicable* for model verification due to insufficient record duration.

Downscaled result of daily rainfall at station scale highly depends on the selected sets of predictors. In this study, a systematic algorithm for predictor selection was developed by integrating statistical approaches. Firstly, backward stepwise regression was applied to extract 8 to 11 NCEP predictors. This method works by including all candidate predictors into the model and consequently removing the least significant predictor out of the model based on *F*-test until there is no further model improvement from the exclusion of remaining predictors. Then, one predictor in a pair of selected predictors which are highly correlated was excluded using a correlation matrix to prevent collinearity. Finally, partial correlation indicating the amount of explanatory power of each predictor was adopted and the top 5 predictors with the highest correlation were selected for rainfall downscaling. The selection of 5 predictors referred to the recommendation of Mullan et al. (2012) to reasonably minimize noise from use of redundant number of predictors for downscaling.

As for model performance, simulated rainfall at Hat Yai station, Kho Hong Agrometeorological station (Kho Hong Agromet), Hat Yai Airport station, Sadao station and Cham Sai Hoi Kong station were evaluated because of their sufficient rainfall record for model calibration and verification as summarized in Table 1. Furthermore, the selection is also considered their spatial distribution covering upstream, midstream and downstream of the basin area. This study assumed that the validity of such stations ensures the model applicability at other stations. Therefore, entire rainfall record of remaining stations were used for model calibration. The evaluation was conducted separately for occurrence and amounts of rainfall. In terms of occurrence, Heidke Skill Score (*HSS*) evaluated the capability of model to regenerate wet day in comparison with the observed one. Whilst, the efficient of determination (R^2) determined the performance of regeneration of monthly rainfall amounts. The validated model at each station then regenerated historical daily rainfall between 1948 and 2017 for further study on change in wet conditions including extreme rainfall characteristics.

2.3 Extreme rainfall indices

The rainfall-related indices of extremes were obtained by reference to Statistical and Regional Dynamical Downscaling of Extremes for European Regions (STARDEX) (STARDEX, 2006). The variables included heavy rainfall threshold at the 90th percentile (pq90), greatest 3-day total rainfall (px3d), simple daily intensity (pint) and heavy rainfall proportion (pfl90). In addition, mean and standard deviation (Stdev) of daily rainfall, and percentage of wet day event (% wet day) were also determined. Likewise, return periods of 3-day rainfall were calculated by fitting annual maximum rainfall values with Gumbel distribution in which the distribution type is recommended by TMD. By considering that all simulations provide realistic variables reflecting climate in the area, the variables derived from calculation of each simulation (100 simulations in total) were averaged to obtain the representative value. Then, the result of each station was subject to weighted average based on area of Theissen Polygon, as illustrated in Figure 2, in order to calculate areal magnitudes. Furthermore, downscaled rainfall was then separated into 3 periods namely, 1948-1970, 1971-1993 and 1994-2017 for investigation of trend driven by climate change.

3. Results and discussion

3.1 Model setup

Selected NCEP predictor set for each rainfall station and their respective model performance are summarized in Table 3. It is apparent that pr_wtr was important for rainfall prediction in which 9 out of 11 stations were adopted pr_wtr in the model. This is due to its representability of water in a column of the atmosphere. In consistence with the finding of this study, Babel et al. (2016) concluded that pr_wtr was one of significant rainfall predictors in Ping Basin in Thailand. It is noteworthy that backward stepwise regression normally selected pr_wtr, prec, r500, r850, rhum and shum as a highly influencing predictor at all studied station. However, they were highly correlated with each other and pr_wtr generally showed the highest explanatory power (partial correlation) among other variables. Considering spatially, geostrophic airflow velocity at 850 hPa (p8_f) was selected for all station located in upstream (Sadao and Khlong La Pang) and downstream areas (Hat Yai, Kho Hong Agromet. and Khlong Wat). Absence of p8_f in midstream (Hat Yai and Hat Yai Airport) may be due to complex urban influence as the midstream is the location of highly concentrated urban area. Furthermore, vorticity at different geopotential heights (p_z and p5_z) also played an important role on rainfall generation in the area, as it was selected at 10 out of 11 stations. In addition, divergence at different potential heights (p_zh and p8zh) was also another significant predictor. Apart from aforementioned predictors, zonal and meridional velocities were selected dispersedly.

In overall, pr_wtr, p8_f, vorticities and divergences were a strong rainfall predictor in the study area. Atmospheric humid variables including pr_wtr highly depends on water vapor saturation in the upper air in which it relates to rainfall occurrrence (Hessami et al., 2008). As for p8_f, vorticities and divergences, there was a wide finding of positive relationships with rainfall occurrence in United Kingdom (Jones et al., 1993; Wilby, 1998; Panziera and Germann, 2010; Maraun et al., 2010).

Table 3. Summary of model performance

No.	Station	Selected predictors	Calibration		Verification	
			HSS	R^2	*HSS*	R^2
1	Hat Yai	p5_z, p8_f, p8_u, p8zh, pr_wtr	0.23	0.51	0.19	0.39
2	Kho Hong Agrometeorological station	p_u, p_z, p_zh, p8_f, pr_wtr	0.29	0.69	0.26	0.43
3	Hat Yai Airport	p5_z, p8_f, p8_u, p8zh, pr_wtr	0.27	0.56	0.26	0.50
4	Cham Sai Hoi Kong	p_z, p_zh, p8_f, p8_u, pr_wtr	0.28	0.55	0.24	0.86
5	Sadao	p_u, p8_f, p8zh, pr_wtr, prec	0.24	0.45	0.17	0.17
6	Khlong Wat*	p_u, p_z, p_zh, p8_f, pr_wtr	0.25	0.58	N/A	N/A
7	Sangwanit School*	p_zh, p5_z, r850, rhum, shum	0.17	0.49	N/A	N/A
8	Ban Bang Sala*	p5_u, p5_z, p8_v, r850, prec	0.23	0.44	N/A	N/A
9	Ban Na Thong Suk*	p_u, p_z, p_zh, 8_f, pr_wtr	0.18	0.60	N/A	N/A
10	Khlong U-Taphao*	p_zh, p5_u, p5_z, pr_wtr, r500	0.27	0.59	N/A	N/A
11	Khlong La Pang*	p_z, p_zh, p5_f, p8_f, pr_wtr	0.28	0.59	N/A	N/A

Remark: * N/A denotes the station is *not applicable* for model verification due to insufficient record duration.

According to *HSS* results, the developed models were considered to be more capable of regenerating wet day than a random process. Similarity of *HSS* values between calibration and verification periods demonstrates the model capability to capture important mechanisms responsible for rainfall occurrence (Fealy and Sweeney, 2007). Nevertheless, the results were not high due to the fact that SDSM has a stochastic component, so simulated series of daily rainfall occurrence would not be expected to match perfectly with observations. In terms of amounts, the model well regenerated at all verified station except for Sadao (Dibike et al., 2008; Souvignet et al., 2010). The low R^2 at Sadao in verification period may be because of its large number of missing value (9.2%). With less missing values, it is believed that the R^2 result will be improved corresponding to other stations. Therefore, with aforementioned *HSS* and R^2 results, it can be concluded that the developed model at all station was acceptable for further use including SDSM application for other unverified stations.

3.2 Extreme rainfall-relating indices

Statistical downscaling of daily rainfall in Khlong U-Taphao Basin is shown in Figure 3. In comparison with 1948-1970, it is apparent that rainfall in the study area dramatically experienced wetter conditions including extreme rainfall events from 1971-1993 through 1994-2018.

Shift in mean and standard deviation implies change in statistical distribution of daily rainfall. In relation to 1948-1970, it was found that mean and standard deviation was respectively increased 1.7 times and 1.4 times in 1971-1993, and 2.1 times and 1.6 times in 1994-2017. Katz and Brown (1992) demonstrated that the frequency of extreme events is more dependent on changes in the variability (square power of standard deviation) than changes in the mean. Thus, change in the variability in this study can indirectly indicate a change in the pattern of extreme events. This phenomenon will be investigated through the estimation of return periods in the following section. As for percentage of wet day, the results were also in consistence with previous variables.

Regarding STARDEX indices, level of pq90, px3d and pint are generally considered to reflect magnitude of extreme rainfall. Since the 90th percentile of a climatic variable is often considered as a threshold for extreme events, especially those related to rainfall, an increase in pq90 indicates an increase in magnitude of extreme rainfall events. Furthermore, according to a historical flood record in the study area, floods were caused by accumulative effect of 3-day rainfall. For instance, 3-day rainfall reached 597.5 mm and 483.3 mm, resulting in devastating floods in the area in 2000 and 2010, respectively. Therefore, higher px3d can indicate an increase in the likelihood and severity of flooding in the area. pfl90 is considered to reflect the frequency characteristics of extreme rainfall events. The downscaled results in 1971-1997 indicated that pq90, px3d and pint were respectively increased 1.8 times, 1.4 times and 1.7 times, while in 1994-2017 they were respectively 2.2 times, 1.6 times and 2.1 times. In case of pfl90, there was a slight decreasing trend from 1971-1997 through 1994-2017. The decrease referred to inconsistent increase in mean and standard deviation in which the former was increased with higher rate than the latter one, resulting in more negative skewness of statistical distribution.

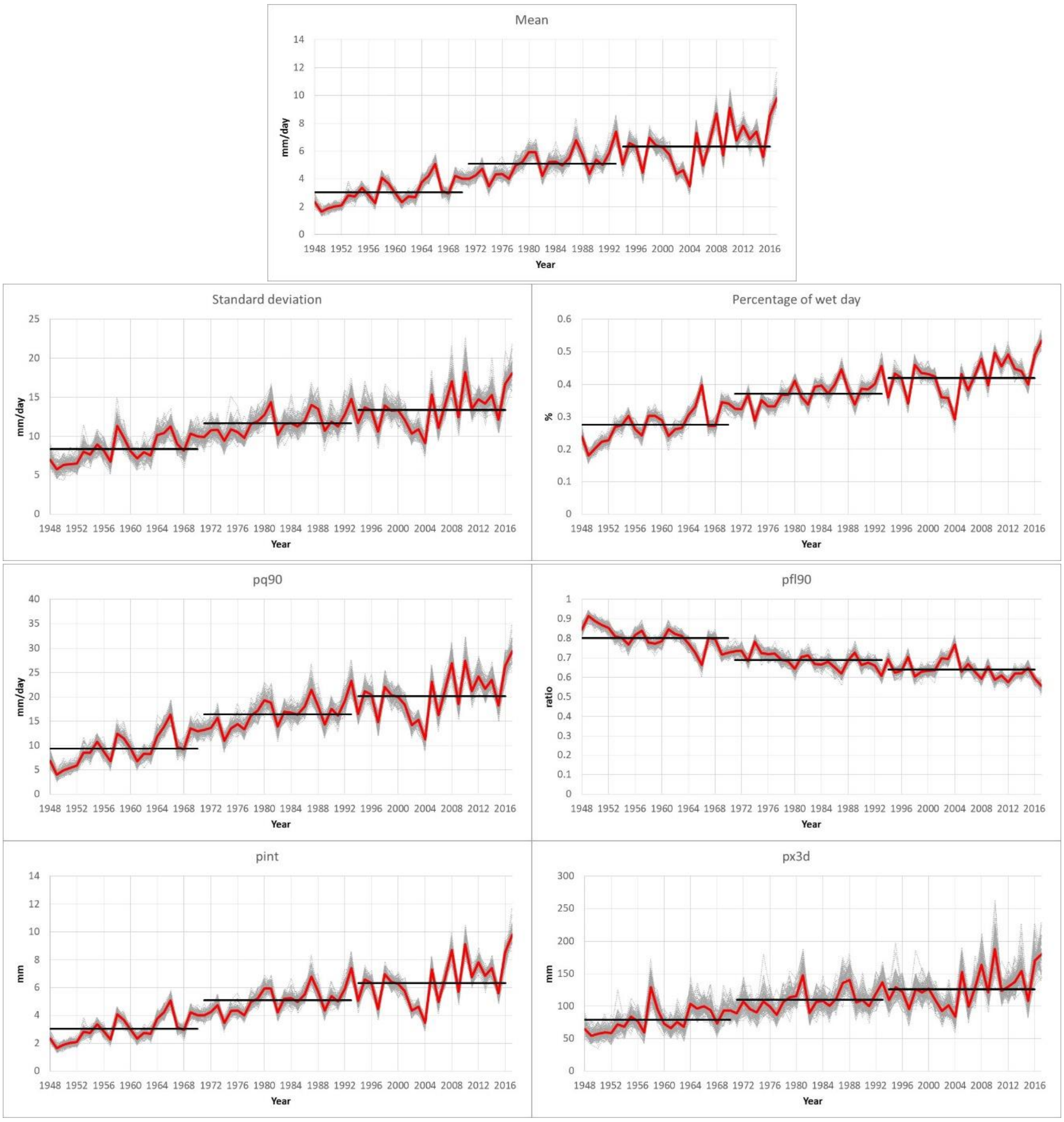

Figure 3. Areal extreme rainfall-relating indices (grey dotted line represents result of each simulation scenario, red thick line shows average over 100 simulations and solid line indicates average over 1948-1970, 1971-1993 and 1994-2017)

3.3 Return periods

Return period values are essential for flood management. They are required as a reference magnitude for risk assessment of the potential damage and benefits gained from the implementation of proposed plans. With these, they can facilitate decision-making processes.

In consistence with results of extreme rainfall-relating indices, 20-year, 50-year and 100-year return periods were respectively increased 18.4%, 14.0% and 11.5% in 1971-1993, and 54.1%, 50.3% and 48.2% in 1994-2017. In other words, 20-year return period in 1948-1970 was equivalent to 8-year return period in 1971-1993 and 3-year return period in 1994-2017. Likewise, 100-year return period in 1948-1970 was approximately 45-year return period in 1971-1993 and only 9-year return period in 1994-2017. These dramatic increases were in consistence with results of downscaled mean, standard deviation and pq90 which influenced magnitude of 1-day extreme rainfall whereas increase in percentage of wet day raised probability that the following days of day one will also be an extreme event, as in this case it was 3-day extreme rainfall. The results of continuously dramatic increase in return period magnitudes were highly concerned, as it can anticipate that characteristics of extreme rainfall event in the study area may be more severe in the future. Tabucanon (2013) demonstrated that the current flood-alleviation measure (as of 2013) in the study area is capable of coping with 20-year return period. Considering the historical trend, additional measures are urgently required to cope with anticipated increasing frequency and magnitude of extreme rainfall event.

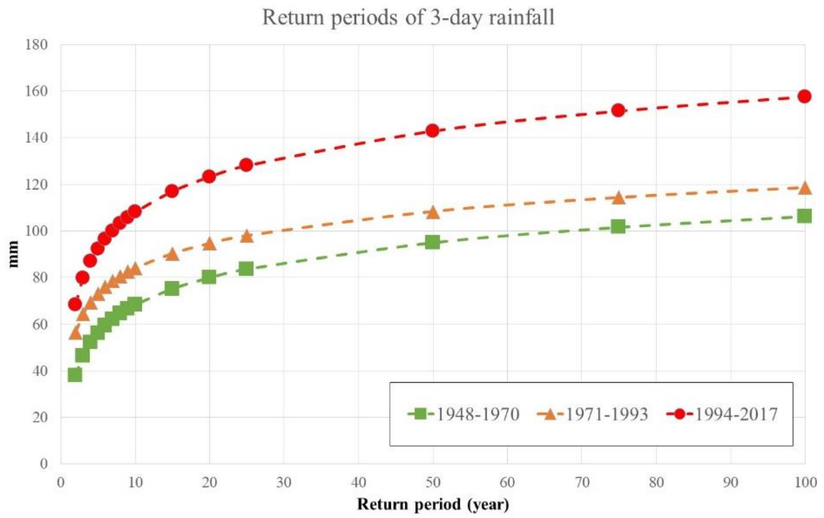

Figure 4. Downscaled areal return periods of 3-day rainfall

4. Conclusions

Historical meteorological record especially rainfall is spatially and temporally limited in developing countries including Thailand. Regarding large uncertainties of future climate change projections, historical record is believed to be more convincible for development of stakeholder's belief and support to government's climate change actions. This study proposes a method to regenerate historical daily rainfall by developing a statistical relationship between large-scale observed meteorological variables and local rainfall at station scale. Khlong U-Taphao Basin in Southern Thailand was selected for demonstration. It was successful to conclude that the area has been experiencing wetter condition including extreme rainfall events since period of 1948 through 2017. The method introduced in this study can apply to any area where a long-term meteorological record including temperature and rainfall is under constraint.

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Evaluating Changes in Flood Regime in Canadian Watersheds Using Peaks Over Threshold Approach

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Abstract

Flood behaviour is likely to alter as a result of the impacts of climate change. This paper investigates the non-stationarity in the flood regime of several Canadian rivers through an analysis of peaks over threshold (POT) data. Identification of thresholds and ensuring the independence of POT events are the two major challenges in the implementation of a POT approach. In the present research, a semi-automatic approach based on the POT package in R – an open source software environment for statistical computing has been used. A total of 127 hydrometric reference streamflow gauging stations that are reasonably free from human intervention have been considered in the present analysis. This ensured the reliability of the trends identified in the flow and timing measures considered herein. The POT data has been extracted from daily streamflow data for each hydrometric station. Four flow and timing measures; (1) duration of POT events, (2) volume of POT events, (3) annual sum of durations in POT events, and (4) annual sum of volumes of POT events were extracted using the daily flow data. The trends in the flow and timing measures were investigated using the Mann-Kendall nonparametric test, and the significance of trends was evaluated using the bootstrap resampling approach. The results of the analysis clearly indicated a predominance of decreasing trends at a greater number of stations in all the four measures of flow and timings considered. It can be concluded that the present analysis indicates a remarkable change in the flood regime of the Canadian watersheds considered herein.

Keywords: Climate/ Change/ Canada/ Peaks/ Threshold

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Kjeldahl Method and Sensory Analyses of a Thornback Ray (*Raja clavata*), Eel (*Anguilla* spp), and Tilapia (*Orechromis mossambicus*) Meat; As a Nutritious Product Alternative

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Abstract

According to data from the Center for the Development of Fisheries and Aquaculture (CENDEPESCA), El Salvador ranks sixth in fish consumption in Central America followed by Belize. Salvadoran population, in recent years has increased the consumption of fish meat. This is why the great restaurants have increased the dishes based on the marine product, in terms of the hamburger industry, the multinational restaurant chain Burger King® has implemented fish meat for their burgers and has apparently been accepted by consumers.

For this investigation three formulations of hamburgers were elaborated with different percentages of Raya, Eel and Tilapia meats as well as beef burgers, which were subjected to a sensory evaluation, searching for the most accepted formula in these products, a bromatological analysis was also made, which included the determination of humity percentage (by drying in a stove), crude fat percentage (by Soxtec method) and total protein percentage (by Micro-Kjeldahl) in a protocol known as proximal analysis.

The results of the bromatological analysis shows that the fish meat burger has a higher percentage of proteins compared to the beef burger, as well as the moisture content; as for fat, the fish burger obtained a lower percentage than the beef burger.

Keywords: Hamburger/ Ray (*Raja Clavata*)/ Tilapia (*Oreochromis Mossambicus*)/ Eel (*Anguilla* spp.)

1. Introduction

The following research paper is an innovative subject, non-studied before and can be used for further research. According to 2013 data from the Center for the Development of Fisheries and Aquaculture (Cendepesca), El Salvador ranks sixth in fish consumption in Central America followed by Belize. In the data of Cendepesca, based on the statistics of production, imports and exports of fishery products for human consumption (including the production of fishmeal and other products of industrial use) it is estimated that in Central America the country that consumes the most fish products and aquaculture is Panama, with 45.7%, followed by Costa Rica and Honduras with 18% and 10.4%, respectively.

The research is aimed at developing three formulations of hamburgers with different percentages of Ray, Eel and Tilapia meats as well as beef burgers, and submit them to a sensory evaluation process with a panel of judges. The bromatological comparison of these hamburgers with a conventional beef burger will be done. The main objective of this research is to know if this new product can become an alternative for a more nutritious burger than the one that is usually consumed.

2. Methodology

2.1 Method

The research was correlational, with the purpose of knowing the relationship between the nature of hamburgers, nutritional value through analytical methods and sensory acceptance by panelists. It was started by gathering bibliographic information related with the topic. A material collection was compiled and then the product was ready to be elaborated.

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A compilation of quantitative and qualitative data was made at the Dr. José Matías Delgado University in the Faculty of Agriculture and Agricultural Research through a sensory analysis with the use of the hedonic scale in which a group of panelists participated.

Later the statistical method was applied, the program used was the Statistical Package for the Social Sciences (SPSS). The coding of frequencies and the analysis of variance (ANOVA) was implemented to determine which formulation differed significantly from each other. We also used a Duncan multiple comparison test; this program was used to calculate the acceptability and variation of the formulations and then the most accepted formulation was determined.

The bromatological analysis was realized in the quality laboratory of the Faculty of Agriculture and Agricultural Research of the Dr. José Matias Delgado University with a sample of beef burger and a burger made of ray, eel and tilapia meat that had the highest acceptance by the panelists.

In this analysis, three determinations were done: humidity percentage (by oven drying), crude fat percentage (by Soxtec method) and total protein percentage (by Micro-Kjeldahl) this was included in a protocol known as proximal analysis.

The research was experimental in nature, since it aims to obtain a functional food through ingredients or snack that can combat the undernourishment, therefore, three formulations were chosen, of which the most accepted through the results of a sensory analysis; in which to sample winner will be carried out its subsequent bromatological study to determine if the formulations met the stated objectives. The study populations was staff and students of the Dr. José Matías Delgado University, from the Agricultural Research Faculty "Julia Hill de O 'Sullivan". The population were of both sexes, i.e., male and female. Almost 15 steps are involved in the production process starting from reception of raw material to packaging, and those are explained in detail in the results and discussion section. A bromatological analysis was performed to the winning sample to further determine moisture, ashes, and proteins.

For the determination of the samples, a sensory analysis was carried out in the laboratory mobile sensorial analysis located within the Faculty of Agriculture and Research Julia Hill of O'Sullivan, taking into account the opinion of 50 judges whose data were tabulated and then an ANOVA analysis of variance was performed to determine Significant differences between the two samples. Finally, bromatological and sensory analysis of the product of the sample was carried out a winner at the Industrial Quality Control Center (CCCI) located in the city of San Salvador. To investigate the results, Analysis of variance, Friedman Test, Correction factor, and ANOVA, and econometric functions were used. Detail of these models/tests is given below.

Correction factor:

$$CF = T2/N$$

Sum of squares for sample=SSF (Sum of Squares Formula)

$SSF=(\Sigma\ MA)\ 2+ (\Sigma\ MB)\ 2+ (\Sigma\ MC)\ 2 -\ FCn$

Mean square samples MSs:

MSs samples=SCm/glm

Variation ratio for samples:

$$Fm = MS\ sample\ /\ MS\ error$$

Hypothesis test process

Hypothesis:

H0: Sample A is superior to Sample B in all terms.

H1: Sample B is superior to Sample A, B in all terms.

H2: Sample C is superior to Sample A, B, C in all terms.

2.2 Meat preparation

The three different meats were mixed and minced, for the ray, the edible part is its fins, so the edible portion of this fish represents approximately 55% (of 100 grams of fish are actually consumed 55). It is a white fish (low in fat) that has few thorns but the most comfortable if we want to consume it, is to buy it already clean. The minced meat was handled with extreme care because it can have bacteria that contaminate the meat and cause food poisoning, as can be caused by Escherichia coli O157: H7. To the meat were added spices such as oregano and cumin to finally cook it at temperature of 90°C. This mixture of fish meat contributes with some vitamins of the B group such as B1 (thiamin), B2 (riboflavin), B3 (niacin) necessary for the conversion of food into energy and also for the correct functioning of the skin, muscles and nerves. It also has small amounts of vitamin A and retinol.

3. Results

3.1 Formulation

Three different formulations were used that vary the amount of meat eel, ray and tilapia. The formula he presents now is the largest significant difference; it is thus the most accepted by the judges.

Table 1. Fish meat formulation

Ingredients	Percentage
Ray meat	14.4
Eel meat	7.2
Tilapia meat	14.4
Fat	24
Water	25.2
Protein	8.21
Salt	1
Egg	5
Oregano	0.05
Pepper	0.10
Cumin	0.03
Dehydrated garlic	0.30
Onion powder	0.10
Liquid smoke	0.10
Total	100

3.2. Bromatological analysis results

The formulation A of the Ray (*Raja clavata*), Eel (*Anguilla* spp.) and Tilapia (*Oreochromis massambicus*) meat compared sensorially with a conventional beef burger, shows that the fish meat has 15.6 g protein/100g, 18.4 g fat/100g and 60.5 g humidity/100 g, in contrast to the beef burger that has 12.8 g protein/100 g, 29.1 g fat/100 g and humidity 52.1 g /100 g. The fish burger has 2.8% more protein than beef, and it also has a 10.7% less fat where theoretically unsaturated fatty acids predominate that bring more benefits and are more precious than the saturated ones like in the beef with 8.4% more water. Based on the bromatological evaluation of the fish burger of formulation A, it represents a food with high nutritional value of macronutrients (proteins and fat) and theoretically has a good supply of B vitamins, so it presents a good innovative opening to the needs and also adds commercial value to resources of hydrobiological origin that are generally not sensory appreciated.

Table 2. Bromatological analysis results

Analysis	Results	Replica	Method
Total fat	18.4g±0.5g/100g	2	Soxhlet Extraction
Humidity	60.5±0.1g/100g	3	Gravimetric Method
Protein	15.6g±0.3g/100g	2	Macro Kjeldahl

3.3 Sensory analysis results

The appearance of the meat was accepted by the 50% of the judges and only 10% indicated that they "liked it moderately". For the color, the 75% of the judges "like it very much", being these the majority. For the smell category, the sample had an acceptance of 40%, which pleased the judges "very much". In the case of the taste, 30% of judges "liked it very much" and just a 10% "disliked it slightly". Half of the judges indicated that they liked the texture of the sample "very much" and the other half liked it "moderately".

The formulation "A" had the results with greater acceptance according to the judges due to the balance in content of meat, protein and water; This formulation gave a better consistency, taste and better appearance to the product, compared to formulation "B" that had 10% more meat content and formulation "C" that had 10% more s protein.

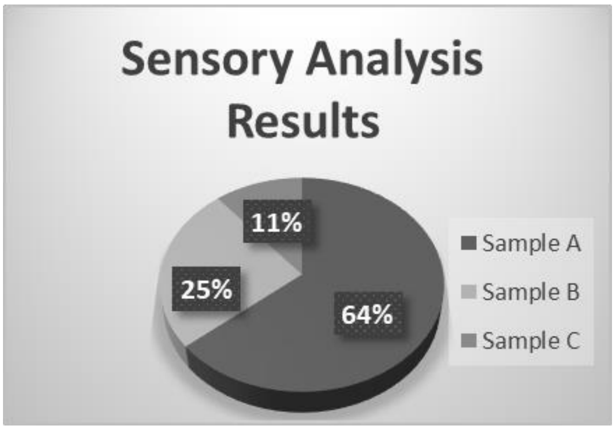

Figure 1. Percentage of acceptance graph

Sensorially both products were accepted by the evaluating judges, it was emphasized that the characteristic fishy smell in these burgers was scarcely or not detected at all.

The discussion of the bromatological analysis was made based on a comparison of the beef burger with the fish meat burger, obtaining the following results:

The amount of protein present in the fish meat burger compared to the beef burger results in a higher protein content, which guarantees that the fish meat burger is a product with better nutritional value.

The total fat content of the fish meat burger is lower compared to the beef burger, which indicates that the fish meat burger is a low-fat burger

The fish meat burger has a higher humidity percentage than the beef burger, therefore this lengthen the shelf life of the product.

Despite the fact that the fish meat was more nutritious than beef there is a limitation, obtaining the raw material is complicated, the ray meat has a low level of commercialization in El Salvador.

4. Conclusions

The assessment of formula A of the Ray (*Raja clavata*), Eel (*Anguilla* spp.) and Tilapia (*Oreochromis massambicus*) meat compared sensorially with a conventional beef burger results in low accepted burger (average of 8.06 on the hedonic scale of 1-9) and the conventional beef burger had an average of 8.37 (on the hedonic scale of 1-9), both results are acceptable.

Fish is classified as a food with many nutritional properties and its use in other products is recommended to increase the nutritional value of a common and highly consumed food like hamburger.

The thornback ray is probably one of the commonest rays encountered by divers. The adult fish can grow to 1m in length although most are less than 85cm. According to WoRMS (World Register of Marine Species), Moreover the Wildlife Trusts Cornwall say is one of the most abundant ray species in Cornish waters, the thornback is a medium sized ray of the skate family growing to cm maximum length. Finally this Rays are not Endangered Species

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Security Food Alternative through Evaluation by Sensory and Bromatological Analyses of Protein from Corn H-59 (*Zea mays*) and Taro Root (*Colocasia esculenta*), in the Elaboration of Tortilla as a Food Supplement

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Abstract

Even though corn has deficiencies in essential amino acids, is one of the most highly consumed foods around the world; mainly as a tortilla. We can combine it with other ingredients changing its sensory properties and making increase its nutritional contents, this is a very important target in food industries; because of this was born the idea of making a taco tortilla from proteic corn and an unknown cultivation as is the Dasheen. Four tortilla formulations were created, each one had different percentage of dasheen and corn. The first formulation had 70% corn flour and 30% dasheen flour, the second one 50% corn flour and 50% dasheen flour, the third one 30% corn flour and 70% dasheen flour and the last one 20% corn flour and 80% dasheen flour. The process started preparing the corn and dasheen flours, proceeded with the formulations described above, the mass was properly weighed, then they were mixed and molded to made a tortilla with 2 to 3mm thickness, both sides of the tortilla were cooked for five minutes. The mixture was cooled and stored using plastic bags under refrigeration. The four samples were put through a sensory analysis, winning the panelist approbation the formulation number two (50% corn flour and 50% dasheen flour).

Keywords: Tortilla/ Taco/ Taro root/ Protein/ H-59 corn

1. Introduction

Corn is one of the most highly consumed foods; mainly in its tortilla form; it represents an important source of carbohydrates and proteins, but the quality of the protein is relatively poor since it has deficiencies in the essential amino acids lysine and tryptophan. An alternative to improve the nutritional value of tortillas, is to use genetically modified corn kernels, as is the case of H-59 corn, which contains about twice as much lysine and tryptophan relative to common corn. This will allow the tortillas prepared with this corn to acquire exceptional nutritional characteristics.

In some cases, roots and tubers are poorly considered as a nutritional food, despite their physical characteristics and nutritional value that's why they have a scarce commercialization. This is the problem of the taro root, it has many qualities that are not used in the food industry.

This research presents the use of corn and taro root for a tortilla having as purpose the combination of these materials to obtain a traditional product with better nutritional characteristics through the substitution of conventional corn and generating a way to encourage production and little food demand extended like the taro.

2. Methodology

2.1 Method

For the elaboration of tortillas, four formulas were used with four different concentrations of taro root.

The grain was cleaned and verified that there was no damage to it, rinsed with water at room temperature and drained.

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Once clean, 5 pounds of corn were placed in a pot, water was added to cover it completely and 5 tablespoons of lime (75 grams) were added, stirring the mixture. It was heated at 80 ° C in a slow fire for 30 to 45 minutes until it boiled, and it was left to boil for 3 minutes. It's was removed from the fire and let the mixture rest for a day. After the time, the cooking liquid was removed and the corn was rinsed until the water came out clean to remove impurities and excess lime; at the same way it was drained and moved to the mill for obtaining dough.

Moreover, the taro root was washed with soap and water using a masque in good condition and cleans. Once clean, the shell was removed and the taro was cut into pieces to facilitate the process. Furthermore, it was placed in a pot with water until it was covered and heated for two hours, then it was drained and crushed to obtain a paste.

According to the formulations, the corresponding amounts of H-59 corn and taro were weighed, mixed and the tortilla was elaborated flattening the paste to a thickness of 2 to 3mm, cooked by both sides for 5 minutes. It was left to cool and stored in refrigeration using plastic bags.

2.2 Sensory analysis

The product was submitted to a sensory analysis made by untrained panelists to determine which is the formulation with greater acceptance based on five characteristics: color, smell, taste, texture and appearance using one of the most used methods for measuring food acceptability, the 9-point hedonic scale, which consist of nine parameters: Like Extremely, Like Very Much, Like Moderately, Like Slightly, Neither Like nor Dislike, Dislike Slightly, Dislike Moderately, Dislike Very Much, Dislike Extremely. Four different tortilla formulations that contained different levels of H-59 corn and taro root were evaluated.

Table 1. Tortilla formulations

Ingredients	Formula 1	Formula 2	Formula 3	Formula 4
H-59 corn	70%	50%	30%	20%
Taro root	30%	50%	70%	80%
Total	100%	100%	100%	100%

Once the response sheets were collected, the data were emptied and later analyzed using a statistical method, for which the ANOVA (analysis of variance) was selected. The level of significance used was 5%. In addition, the data was presented through a descriptive statistic graph.

The tortilla with the best parameters evaluated in the sensory analysis, was subjected to a method used to determine the percentage of total protein, called "Micro Kjeldahl method".

3. Results and Discussion

3.1 Sensory analysis

From the comparison of the evaluated parameters for four elaborated formulations, the following results can be extracted.

Formula 1 was the best evaluated in terms of color (7.3), smell (6.9) and appearance (6.75). Regarding the parameters of texture and flavor formula 2 obtained the best results, 6.4 and 6.85 respectively.

In the global analysis of the four formulations where all the parameters were evaluated, the following results were obtained: formula 2 obtained the best results with a 6.76, followed by formula 1 with a 6.73, thirdly, formula 3 has a result of 6.35 and finally in the last place is the formula 4, with 80% taro root and 20% of corn, which obtains results of 6.09.

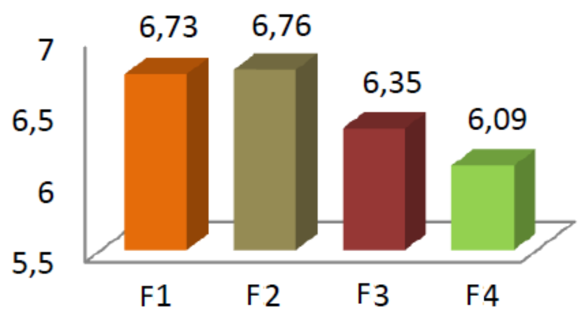

Figure 1. Sensory analysis results

3.2 Statistical analysis

There is no significant difference at 5% between formulations F1, F2, F3 and F4. All the samples presented the same level of "sensory acceptance.

3.3 Bromatological analysis

If we compare the percentages of protein from tortillas made in this research with the most popular tortillas (Milpa Real), we can observe a minimal difference since the Milpa Real tortillas have 1.5 g of protein and the tortillas of corn and taro root have 1.23 g of protein; so, we can conclude that the tortilla obtained is satisfactory.

Although the tortillas for tacos were made with unconventional materials, the acceptance of the product in front of the tasting panel was positive. The results obtained between each sample did not present significant differences, all parameters evaluated (color, smell, taste, texture, appearance) were well rated, being in the range of 6.09-6.76. The sample that received the greatest acceptance was formulation 2 of 50% corn and 50% taro root.

The result of the bromatological analysis obtained as a protein data: 5.58 g/100 g, which indicates a contribution similar to the commercial tortillas.

Table 2. Protein analysis

Analysis	Results	Replica	Method
Protein	5.58±0.27 g/ 100 g	4	Micro Kjendhal

4. Conclusions

It is feasible to make a tortilla for tacos based on corn and taro, a new alternative use for the non-traditional cultivation of taro root.

The tortillas for tacos made only of corn have a brittle texture, which is intensified by adding the common ingredients for tacos, so, the corn in combination with the taro root provide desirable rheological characteristics such as malleability and softness to the tortilla.

If it is desired to obtain tortillas for tacos with better rheological properties, the use of taro dough in proportions greater than 50% is adequate.

Through the sensory analysis, it was identified that the dough of maize and malanga was of satisfactory acceptance among the public.

5. Acknowledgement

We appreciate the Faculty of Agriculture Investigation Agricola, Dr. Jose Matias Delgado University and Faculty of Environment and Resource Studies, Mahidol University for the weather data sets used in this study. We are grateful for those anonymous reviewers for their thoughtful comments, which helped considerably to improve the presentation of our work.

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Bromatological and Sensory Analysis of a Corn (*Zea mays*) Flour Fortified with Moringa (*Moringa oleífera*), to Increase Its Nutritional Value

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Abstract

This research details an effort to develop from the traditional flour into a fortified and nutritious flour from Zea mays and Moringa oleifera. Due of levels of malnutrition in El Salvador which is a problem, mainly in the areas of extreme poverty. Having cleared the problem previously mentioned, it becomes of great importance to look for viable solutions to increase the intake of micro nutrients, in the daily diet of the population.

The moringa tree (Moringa oleifera) commonly called "teberinto", is a tree that can be consumed and used in its entirety (fruit, roots, stem and leaf). It has a high nutritional level in different nutrients and vitamins such as phosphorus, magnesium, vitamins B1, B2, B3 and B6, as well as proteins, calcium and iron, which are the three properties that have been considered in the present investigation. It has merged the need for nutrients from the popular diet with the various benefits that the moringa leaf provides, thus managing to implement this natural resource in a mixture with complementary proportions between moringa leaf powder and corn flour, acceptable to the palate and beneficial to the organism, the production of tortillas is destined that by means of general information on the ingredients to be used and a nutritional study carried out in FUSADES (Salvadoran Foundation for Economic and Social Development), the benefits it presents through its proteins, calcium and iron reestablished in an accurate way.

Keywords: Moringa/ Fortified flour/ Micro nutrients/ Malnutrition

1. Introduction

In El Salvador, the sources of basic grains such as beans, corn, rice and wheat are essential for the nutrition of the population, with corn being one of the most consumed crops in the country; due to the large number of derivative products that can be obtained to a greater extent, such as corn flour.

According to FAO research, El Salvador has had three consecutive decades of malnutrition in the population, especially in the population that is less than five years old, as a way to counteract the lack of nutrients in food, fortification programs have been created, to ensure that consumers can absorb the necessary nutrients in their daily food intake. Like many countries in the world, corn is considered as one of the fundamental and basic foods of the daily diet, and for Salvadorans it is not the exception.

For this reason, corn flour fortified with moringa powder, a crop of great potential, will be elaborated as a supplier of valuable products that has not been fully exploited, although it contains most of the essential nutrients that the human body needs and all the parts of the tree can be perfectly exploited.

2. Methodology

Two formulations were made by mixing the corn flour and the moringa powder and subject to a sensory, bromatological and microbiological analysis.

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The best grains with no physical damage were selected and weighed, in order to avoid affecting the final product, a cleaning is also realized with the idea of detection and elimination of damaged or rotten corn. The resulting paste of the ground process is separated from the water that it can contain using a drum desiccator. With an initial temperature that is dispersed in a range of between 270 to 275º C and a final temperature of 40º C, to be able to eliminate the humidity of the flour, and finally get a product with a humidity of 8 -10%.

Once the corn is clean, this is cooked by direct heat, add 2 liters of water / Kg corn, at 1% lime, for 30 minutes, then let stand for 12 hours.
The water is separated from the corn and is washed with water as a function of dissipating the lime and the detached pericarp. The paste resulting from process is separated from the water by a drum desiccator and take it to a final temperature of 40º C.

Finally, 70% of corn flour was added, according to the formulation established above. The flours should be of a fine and uniform granular size. The moringa powder should be a fine powder and 30% should be added according to the established formulation.The research has a descriptive design, because the data is taken and observed directly from the reality with a sensory analysis. The results obtained after the sensory evaluation will be evaluated with the Analysis of variance ANOVA, this method is used to compare two or more sample means. The level of significance that will be used will be 5%.

Table 1. Content of corn and moringa for the A and B samples

Ingredients	Sample A	Sample B
H-59 corn	70%	50%
Moringa	30%	50%
Total	100%	100%

Table 2. Results of the Bromatology Analysis for sample A

Analysis	Results	Method
Protein	10.49g/100g	Block digestion
Total fat	3.60g/100g	Soxhlet
Fiber	2.18g/100g	Gravimetric
Calcium	400.30mg/100g	Spectrophotometry
Carbohydrates	30.11%	Calculation
Humidity	8.53%	Halogen analyzer

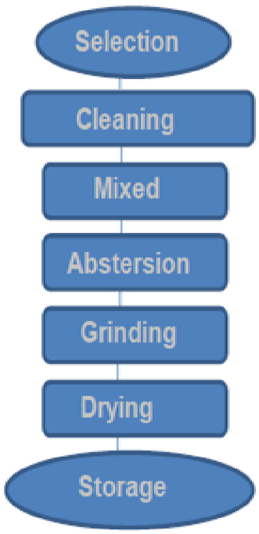

Figure 1. Diagram of the elaboration of the flour

3. Results and Discussion

3.1 Statistical analysis

According to the evaluating judges, both samples were approved but sample B had lower results than sample A. The taste of formula B was accepted with an average of 7.3. In general terms it is always the sample "A" with an average of 8.6 the best evaluated.

3.2 Bromatological analyses

Regarding protein content, it is observed that the commercial brand of corn flour has a significantly lower percentage than corn flour fortified with moringa powder.

The results obtained of humidity content of the flour were lower compared to the other brand, but the Codex Alimentarius standard for flours establishes that the product should not contain more than 15% humidity, achieving a fairly acceptable percentage.

Table 3. Nutritional content comparative between the best evaluated Formula (A) and a commercial brand of flour (MASECA)

Analysis	Sample A	MASECA
Protein	10.49g	8.30g
Total fat	3.60g	4.10g
Fiber	2.18g	1.10g
Calcium	400.30mg	29.0mg
Carbohydrates	30.11%	25%
Humidity	8.53%	10.8%

3.3 Microbiological analysis

Microbiological analyzes resulted in a low number of coliforms total (30 CFU/g). According to the MSN (Mandatory Salvadoran Norm), the maximum number of total coliforms is 100 CFU/g so we conclude

That the corn flour fortified with moringa powder is suitable for human consumption because it's a harmless product and fulfill the parameters established in the standard.

3.4 Sensory Analysis

The formulation "A" (moringa powder at 30% and 70% corn flour), and the second one shows "B" (moringa powder at 50% and 50% corn flour), were evaluated by judges through a sensory analysis, considering the following variables: color, smell, taste, appearance and texture in this way obtained better results in the sample "A" than in the sample "B". Where the results show that the taste and texture are better qualified in The "A" sample. The appearance of the tortilla was the same as that of an artisan tortilla and the moringa slightly changed the color of the dough, so it was better qualified than the sample "B" that its color was darker and appearance was not very pleasant for the judgements.

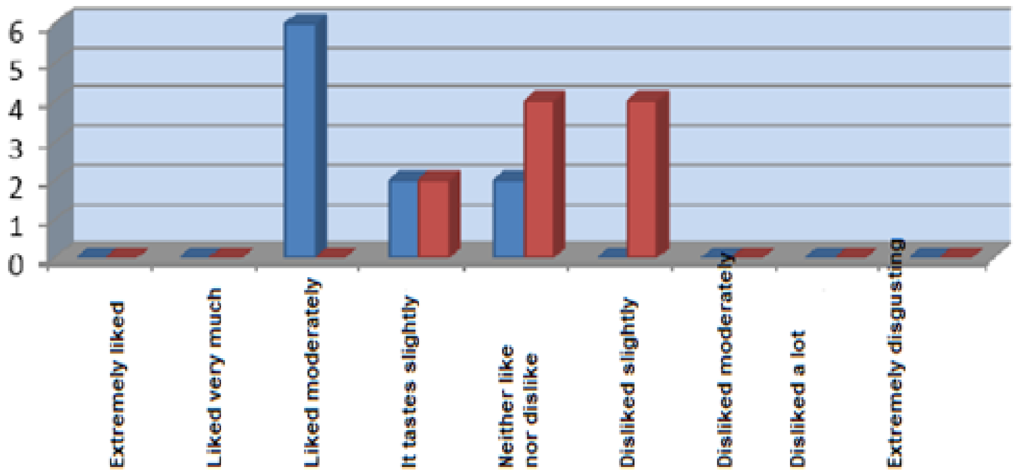

Figure 2. Level of acceptance of the jury

4. Conclusions

The fortification of corn flour with moringa powder yielded results much more rewarding than expected, these analyzes were made at FUSADES show a higher nutrient index in the "A" sample (30 % moringa powder and 70% corn flour). Of which there was a better acceptance by the judges in the sensory analysis. Compared with the commercial sample (MASECA), it shows a considerable protein increase in the sample "A" of 2.19 g/100 g sample compared with (MASECA). We can also appreciate an increase of calcium in sample "A" (371.3mg/100g) compared with (MASECA).

The fortified flour provided also shows a higher nutrient index in the "A" sample (30 % moringa powder and 70% corn flour. This sample had a better acceptance by the judges in the sensory analysis.

5. Acknowledgement

We appreciate the Faculty of Agricultu e Investigacion Agricola, Dr. Jose Matias Delgado University and Faculty of Environment and Resource Studies, Mahidol University for the weather data sets used in this study. We are grateful for those anonymous reviewers for their thoughtful comments, which helped considerably to improve the presentation of our work.

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Characterization of Manganese Oxide-Biomineralization by the Psychrophilic Marine Bacterium, *Arthrobacter* sp. Strain NI-2 and Its Spontaneous Mutant Strain NI-2'

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Abstract

Metal pollution and metal shortage are a growing threat to the global environment and world high-tech industry. One of the promising strategies for removing and recycling metal-elements from the environments is by using Metal-Biotechnology based on metal-related biological activities, which include bioaccumulation, bioadsorption, and biomineralization. In this study, focusing on Manganese (Mn) pollution, we have isolated and analyzed the Mn(II)-oxidizing marine bacterium, *Arthrobacter* sp. NI-2 strain, from Imari Bay, Imari-shi, Saga, Japan. We have also isolated a spontaneous mutant, *Arthrobacter* sp. NI-2' strain with enhanced Mn(II)-oxidizing activity. Under the liquid culture condition at 30°C, *Arthrobacter* sp. NI-2' strain could efficiently remove more than 96% of Mn(II) from the liquid culture media containing 0.4 mM Mn(II). Although Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 strain is suppressed under the low temperature conditions, the increased Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2' strain was maintained when the growth temperature was shifted from 30°C to 10°C. Therefore, the *Arthrobacter* sp. NI-2' strain would be useful as a tool for Mn removal form low-temperature water, such as groundwater around the mining area.

Keywords: Metal-biotechnology/ Biomineralization/ Manganese(II)-oxidizing activity/ Biogenic manganese oxide/ *Arthrobacter* sp./ Low temperature

1. Introduction

Metal pollution and metal shortage are a growing threat to the global environment and world high-tech industry. As summarized in Figure 1, using metal-related biological activities (i.e. biosensor, bioaccumulation, bioadsorption, biomineralization, and chemisorption), Metal-Biotechnology would be one of the promising strategies for monitoring, removing and recycling metal-elements in polluted environments (Ike et al., 2011).

As Manganese (Mn) is one of the important elements for both the ecosystem and the industry, environmental Mn-pollution and industrial Mn-shortage are the matter of concern. In the environment, Mn(III, IV) oxides, found in various solid forms, play important roles in the global cycling of many major elements (C and S) and trace elements (Fe, Co, Pb, Cu, Cd, and Cr) as an oxidation catalyst and a metal scavenger (Namgung et al., 2018; Tebo et al., 2004). In water ecosystem, Mn(II) found as Mn^{2+} ions are further oxidized for the formation of Mn(III, IV) oxides mainly by the activity of microorganisms, because the rates of Mn(II) oxidation catalyzed by microorganisms are much faster than that of the abiotic Mn(II) oxidation (Nealson et al., 1988; Tebo et al., 2004). The biogenic Mn-oxides formation system, i.e. Mn-oxide biomineralization, could be a good tool for Mn removal from industrial wastewater (Barboza et al., 2016; Ike et al., 2011). Moreover, metal scavenger property of Mn oxides could be an additional tool for multiple heavy-metal removal for industrial wastewater (Figure 2, Chemisorption). Mn(III/IV) oxides are also recognized as powerful oxidants that are capable of oxidizing a wide range of compounds including organic contaminants (Remucal and Ginder-Vogel, 2014).

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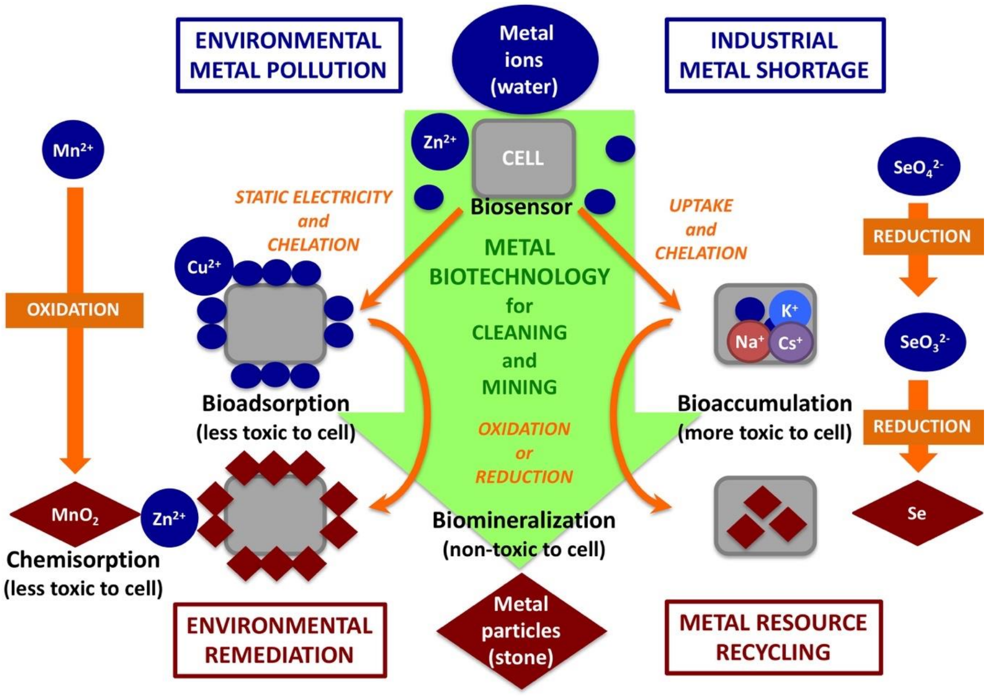

Figure 1. The overview of Metal Biotechnology including biosensor, bioaccumulation, bioadsorption, biomineralization, and chemisorption strategies.

As summarized in Figure 2, however, biogenic Mn(II)-oxidizing activities may be inhibited by environmental stresses such as osmotic stress caused by high salinity and thermal stress caused by dynamic changes of cold/hot weather. Therefore, in order to overcome the weakness of the biogenic Mn(II)-oxidation systems, we set out to isolate robust Mn(II)-oxidizing marine bacteria with ability to maintain the Mn(II)-oxidizing activities under high-salinity and/or low-temperature conditions. Previously, two Mn(II)-oxidizing marine bacteria strains NI-1 and NI-2 were isolated from Imari Bay, Imari-shi, Saga, Japan (Nakayama and Ikegami, 2009). As the NI-1 strain belonging to *Bacillus* sp., which exhibits Mn(II)-oxidizing activity even at 3% and 6% NaCl conditions, the *Bacillus* sp. NI-1 became good candidate for Mn-bioremediation of high-salinity wastewater such as contaminated seawater and concentrated seawater generated by desalination. Although the NI-2 strain does not possess Mn(II)-oxidizing activity under high-salinity conditions, we found that it thrives at low temperatures of 4 and 10°C as a psychrophilic marine bacterium. As cold stress may inhibit Mn oxide biomineralization system in cold wastewater such as drainage water of Mn mines in winter season or at cold climate regions, we further characterized NI-2 strain and its properties including Mn(II)-oxidizing activity under low-temperature conditions.

In this study, we have identified NI-2 strain as *Arthrobacter* sp. based on sequence analysis of the 16S rRNA gene in its genome. Moreover, we have successfully isolated the spontaneous mutant *Arthrobacter* sp. NI-2' strain, which exhibits enhanced ability of Mn oxide biomineralization at 10°C condition. We conclude that the *Arthrobacter* sp. NI-2' strain would be useful for bioremediation of metal-contaminated wastewater even at low temperature conditions.

2. Methodology

2.1. Isolation of Mn(II)-oxidizing bacteria

In order to isolate Mn(II)-oxidizing bacteria (MOB), seawater and seafloor sediment samples collected from Imari bay, Imari-shi, Saga, Japan (Nakayama and Ikegami, 2009) were diluted in 3% NaCl, and 100 μL of each dilution was spread onto solid LEPT media (Boogerd and de Vrind, 1987) containing 0.2 mM $MnCl_2$ at pH 7.5 and incubated for 14 days at 30°C in the dark.

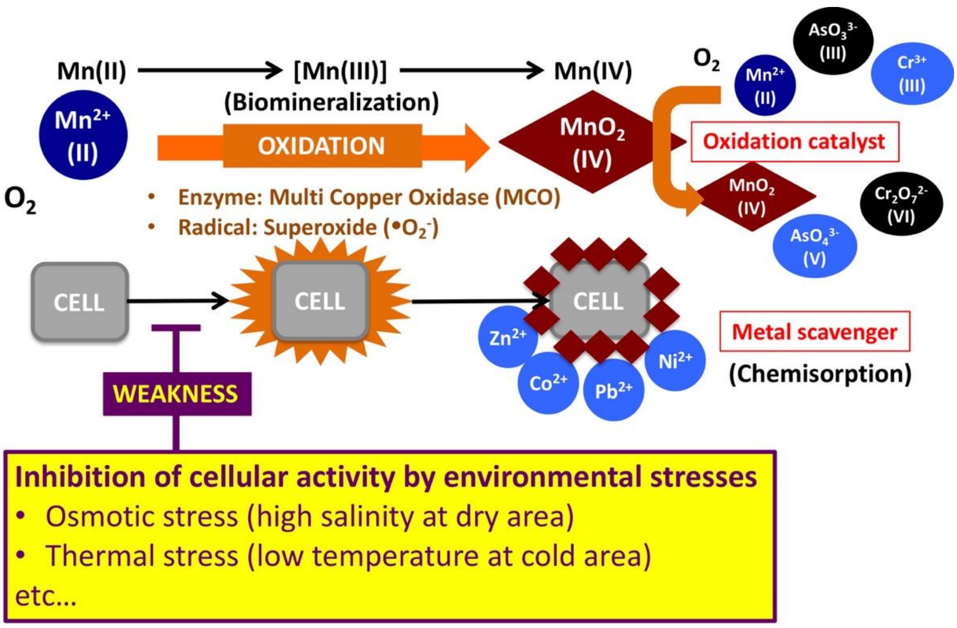

Figure 2. Mn oxide biomineralization and chemisorption as a tool for heavy-metal removal from polluted water. Oxidation of Mn(II) to Mn(III, IV) is mediated by microbial cell activities, which include enzyme reaction by multi copper oxidase (MCO) and/or radical reaction by superoxide (O_2^-).

MOB were identified by using leucoberbelin blue I (LBB), which reacts with Mn(III/IV) oxides and the LBB's color is changed to blue in colorimetric assays (Krumbein and Altmann, 1973). In brief, a drop or spray of the LBB reagent was applied directly to a brownish-black colony or to the whole surface of the growth medium, and the mixture was incubated for 5 min at room temperature in the dark prior to a visual inspection for color change. LBB-positive colonies were transferred and streaked for single colony isolation at least 3 times.

In this study, two isolates named NI-1 and NI-2 strains were used. The NI-1 strain, which was identified as *Bacillus* sp. (Nakayama & Ikegami, 2009) with Mn(II)-oxidizing activity is used as a positive control strain for Mn(II)-oxidizing activity. *Escherichia coli* DH5α, which possesses no Mn(II)-oxidizing activity is used as a negative control strain.

Spontaneous mutant of NI-2 strain with enhanced Mn(II)-oxidizing activity, named NI-2', was isolated by chance during routine subculture periods.

2.2 Identification of MBO isolates by 16S rRNA gene analysis

In order to identify MOB isolates, total genomic DNA was extracted and purified with ISOPLANT II kit (Nippon Gene Co., Ltd., Tokyo, Japan). The almost full length of 16S rRNA gene fragments (about 1.5 kbp) were PCR-amplified using the universal primer set, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAAGGAGGTGATCCAGCC-3'), in a Takara Thermal Cycler (Takara, Shiga, Japan). The PCR products were subcloned with the Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen™, Thermo Fisher Scientific, Waltham, MA) following the manufacturer's specifications. The sequence of the subcloned 16S rRNA gene fragment was determined using universal primer set, M13 Forward (-20) (5'-GTAAAACGACGGCCAG-3') and M13 Reverse (5'-CAGGAAACAGCTATGAC-3'), provided in the Kit. The sequences of the fragments were subjected to a homology search in the APORON database (Techno Suruga Laboratory, Shizuoka, Japan) and phylogenetic trees were constructed to ascertain the phylogenetic positions of the isolates.

2.3 Bioassay for Mn(II)-oxidizing activities under various conditions.

In order to evaluate the effect of NaCl stress on the cell growth and biogenic Mn-oxide formation, a series of solid LEPT media with 3% or 6% NaCl, and with or without 0.2 mM $MnCl_2$ were prepared. Then bacterial cells were streaked on the media and incubated for 14 days at 30°C in the dark as a bioassay for Mn(II)-oxidizing activities under NaCl-stress conditions. In addition, to evaluate the effect of cold stress on the cell growth and biogenic Mn-oxide formation, bacterial cells were streaked on the media and incubated for 14 days at 4°C, 10°C, or 30°C in the dark as a bioassay for Mn(II)-oxidizing activities under cold-stress conditions.

2.4 Quantification of Mn in liquid culture media by ICP-OES analysis.

In order to quantify Mn concentration in liquid culture media, bacterial cells were cultured in 6 mL of the half strength of liquid LEPT media (1/2 LEPT media) with or without 0.4 mM $MnCl_2$ for 14 days and 5 mL of liquid samples were collected from supernatant of the cultures after centrifugation at 3,000×g at room temperature for 10 min to pellet bacterial cells and Mn oxides. The liquid samples were transferred to 50 mL Polypropylene tubes with watch glasses as a lid (DigiTUBE, SCP Science, Quebec, Canada) and the tubes were settled in a digestion block (DigiPREP jr., SCP Science, Quebec, Canada). Digestion was done by adding 25 mL of Milli-Q water and 5 mL of 70% HNO_3 (Nacalai Tesque Inc., Kyoto, Japan) to each sample and heated the mixtures to 65°C, maintained for15 min, then heated up to 105°C and maintained for 120 min before letting the mixture cool down to room temperature. After cooling down, 0.5 mL of 30% H_2O_2 (Fujifilm Wako Pure Chemical, Ltd., Osaka, Japan) were added to the mixtures and the digestion block was again heated to 105°C and maintained at the temperature for 60 min. The digested solution samples were cooled down and filtered through 0.45 µm-pore-size Teflon® membrane filter (DigiFILTER, SCP Science, Quebec, Canada). After rinsing the filter with Milli-Q water, volume of each filtered samples were adjusted to 50 mL by adding Milli-Q water. Measurements were conducted on an ICP-OES (ICPS-7500, Shimadzu Corporation, Kyoto, Japan) following the instruction provided by the manufacturer.

3. Results and Discussion

3.1 Identification of Mn(II)-oxidizing marine bacterium NI-2 strain

As shown in Figure 3, two Mn(II)-oxidizing marine bacteria strains NI-1 and NI-2 were isolated from seawater and seafloor sediment samples collected from Imari Bay, Imari-shi, Saga, Japan (Nakayama & Ikegami, 2009). We found that when Mn(II) is present in the medium, both NI-1 and NI-2 strains produce visible brown-colored particles (Figure 3A and Figure 7). Production of biogenic Mn oxide was confirmed by blue color formation after LBB spray treatment (Figure 3B).

Based on 16S rRNA gene sequencing, the NI-1 strain was previously classified as *Bacillus* sp. (Nakayama & Ikegami, 2009), and the NI-2 strain is classified as *Arthrobacter* sp. in this study (Figure 4). The closest relative species of *Arthrobacter* sp. NI-2 strain is *Arthrobacter subterraneus* CH7^T, which was isolated as a new species from deep subsurface water of the South Coast of Korea (Chang et al., 2011). In the previous report, *A. subterraneus* CH7^T was found to grow under low temperature conditions similar to deep sea environment (Chang et al., 2011). As shown in Figure 5, we found that *Arthrobacter* sp. NI-2 strain grow well under cold-stress condition (4°C), while *Bacillus* sp. NI-1 strain cannot grow under this condition. The result indicates that *Arthrobacter* sp. NI-2 is a psychrophilic marine bacterium.

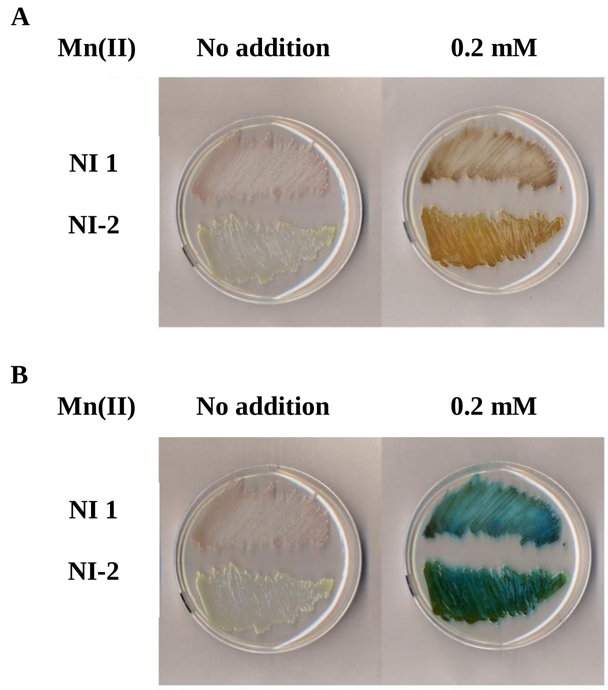

Figure 3: Mn(II)-oxidizing marine bacteria strains NI-1 and NI-2 isolated from Imari Bay, Imari-shi, Saga, Japan. **A**, Both NI-1 and NI-2 stains were cultured for 14 days at 30°C on LEPT media with or without $MnCl_2$ (pH 7.5). **B**, Same samples as A, 5 min after LBB spray treatment.

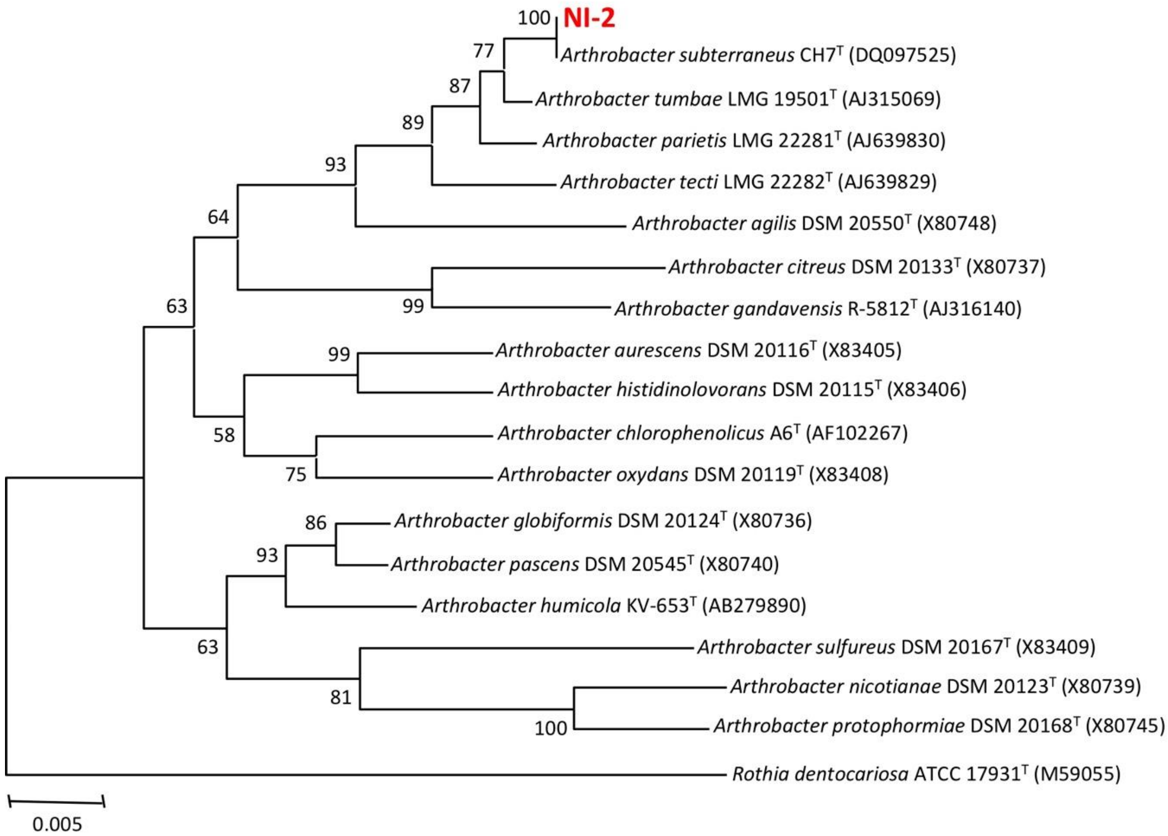

Figure 4. NI-2 strain is classified as *Arthrobacter* sp. in phylogenetic tree based on 16S rRNA gene sequencing by neighbor joining method.

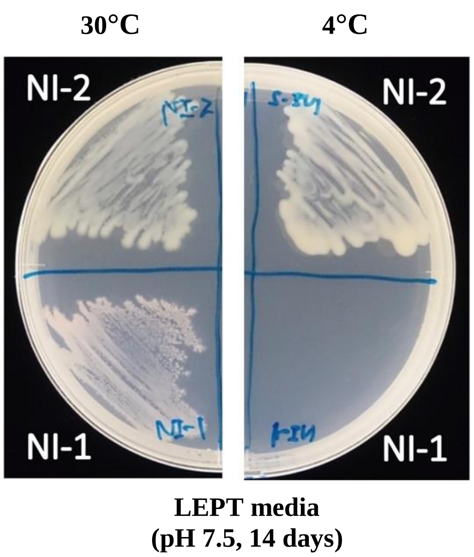

Figure 5. Growth of *Bacillus* sp. NI-1 and *Arthrobacter* sp. NI-2 under cold-stress condition.

3.2 Isolation of NI-2 derivative mutant with increased Mn(II)-oxidizing activity

Recently, *Arthrobacter* sp. strain QXT-31 was identified as a non-Mn(II)-oxidizing strain in monoculture condition, however, interaction (co-culture) with another non-Mn(II)-oxidizing *Sphingopyxis* sp. QXT-31 strain could induce cooperative Mn(II) oxidation by *Arthrobacter* sp. strain QXT-3 in an aquatic environment (Liang et al, 2016). Interestingly, during our screening and isolation process, we have also observed that *Arthrobacter* sp. NI-2 strain displayed improved Mn(II)-oxidizing activity when interacts with an unidentified non-Mn(II)-oxidizing bacteria (Figure 6). However, in contrast to *Arthrobacter* sp. QXT-31 strain, *Arthrobacter* sp. NI-2 strain showed Mn(II)-oxidizing activity even in monoculture at 30°C (Figure 7). This observation implicated that *Arthrobacter* sp. NI-2 strain is probably equipped with endogenous auto-activation system for Mn(II)-oxidizing activity.

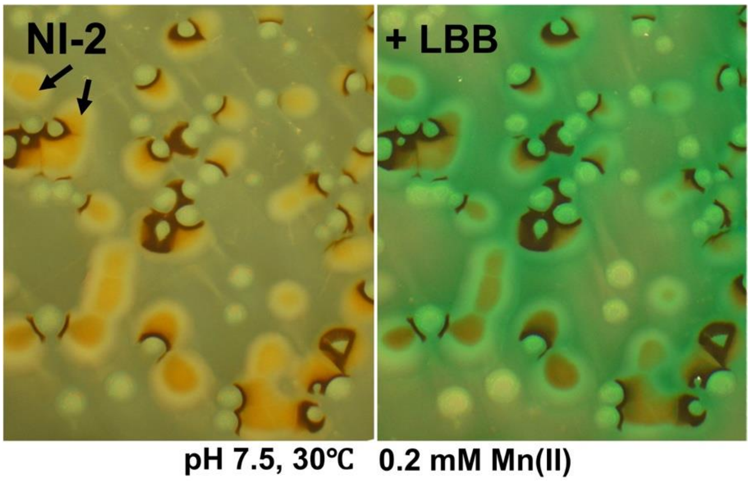

Figure 6. Enhanced Mn(II)-oxidizing activity by interaction with non-Mn(II)-oxidizing bacteria in *Arthrobacter* sp. NI-2 strain.

In this study, *Bacillus* sp. NI-1 and *E. coli* DH5α strains were used as a positive control strain and a negative control strain, respectively, for bioassay of Mn(II)-oxidizing activity. During the routine sub-culturing of NI-2 strain, we have identified a spontaneous mutant colony, which shown a faster and more intense accumulation of Mn oxide (brown-colored particles). As shown in Figure 7, this spontaneous mutant, which we named *Arthrobacter* sp. NI-2' possesses enhanced Mn(II)-oxidizing activity on the Mn(II)-containing LEPT medium at 30°C.

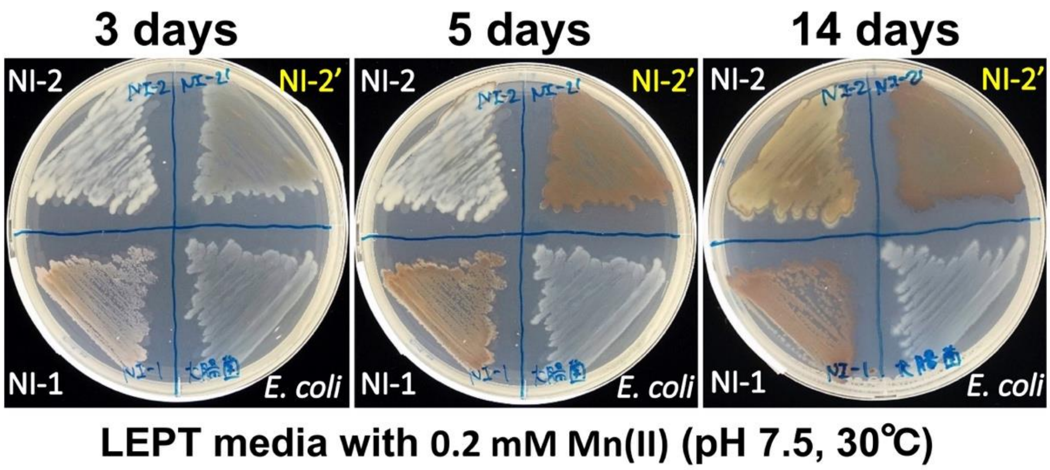

Figure 7. Mn(II)-oxidizing activity in *Arthrobacter* sp. NI-2 and its derivative mutant, *Arthrobacter* sp. NI-2'. *Bacillus* sp. NI-1 and *E. coli* DH5α were used as a positive and a negative control strains for Mn(II)-oxidizing activity, respectively.

3.3 Mn(II) removal by Mn-oxide biomineralization in liquid culture system

In order to evaluate ability of Mn(II) removal by Mn-oxide biomineralization in liquid culture system, *Bacillus* sp. NI-1, *E. coli* DH5α, and *Arthrobacter* sp. NI-2' were subjected to bioassay of Mn-oxide biomineralization. Mn(II)-oxidizing activity seems to be mediated by multi copper oxidase (MCO) on the surface of spore of *Bacillus* sp. NI-1 and is found to be induced during spore formation triggered by starvation in other Mn(II)-oxidizing *Bacillus* sp. strains such as SG-1 strain (de Vrind et al.,1986; Francis & Tebo, 2002). Therefore, our bioassay was performed in half strength of liquid LEPT (1/2 LEPT) culture media, in which nutrients were reduced to accelerate the entering to the starvation phase. As shown in Figure 8A, both *Bacillus* sp. NI-1 and *Arthrobacter* sp. NI-2' were able to form brown-colored particles of Mn oxide efficiently in the culture medium containing 0.4 mM $MnCl_2$ (approx. 20 ppm Mn; doubled the concentration of Mn allowed in drainage water by Uniform Effluent Standard in Japan). Moreover, more than 96% of Mn(II) could be removed from liquid culture media through Mn-oxide biomineralization by *Arthrobacter* sp. NI-2' and *Bacillus* sp. NI-1 (shown in Figure 8B). While only 20% could be remove through biosorption or bioaccumulation by *E. coli* DH5α, which possessed no Mn(II)-oxidizing activity. The results suggest that both *Arthrobacter* sp. NI-2' and *Bacillus* sp. NI-1 strains would be useful for Mn-bioremediation of wastewater at 30°C. As *Arthrobacter* sp. NI-2' was expected to grow under cold-stress conditions similar to the original *Arthrobacter* sp. NI-2 strain, we further investigated Mn(II)-oxidizing activity of each strain under cold-stress conditions.

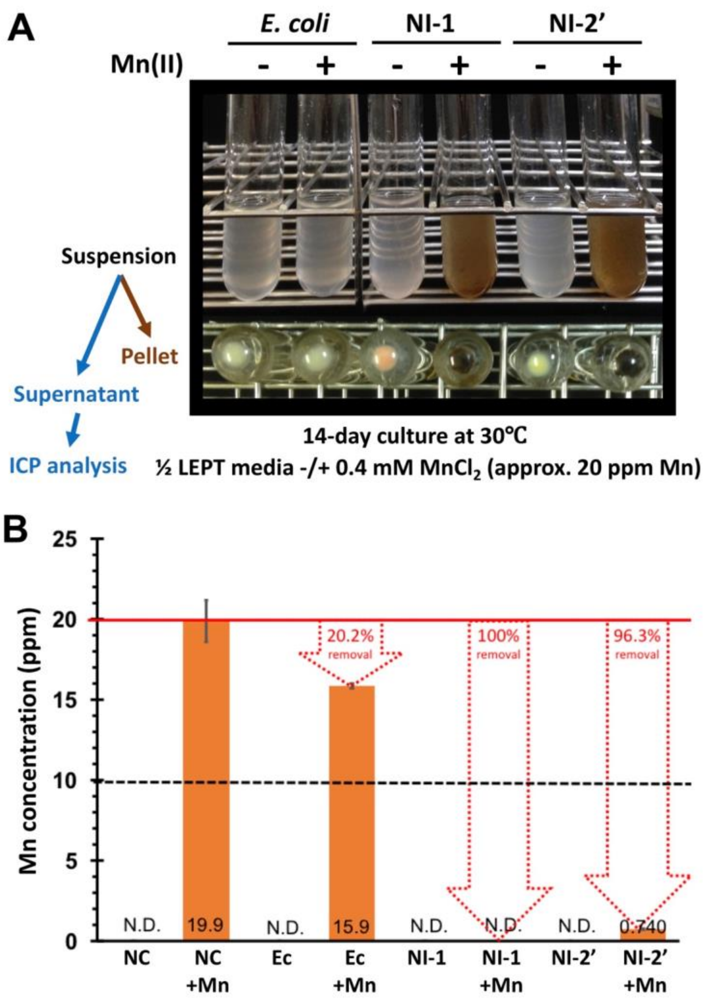

Figure 8. Bioassay of Mn-oxide biomineralization in liquid culture media using *E. coli* DH5α (Ec), *Bacillus* sp. NI-1 (NI-1), and *Arthrobacter* sp. NI-2' (NI-2'). **A**, cell-suspension cultures are separated into pellets and supernatants by centrifugation and each supernatant was subjected to ICP analysis. **B**, ICP-OES analysis of supernatant samples of suspension cell-cultures. N.D. indicates not detectable levels by ICP-OES because of low Mn concentration. NC indicates sample with no bacterial cells (no inoculation) as a control.

3.4 Mn(II)-oxidizing activity under NaCl- or cold-stress conditions

Due to the fact that *Arthrobacter* sp. NI-2 and NI-2' strains can grow well under NaCl-stress condition similar to *Bacillus* sp. NI-1, we tested Mn(II)-oxidizing activities of both *Arthrobacter* sp. NI-2 and NI-2' strains in medium containing 3% (sea water level) and 6% NaCl. Interestingly, Mn(II)-oxidizing activities of both strains were completely suppressed under these conditions (Figure 9). The results indicate that sea-water level of NaCl strongly affects Mn(II)-oxidizing activity of both

Arthrobacter sp. NI-2 and NI-2' strains but not that of *Bacillus* sp. NI-1. Thus, *Bacillus* sp. NI-1 would be a useful strain for Mn removal in high-salinity wastewater including seawater and concentrated seawater as byproduct of desalination (Nakayama And Ikegami, 2009). Therefore, we decided to further investigate the ability of Mn-oxide biomineralization in *Arthrobacter* sp. NI-2' under low-temperature condition without NaCl stress.

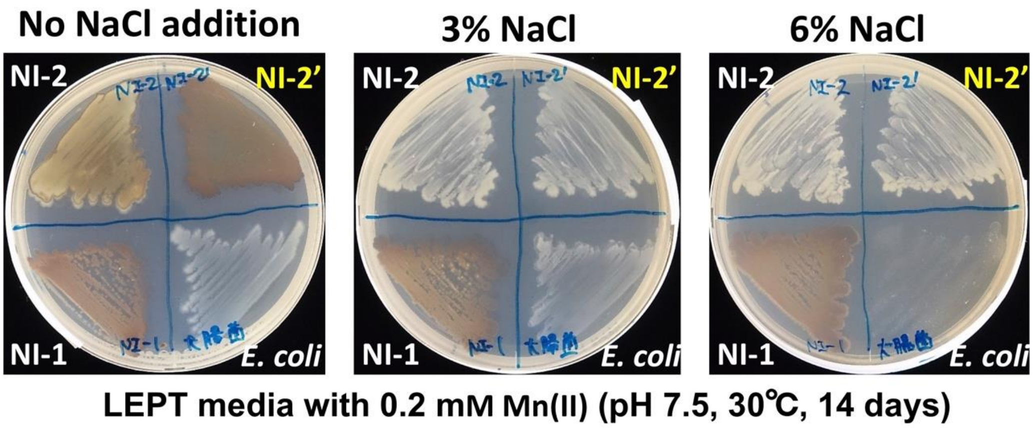

Figure 9. Effect of NaCl stress on cell growth and Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 and its derivative mutant, *Arthrobacter* sp. NI-2'. *Bacillus* sp. NI-1 and *E. coli* DH5α were used as a positive and a negative control strains of Mn(II)-oxidizing activity, respectively. The control plate (No NaCl addition) is exactly the same as the 14-days plate shown in Figure 7.

In order to evaluate Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 and NI-2' under cold-stress conditions, these 2 strains together with *E. coli* DH5α and *Bacillus* sp. NI-1, were subjected to bioassay of Mn-oxide biomineralization on the Mn(II)-containing LEPT medium incubated at 30°C, 10°C, or 4°C. As shown in Figure 10, the growth of *E. coli* DH5α and *Bacillus* sp. NI-1 strains were completely inhibited at 10°C and 4°C because of their sensitivity to cold stress, while both *Arthrobacter* sp. NI-2 and NI-2' strains thrived under these cold-stress conditions. Remarkably, *Arthrobacter* sp. NI-2' strain showed strong Mn(II)-oxidizing activity at 10°C condition while *Arthrobacter* sp. NI-2 strain did not. These results suggest that Mn(II)-oxidizing activity may connected to thermal-responsive signal transduction in *Arthrobacter* sp. NI-2 and NI-2'. Moreover, the *Arthrobacter* sp. NI-2’ strain could be a superior strain for Mn-bioremediation of cold wastewater above 10°C.

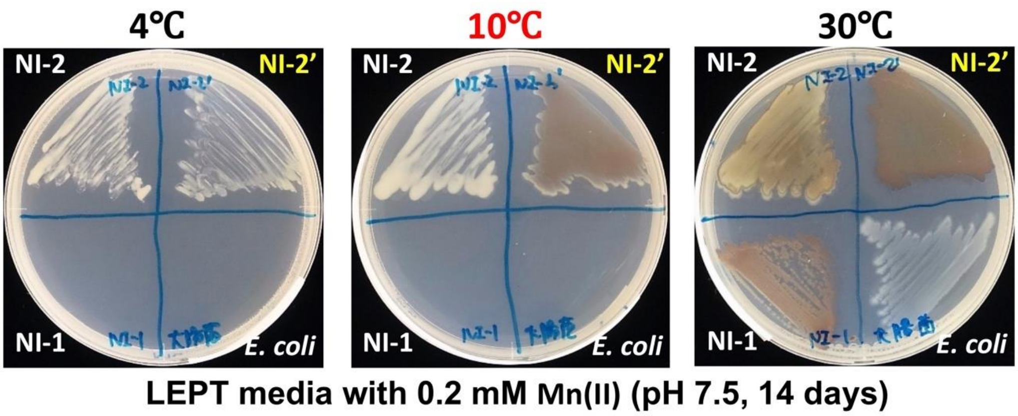

Figure 10: Effect of cold stress on cell growth and Mn(II)-oxidizing activity of *Arthrobacter* sp. NI-2 and its derivative mutant, *Arthrobacter* sp. NI-2'. *Bacillus* sp. NI-1 and *E. coli* DH5α were used as a positive and a negative control strains of Mn(II)-oxidizing activity, respectively. The control plate (30°C) is exactly the same as the 14-days plate shown in Figure 7.

4. Conclusions

In the study, we identified and characterized Mn(II)-oxidizing marine bacteria, *Arthrobacter* sp. NI-2, and its derivative mutant *Arthrobacter* sp. NI-2' with an enhanced Mn(II)-oxidizing activity. In contrast to the previously identified *Bacillus* sp. NI-1, both NI-2 and NI-2' strains did not show Mn(II)-oxidizing activity under NaCl-stress conditions. However, under cold-stress condition (10°C), which inhibited the growth of *Bacillus* sp. NI-1 strain, the *Arthrobacter* sp. NI-2' strain can grow and actively oxidize Mn(II). Therefore, we conclude that, while *Bacillus* sp. NI-1 is a good strain for Mn biomineralization under high salinity environments, the *Arthrobacter* sp. NI-2' strain can be used for Mn bioremediation in low temperature environments, such as drainage water of Mn mines in winter or at cold climate regions.

To clarify molecular mechanisms underlying the activation of Mn(II)-oxidizing activity in *Arthrobacter* sp. NI-2' strain, we have currently established a transposon mutagenesis system for *Arthrobacter* sp. NI-2' strain with a modified method based on the methods previously described (Gartemann and Eichenlaub, 2001; Zhang et al., 2011). The transposon-insertional mutants with suppressed Mn(II)-oxidizing activity obtained from the population will facilitate the identification of key genes in the mechanisms.

Chemisorption and oxidation catalyzing properties of Mn oxide (Figure 2) make Mn biomineralization applicable for removal of multiple heavy metals and a wide range of compounds including organic contaminants from the environments (Remucal & Ginder-Vogel, 2014). The finding of the key genes and factors in the mechanisms underlying the activation of Mn(II)-oxidizing activity will contribute to the development of efficient bioremediation technology for decontamination of wastewater even at low temperature conditions.

5. Acknowledgements

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Optimization Studies Using Response Surface Methodology for Cr(VI) Adsorption on Graphite Oxide-Plaster Composite

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Abstract

This study aimed to examine an optimized condition for hexavalent chromium (Cr(VI)) removal after secondary treatment system using a recent developed adsorbent, graphite oxide-plaster (GOP) composite. The response surface methodology involving central composite design was applied to investigate ordinary factors that influencing adsorption process versus the removal efficiency of Cr(VI) from wastewater using GOP composite adsorbent. The three typical controllable parameters, such as adsorbent dose, contact time and adsorption temperature, were included in the study. In order to observe a possibility to use GOP adsorbent in wastewater treatment system, wastewater from electroplating industry was used throughout the study. The results showed that the increase of GOP dose can always enhance Cr(VI) removal efficiency as well as adsorption time, its removal efficiency seemed to be a predictable tendency. The optimum condition was found at GOP dose of >10 g/L and adsorption time of >5 h. The results also signified a good benefit of temperature to the enhancement of adsorption efficiency at >30°C, which is beneficial for the installment of outdoor wastewater treatment system in a tropical weather.

Keywords: Chromium/ Graphite/ Plaster/ Wastewater/ Response surface methodology

1. Introduction

Among several heavy metal pollutants, chromium is one of a toxic metal compounds that generated from industrial process and discharged to natural water sources. Chromium is well known that can be existed in two forms as; hexavalent chromium Cr(VI), which is greater toxic than that of trivalent chromium Cr(III) (Donmez and Kocberber, 2005; Ramakrishna and Susmita, 2012). However, the industries were obligated to provide wastewater treatment facility in order to reduce exceeded contaminants as well as heavy metals like chromium from wastewater before discharging, regarding to industrial effluent standard of Thailand. The restriction of Cr(VI) contamination in effluent wastewater must not exceed 0.25 mg/L, while Cr(III) must not exceed 0.75 mg/L. Regardingly, it is unneglectable that the wastewater treatment system shall own sufficient efficiency in order to remove those toxic metals. Hence, the ideas to reduce toxic contaminants, especially heavy metals, has gained a great attention from experimental to practical scales in order to find the most cost effective, less energy consumption and compatible in wide range.

Adsorption technology is one of the most flexible and practical methods that has been applied for chromium ions removal from industrial effluents (Babel and Kuniawan, 2003; Ramakrishna and Susmita, 2012) using adsorption process. Adsorbent plays an important role in chromium adsorption, as it owns novel porosity, high adsorptive capacity and low cost (Mohan et al., 2005; Ekpete and Horsfall, 2011; Nwabanne and Igbokww, 2011). Up to date,

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various carbon materials were investigated the feasibility and capacity for chromium removal from aqueous solution through adsorption methods. Graphite oxide, a graphene derivative, has been reported its potential on pollutants adsorption, including hexavalent chromium (Deng et al., 2010; Bin et al., 2011; Wu et al., 2013).

In this study, we synthesized graphite oxide (GO) with Tours Method and composited with plaster (P) in order to produce composite adsorbent GOP for Cr(VI) adsorption. The experimental design and interpretation was performed using statistical program to the extent of response surface method (RSM) for operational optimization. The contour and 3D plots were applied in order to better understand the interactions between operational parameters and Cr(VI) removal efficiency.

2. Methodology

2.1. Preparation of graphite oxide (GO)

The GO was synthesized using modified Tours' method (Marcano et al., 2010). The 3 g of graphite obtained from jewelry industry waste was mixed with 18 g of $KMnO_4$ powder and carefully added the mixture of concentrated H_2SO_4/H_3PO_4 (360 mL/40 mL) while storing in ice bath. After mixing, the mixture was heated with stirrer to 50°C and maintained for 9 h. After 9 hours, the mixture was cooled at room temperature and slowly added iced 400 mL DI water and 30% H_2O_2 3 mL and stirred the mixture homogeneously. The residual burnt graphite was removed from the mixture using testing sieve, Retsch® 300 µm, and consequently filtered using Whatman No.2 filter paper. The suspension GO cake was collected on the filter paper and was later washed with DI water, 30% HCL, and ethanol, respectively. Carefully rinse GO suspension from the filter paper and collected in a glass tray before dried overnight at 50°C in the oven. The dried GO flake was harvested and placed in a zip lock bag and kept in de-humidified cabinet. The GO flake was grounded with mortar and sieved through 420 µm sieve before using.

2.2. Preparation of graphite oxide-plaster composite (GOP)

The powdered GO was mixed with DI water to dissolve, while plaster was consequently added before applied sonication to the mixture. The ratios of GO/P were varied to 1:3, 1:4, 1:5 and 1:10. After 1 hour, the sonicated mixture was dried in 70°C oven before glass-ball milling the mixture into a homogeneous mixing, thus the GOP was ready for Cr(VI) adsorption experiment.

2.3 Characterization of GOP

The morphologically characterization of GOP was investigated using scanning electron microscope (SEM) at 15 kV (JEOL, JSM-6400, Japan). The functional groups alteration of GOP powder was examined using Fourier-transform spectroscopy (FTIR), (Thermo Scientific Nicolet iS5, United States). The adsorbent crystalline structure was examined using X-ray Diffraction (XRD) (Bruker AXS, Germany) in the condition of Cu-K_α radiation in 5-80 degree range.

2.4 Sorption experiment

The adsorption test was carried out in the shake-flask system. The stock solution was prepared from the concentrated chromium contamination of electroplating wastewater using dilution method. The final Cr(VI) was adjusted between 7 mg/L and 40 mg/L, respectively, representing possible concentration that found in secondary treated wastewater. Batch adsorption studies were performed using Erlenmeyer flasks contained 50 mL Cr(VI) solutions. Three different factors were determined in the study which; different dosage, temperature and adsorption time, the experimental design was assisted using statistical analysis program (Minitab® 18.1). The flasks were shaken under the designated conditions with continuous shaking incubator (FTSH-501, SCI Finetech) at 120 rpm. The concentration of Cr(VI) was determined following colorimetric standard procedure by UV-VIS spectrophotometer (Shimadzu, UV 1201) at 540 nm. The removal percentage of Cr(VI) were calculated according to equation (1).

$$\text{Adsorption (\%)} = \frac{(C_0-C_f)}{C_0} \times 100 \qquad (1)$$

where, C_0 is the initial concentration and C_f is the final concentration of Cr(VI) ions. The experiments were duplicate carried out in every batch.

Table 1. Experimental range and levels of independent variables

Cr(VI) concentration	Factors	Coded symbol	Range	
			Low	High
7 mg/L	Dosage	A	0	0.5
	Temperature	B	20	45
	Time	C	0	24
40 mg/L	Dosage	A	0.1	1.0
	Temperature	B	15	45
	Time	C	0	24

2.5 Factors for experimental design

Modeling of adsorption process of Cr(VI) on GOP adsorbent was designated depending on three controllable factors to optimize the condition towards using GOP for Cr(VI) removal from electroplating wastewater. Three factors consisted of dosage (A), temperature (B), and time (C). Regarding to different initial concentrations was considered, hence, two experimental sets were carried out for different concentrations (7 mg/L and 40 mg/L). Standard RSM design was used to determine the interaction effects of all process parameters. The low and high levels and ranges of all factors were given in Table 1. The values of variables and ranges were chosen based on the preliminary experiment. Twenty experiments of each batch were conducted for each initial concentration. The optimum values of the variables were obtained by solving the regression equation and analyzing the contour plots.

3. Results and Discussion

3.1 Characterization of adsorbent

The morphology of GOP 1:4, the optimum ratio for Cr(VI) adsorption, was investigated by SEM. As seen in Figure 1 (a), particular fraction of plaster (P) can be clearly observed on a smooth surface, presented in various shapes and sizes. At low magnification, graphene oxide was unable to be observed. We magnified the observation to examine graphite oxide (GO), Figure 1 (b). GO was presented in aggregated form in which attached on the surface of plaster particles. As seen from the SEM images, the GOP adsorbent attributed from a composition of GO and P, that GO is mostly attached on the surface of P as coating, flake and aggregating forms.

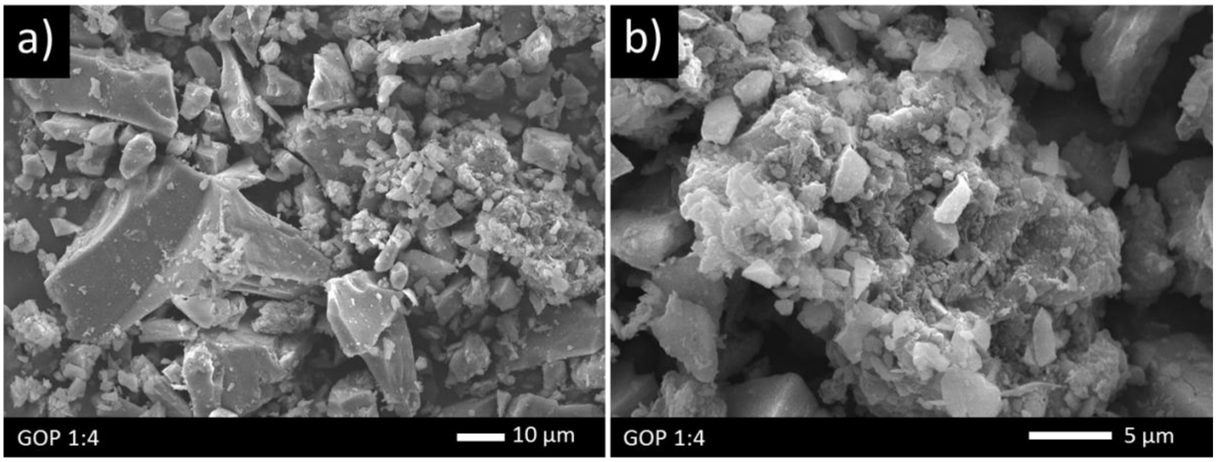

Figure 1. SEM images of GOP 1:4 at (a) low (×1,000) and (b) high (×3,500) magnification.

The morphology of GOP 1:4, the optimum ratio for Cr(VI) adsorption, was investigated by SEM. As seen in Figure 1 (a), particular fraction of plaster (P) can be clearly observed on a smooth surface, presented in various shapes and sizes. At low magnification, graphene oxide was unable to be observed. We magnified the observation to examine graphite oxide (GO), Figure 1 (b). GO was presented

in aggregated form in which attached on the surface of plaster particles. As seen from the SEM images, the GOP adsorbent attributed from a composition of GO and P, that GO is mostly attached on the surface of P as coating, flake and aggregating forms.

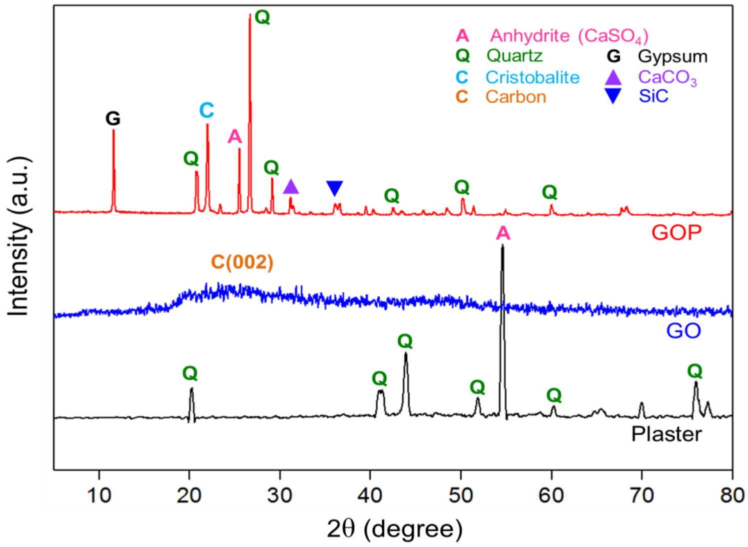

Figure 2. XRD patterns of three materials; plaster, GO and GOP.

In order to understand the crystallinity properties of GOP, we studied XRD in order to obtain the examination. As shown in Figure 2, the XRD patterns of plaster presented the consistence of quartz and anhydrite ($CaSO_4$) as a main crystalline structure, while GO was determined amorphous structure and consisted of crystalline carbon that can be observed from the peak at 2θ=26° (JCPDS card no. 19-0629). The patterns of XRD spectrum of GOP revealed the composition of 2 materials in which quartz was determined as a major crystallinity structure of GOP following the peak position that presented in plaster. However, there were several peaks of GOP that unresonated to the peaks positions of GO and plaster, such as the peaks at 2θ=31.2°and 36°. These peaks are possibly be induced after the composition of GO and plaster in which corresponded to $CaCO_3$ (M. Singh et al., 2016) and SiC (He et al., 2015), where the carbon source is graphite oxide that combines to SiO_2 in plaster.

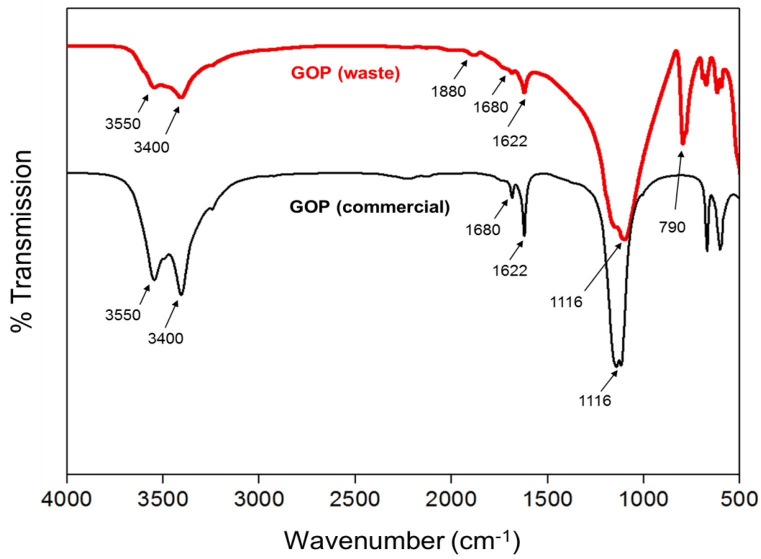

Figure 3. FTIR spectra of GOP (synthesized from commercial material) and GOP (synthesized from waste).

The FT-IR spectra of GOP adsorbent (synthesized from jewelry industry waste) was compared with the spectra of GOP (synthesized from commercial graphite and plaster) in order to examined surface functional groups of GOP. Ordinary surface functional groups were presented at 3,550 cm^{-1} and 3,400 cm^{-1} corresponding to the stretching vibrations of hydroxyl, C-OH groups (Bourlinos et al., 2003). The spectrum of transmission bands presenting in the range of 1620-1730 cm^{-1} corresponding to the vibration of carboxylic acid, in which commonly found in graphite precursors (Cote et al., 2009; Kaniyoor et al., 2010). The functional groups that vibrated in the range of 500-1,000 cm^{-1} had similarities with both samples of GOPs. The strong band at 1,100 cm^{-1} attributed to phenyl hydroxyl (C-O) stretching vibrations (Bourlinos et al., 2003; Cote et al., 2009). The peak at 790 cm^{-1} vibrated differently from GOP (commercial) corresponded to the out-of-plane blending of C-H (Feng et al., 2011) that is unresonated with ordinary peak that found in graphite and graphene oxide.

3.2 Response surface methodological approach

The experiments were performed under specific combinations of physical parameters using statistically designed experiments in order to study the interaction of effects of all variables. The main affects that taken into consideration as shown in figure 3 obviously represented that the variables that involved the study played an important role in the adsorption efficiency of Cr(VI), since each variables significantly contributed to the efficiency of Cr(VI) removal. The main effect indicated the mean response changed across the level of factors, these plots were considered when multiple factors are involved (Ponnusamy and Subramaniam, 2013). Regarded to the selected variables included in the study, it clearly revealed that GOP dosage, temperature and adsorption time are dominant factors influencing the percentage of Cr(VI) removal which is un-unneglectable.

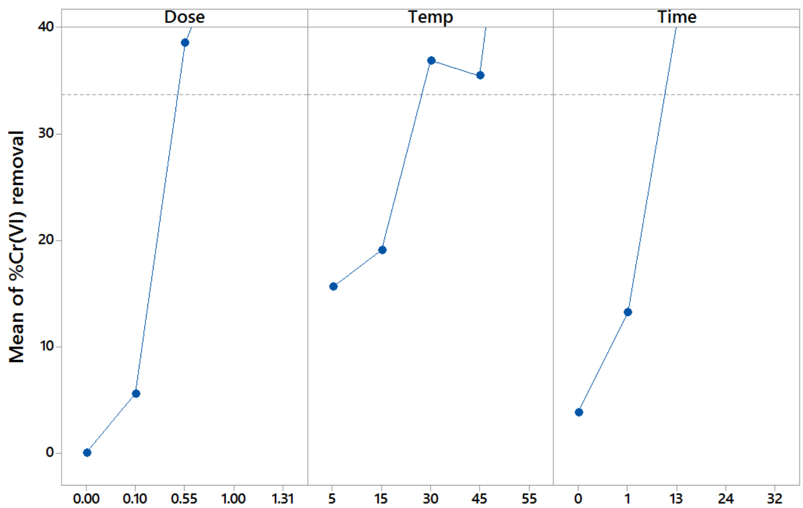

Figure 3. Main effect plots of parameters on Cr(VI) removal from electroplating wastewater

3.2.1 Effect of interactions and optimization at low Cr(VI) concentration

In order to visualize the effects of interaction factors towards Cr(VI) removal efficiency of GOP, we plotted contour graph of the main interaction factors that influenced the responses (Removal efficiency of Cr(VI)). The two dimensional contour plots presented in figure 4 performed at low concentration of Cr(VI) (7 mg/L). Figure 4 (a)-(c) represented the Cr(VI) removal efficiency responses of the different interaction factors. The most possible optimum condition towards removal efficiency was higher than 80% influencing by the amount of GOP dosage 2.5-5 g/L at temperature that higher than 32°C. The narrow region of optimum condition presented interesting results, which at the dosage greater than 5 g/L and high temperature over 40°C, the greatest efficiency could be found. These particular results supported the interaction between 3 variables; GOP dosage, time and temperature are critical to the adsorption efficiency. The response surface plot in Figure 4(d)) represented the plot of two main factors (GOP dosage and temperature), which attained the visualization when Cr(VI) removal efficiency elevated by the two influential factors. However, at low contamination of Cr(VI), minimum amount of GOP is desired cooperated with a preference of higher temperature to achieve good Cr(VI) removal efficiency.

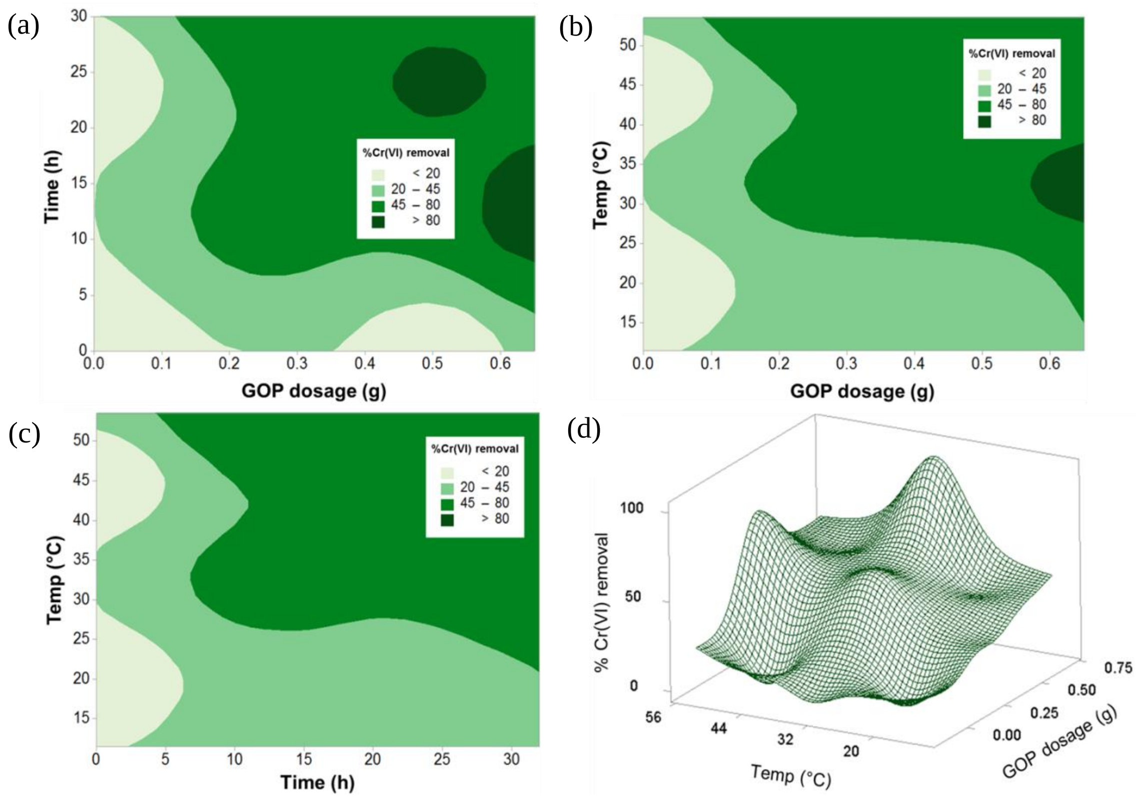

Figure 4. Contour plots interaction of a) GOP dosage and adsorption time, b) GOP dosage and temperature, c) adsorption time and temperature, and d) 3D surface plots effects of GOP dosage and temperature towards 7 mg/L Cr(VI) removal efficiency.

3.2.2 Effect of interactions and optimization at high Cr(VI) concentration

The experiment design using statistical program was similarly performed in order to examine the response of Cr(VI) removal efficiency of GOP when the concentration of Cr(VI) was raised. The collective data was analyzed in contour plotting, Figure 5 (a)-(c). The 2D contour plots showed an optimum condition to enhance the removal efficiency of Cr(VI) concentration in wastewater over 80% required the amount of GOP 11 g/L in 30°C surrounding temperature. The surface 3D plot in Figure 5 (d) clearly supported the optimum condition as the surface graph revealed Cr(VI) removal efficiency enhanced when GOP dosage was high. This result expressed vice versa with the results of low Cr(VI) contamination. The higher amount of GOP adsorbent used, required lower reaction temperature but we could observe an obvious 3D plot tendency for Cr(VI) removal efficiency.

We examined leaching potential of Cr(VI) in water by using GOP after adsorbed 7 mg/L Cr(VI) for one month. As shown in Figure 6, the concentration of leached Cr(VI) from GOP was observed in a least amount (<0.001 mg/L), even though the adsorbent had been used for Cr(VI) adsorption over a month. This primary test enlightened a possibility to reuse GOP for other application or dispose the material with the conventional methods.

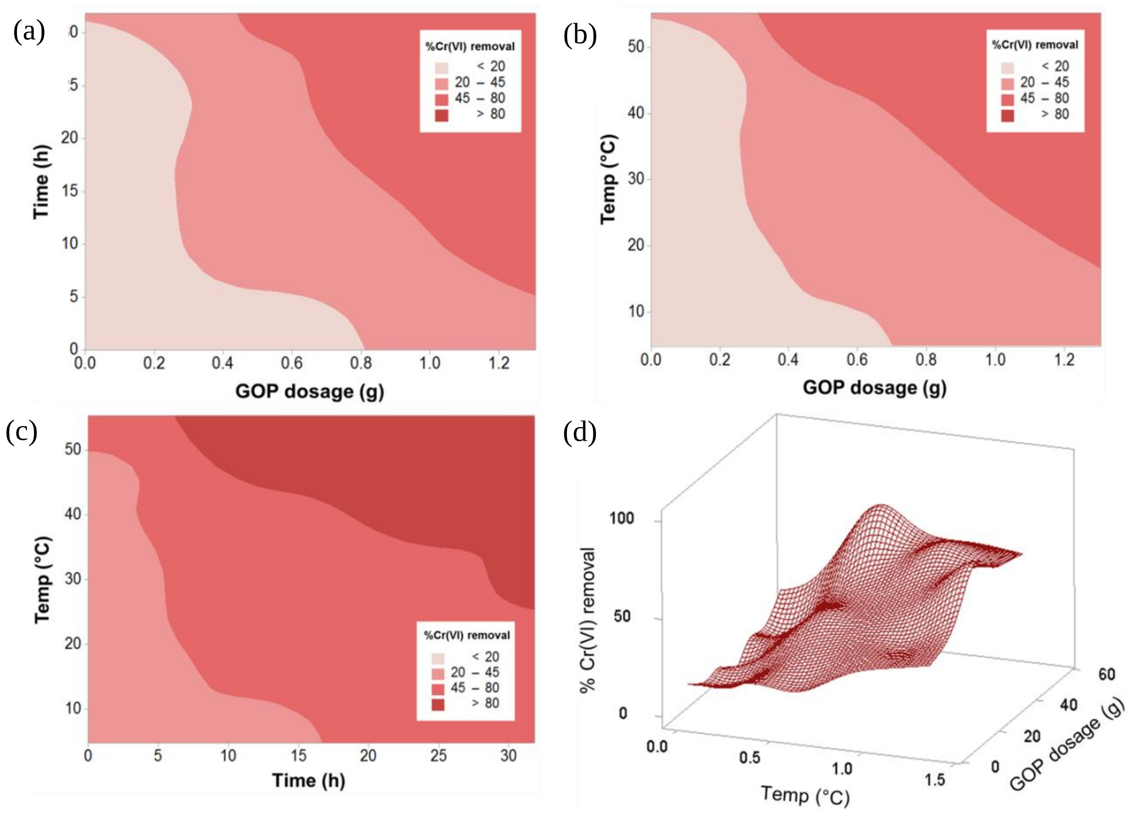

Figure 5. Contour plots interaction of a) GOP dosage and adsorption time, b) GOP dosage and temperature, c) adsorption time and temperature, and d) 3D surface plots effects of GOP dosage and temperature towards 40 mg/L Cr(VI) removal efficiency.

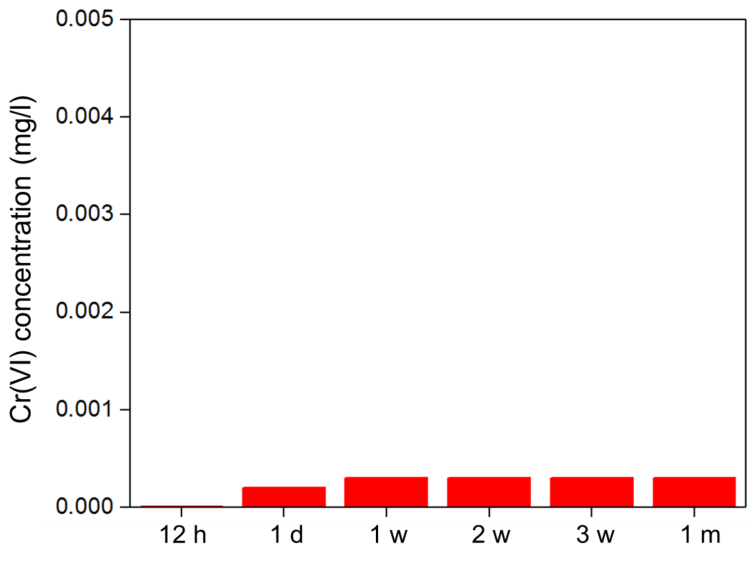

Figure 6. Leaching test for GOP after adsorbed 7 mg/L Cr(VI) over 18 days.

4. Conclusions

A highly efficient adsorbent, Graphite oxide/Plaster (GOP), for Cr(VI) removal were successfully synthesized from the composition of graphite oxide (GO) and plaster (P) from jewelry industry waste. The morphology of GOP as observed from SEM presented in various shapes and sizes, while GOP mostly presented in aggregation form attached on the surface of plaster particle. The XRD analysis revealed the dominant crystalline structure of GOP was influential by the crystallinity of plaster, while the functional groups on GOP surface was dominantly resulted from GO, confirmed by FT-IR. The GOP adsorbent was applied to remove Cr(VI) ions from electroplating wastewater at different concentrations, low concentration (7 mg/L) and high concentration (40 mg/L). The optimization study used statistical program to design the experiment. The main factors that influenced the Cr(VI) removal efficiency were GOP dosage and temperature. At low Cr(VI) contamination, 5 g/L of GOP dosage was required under 32°C reaction temperature in order to maintain 80% removal efficiency, while at high Cr(VI) contamination 11 g/L of GOP dosage at 30°C was preferences for good removal efficiency.

5. Acknowledgements

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Isolation and Screening of Alkaliphilic Bacteria for Biosurfactant Production Using Agricultural/Agro-Industrial Wastes as Substrate

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Abstract

Biosurfactants have been used considerably as alternatives to chemical surfactants due to their high surface activity and low toxicity. In this study, alkaliphilic bacteria were isolated from alkaline contaminated soils collected from a rice bran oil refinery plant using crude rice bran oil, soapstock and wax as substrates. Among all isolates, three bacterial strains showed great results of surface tension reduction and were identified as *Brevibacterium casei* NK8 (28.31 mN/m), *Microbacterium paraoxydans* NK22 (29.92 mN/m) and *Pseudomonas mendocina* NK41 (29.45 mN/m). Moreover, cell-free broth of *Brevibacterium casei* NK8 had high emulsification index (45.83 %), oil displacement (73.33 %) and critical micelle dilution (18.5 times) values. The potential of an alkaliphilic *Brevibacterium casei* NK8 to utilize different agricultural/agro-industrial wastes for biosurfactant production was evaluated. The wastes were hydrolyzed by alkali during sterilization process. Production of biosurfactant was found to be the highest with defatted rice bran (1.86 g/L) as compared to rice husk, durian shell and corn husk. The using of low-price lignocellulosic substrates enhance potential economical production of biosurfactant on further industrial scale.

Keywords: Alkaliphilic bacteria/ Biosurfactant/ Agricultural/agro-industrial wastes/ Lignocellulosic wastes/ Alkaline pretreatment

1. Introduction

Biosurfactants, amphipathic products of biological origin, have generated wide interest as potential nontoxic alternative surface-active agents (Gutnick and Bach, 2017). Biosurfactant have the unique property of reducing the surface and interfacial tension of liquids, resulting to their many applications in the field of agriculture, petroleum, microbial enhanced oil recovery, biomedical sciences, cosmetics, food processing and pharmaceuticals. Chemically, the biosurfactants are classified into lipopeptides, glycolipids, neutral lipids, fatty acids, phospholipids, polymeric and particulate compounds (Chen et al., 2015).

Many biosurfactants are produced by microorganisms during aerobic fermentation at neutral condition. To prevent contamination and enhance biosurfactant solubilization, this research interest in the production of biosurfactant by alkaliphilic bacteria. Moreover, alkaliphilic exoenzymes produced during fermentation, such as alkaline cellulases and/or alkaline proteases, can enhance biosurfactant effectiveness (Horikoshi, 1996; Satyanarayana et al., 2005). Alkaliphiles can be isolated from normal environments such as garden soil, while they can be found higher in alkaline environments. Highly alkaline environments for microorganisms include alkaline contaminated soils and industrial-derived alkaline effluents.

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A major limitation of the commercialization of biosurfactant has always been their cost, as they are more expensive than petroleum-based surfactant. To overcome this obstacle, agricultural/agro-industrial wastes comprised of lignocellulosic biomass are inexpensive, renewable and abundant, thus provides a unique natural resource for large-scale and cost-effective biosurfactant production. Lignocellulosic biomass is a complex matrix that is relatively resistant to degradation. Sugars are locked in a recalcitrant structure that requires a pretreatment step to release them (Hassan et al., 2018). Many conventional methods are currently used to pretreat lignocellulosic biomass such as steam/steam explosion, grinding/milling, hot water/autohydrolysis, acid treatment and alkali treatment (Amin et al., 2017) . In this research, agricultural/agro-industrial wastes were pretreated during sterilization process in Horikoshi alkaline medium. Then, alkaliphilic biosurfactant-producing bacteria could be cultivated subsequently without pH adjustment. The advantage of alkali pretreatment is carried out under milder condition than acid method by using non-polluting and non-corrosive chemicals such as ammonia, sodium hydroxide, sodium carbonate, and calcium hydroxide. Alkali pretreatment have been identified as methods that release low concentrations of inhibitors (e.g., acetic and formic acid, furfural, 5-hydroxymethylfurfural (HMF), and phenolic compounds) that are potentially inhibitory to microbes (Koppram et al., 2014).

The objective of this work was to screen the effective alkaliphilic bacteria and investigate the potential agriculture residues as substrate for biosurfactant production. Firstly, alkaliphilic biosurfactant-producing bacteria were isolated from alkaline oil contaminated soils from vegetable refinery plant using oily-byproducts as substrate. Secondly, several parameters were used to test surface activity of isolated strains such as surface tension, oil displacement, emulsification index and critical micelle dilution. Thirdly, agricultural/agro-industrial wastes in Thailand were hydrolyzed by alkali and utilized by the isolated alkaliphilic bacteria to produce biosurfactant. The pretreated agricultural/agro-industrial wastes contain soluble sugar and oil that could be used as substrates instead of oily-byproduct from crude rice bran oil refinery.

2. Methodology

2.1 Soil samples and oily by-products

Soil samples and oily by-products were collected from rice bran oil refinery plant in Thailand. Soil samples were collected at a depth of 15 cm and stored at 4°C. The pH of all soil samples was about 10. Oily by-products were obtained from CEO Agrifood Co. Ltd, included crude rice bran oil, soapstock and wax.

2.2 Isolation and screening of alkaliphilic bacteria

Soil samples were inoculated into Horikoshi medium (HM) of pH 10 for enrichment of bacteria using oily-byproducts as substrate. Enriched samples were diluted and spread on the HM agar plates containing (g/L) glucose 10, peptone 5, yeast extract 5, K_2HPO_4 1, $MgSO_4 \cdot 7H_2O$ 0.2, Na_2CO_3 10 and agar 20, pH 10. Plates were incubated at 30°C for 24 h and individual colonies were picked up and further streaked on HM agar plate in order to obtain pure culture. All the bacterial isolates were maintained on HM agar at 4°C for subsequent experiments.

To study biosurfactant production potential, each isolate was cultured in HM broth using oily-by products as substrate. The culture medium samples were autoclaved at 110°C for 10 min and centrifuged at 8,000 rpm for 30 min to remove bacterial cells. Various methods were used to study surface activity of cell-free broth such as surface tension, emulsification index and oil displacement. The surface tension was measured by digital tensiometer (Kruss, BP100) at 25°C using the plate method.

The emulsification index was determined by vortexed an equal volume of rice bran oil and cell-free broth for 2 min. Then, left the mixture to stand for 24 h. The emulsification index was determined as the percentage of the height of the emulsified layer (mm) divided by the total height of the liquid column (mm).

The oil spreading assay was performed by added 20 mL of distilled water to glass petri dishes, followed by 10 μL of rice bran oil on the surface of the water. Then, 10 μL of the cell-free broth was dropped to the center of the oil layer. The diameter of the clear zone formed by the oil displacement was measured and calculated compared to the diameter of the oil layer.

2.3 Selection of alkaliphilic bacteria for bio-surfactant production

Critical micelle dilution (CMD) value was used to selected alkaliphilic bacteria for further study. The culture medium samples were autoclaved at 110°C for 10 min and centrifuged at 8000 rpm for 30 min to remove bacterial cells. Cell-free broth was diluted with distilled water from 2 to 512 times. Then, the diluted cell-free broth was measured the surface tension. The CMD value was determined from plotting dilution versus surface tension.

2.4 Molecular identification of the selected alkaliphilic bacterial strains

Pure culture plate of the selected strains was sent to a sequencing service by Macrogen Inc (Seoul, South Korea). The 16S rRNA gene sequences were compared with GenBank using the BLASTn program.

2.5 Biosurfactant production using agricultural/ agro-industrial wastes under alkaline condition

Several agricultural/agro-industrial wastes in Thailand was examined as alternative substrate for biosurfactant production: rice husk, durian shell, corn husk and defatted rice bran. Rice husk, durian shell and corn husk were collected from agricultural area in Phitsanulok province. Defatted rice bran was supported by CEO Agrifood Co. Ltd. All agricultural/agro-industrial wastes were dried and mashed into size of 1 mm. For inoculum preparation, a loopful of bacteria was transferred to HM broth and incubated for 48 h at 30°C with agitation of 200 rpm. The optical density of inoculum was adjusted to 1.0 at 600 nm. Biosurfactant was produced by culturing 10% (v/v) inoculum in HM broth without glucose containing 1.0% (w/v) mashed agricultural/agro-industrial wastes for 4 days at 30°C with agitation of 200 rpm. Amount of produced biosurfactant was analyzed by acid precipitation and solvent extraction method. The cell number was analyzed by the plate count technique.

2.6 Biosurfactant yield analysis

The produced biosurfactant was extracted from culture broth by using the same procedure as Khondee et al. (2015). Briefly, the cell-free broth was adjusted pH to 2.0 by using 6 M HCl to precipitate the biosurfactant. Then, an equal volume of chloroform:methanol (2:1) was added to the acidic cell-free broth and shaking for 30 min. The solvent mixture was separated and evaporated by a rotary evaporator at 45°C. The product was dissolved in methanol and separated from remaining oil. The solvent was evaporated at 60°C. Dry weight of crude product was measured to calculate biosurfactant yield.

3. Results and discussion

3.1 Isolation of biosurfactant-producing alkali-philic bacteria

A total of 41 isolates with different morphological types were isolated from alkaline contaminated soils collected from rice bran oil refinery plant. All isolated were identified surface activity by various methods which are surface tension, emulsification index and oil displacement. Three strains were selected from each oil inducers: NK8, NK22 and NK41 (Formerly known as NKHM8, NKHM22 and NKHM41). All three strains could reduce surface tension of HM broth from 37.55 to below 30.00 mN/m. Emulsification index of these strains were in the range from 45 to 50%. However, oil displacement of bacterial strain NK8 and NK41 showed much higher than NK22. The residual wax used as inducer for NK22 might affect to oil displacement activity. Jain et al. (2012) reported that the cell-free broth of alkaliphilic *Cronobacter sakazakii* RJ-06 showed the highest surface tension reduction to 46.07 mN/m, while emulsification index with vegetable oils were in between 90% to 100%. The uronic acid and proteinaceous component of bacterial biosurfactant played an important role in the emulsification of hydrocarbons and oils, apart from functional groups (acetyl) present in the biopolymer, which also provide hydrophobicity, imparting enhanced emulsifying activity (Bramhachari et al., 2007). Therefore, different chemical structure of biosurfactant from diverse bacterial strain affect to the variation of surface activities.

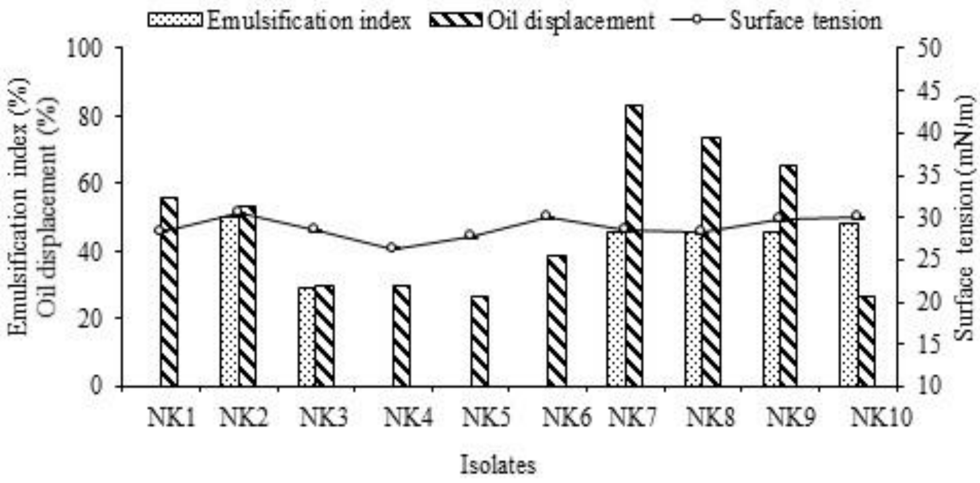

Figure 1. Cell-free broth surface tension (mN/m), emulsification index (%) and oil displacement (%) of the isolated strains cultivated in HM using crude rice bran oil as carbon source.

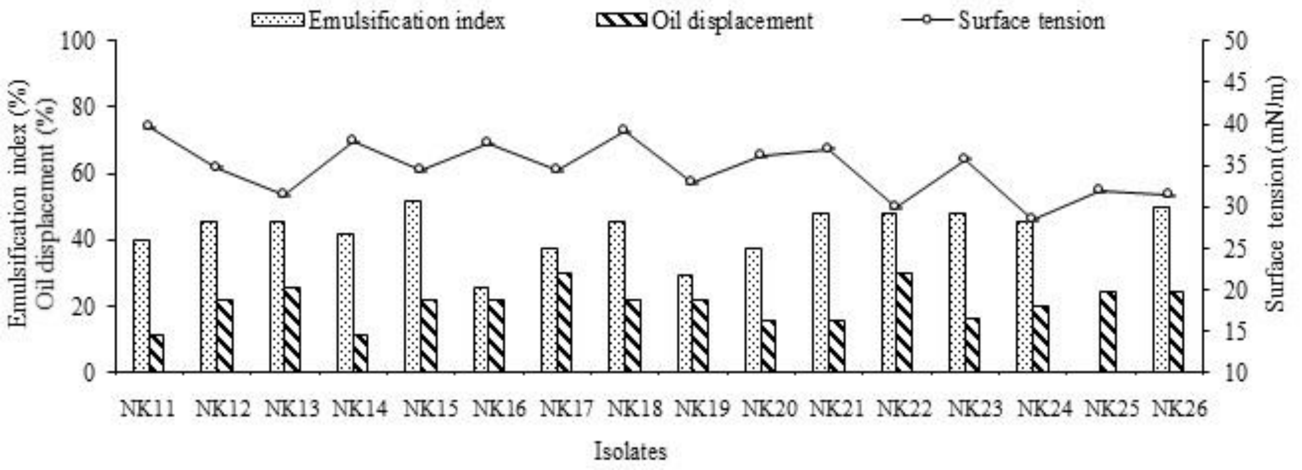

Figure 2. Cell-free broth surface tension (mN/m), emulsification index (%) and oil displacement (%) of the isolated strains cultivated in HM using wax as carbon source.

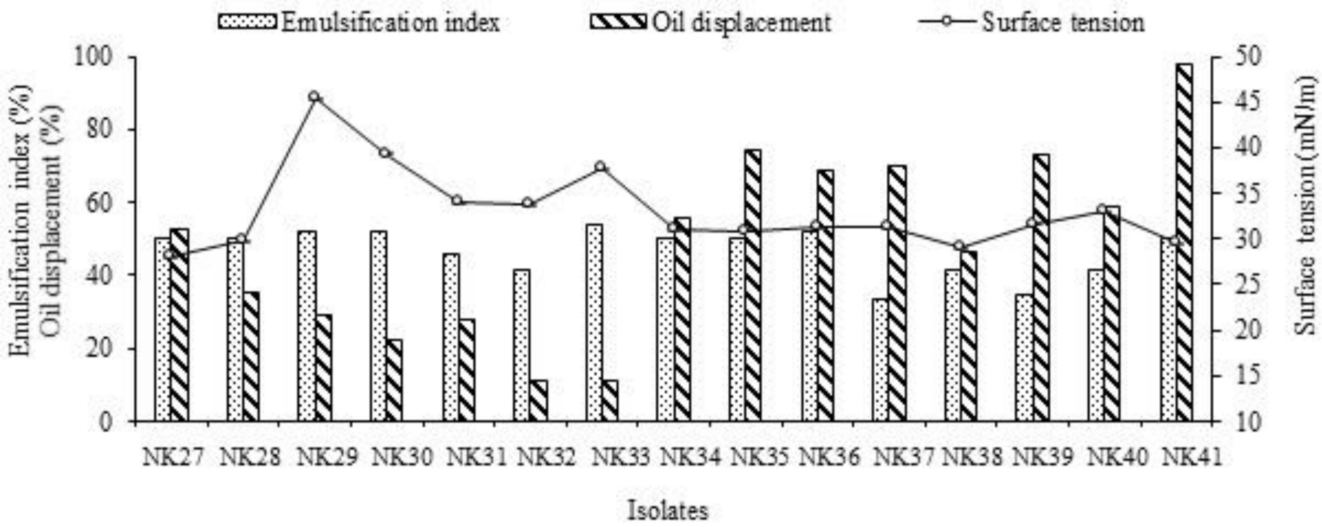

Figure 3. Cell-free broth surface tension (mN/m), emulsification index (%) and oil displacement (%) of the isolated strains cultivated in HM using soapstock as carbon source.

3.2 Selection of alkaliphilic bacteria for bio-surfactant production

CMD value was used to evaluate surface activity of the produced biosurfactant in cell-free broth. It was determined by calculating graph intersection from two equations showed in Figure 4. The high CMD value indicated that the produced biosurfactant provided great interfacial tension reduction between air-water interface. NK8, NK22 and NK41 had CMD values of 18.5, 6.0 and 23.0 times respectively. Molecular identification of the isolates was performed with NK8, NK22 and NK41 strains by comparing 16S rRNA gene sequencing from Macrogen Inc to the database of known 16S rRNA sequences. The molecular identification of these strains show that these strains belongs to: *Brevibacterium casei* (Strain NK8), *Microbacterium paraoxydans* (Strain NK22) and *Pseudomonas mendocina* (Strain NK41). *Pseudomonas* spp. have been in the pathogens list of Department of Medical Sciences, Ministry of Public Health, Thailand. Therefore, *Brevibacterium casei* NK8 was selected for further study.

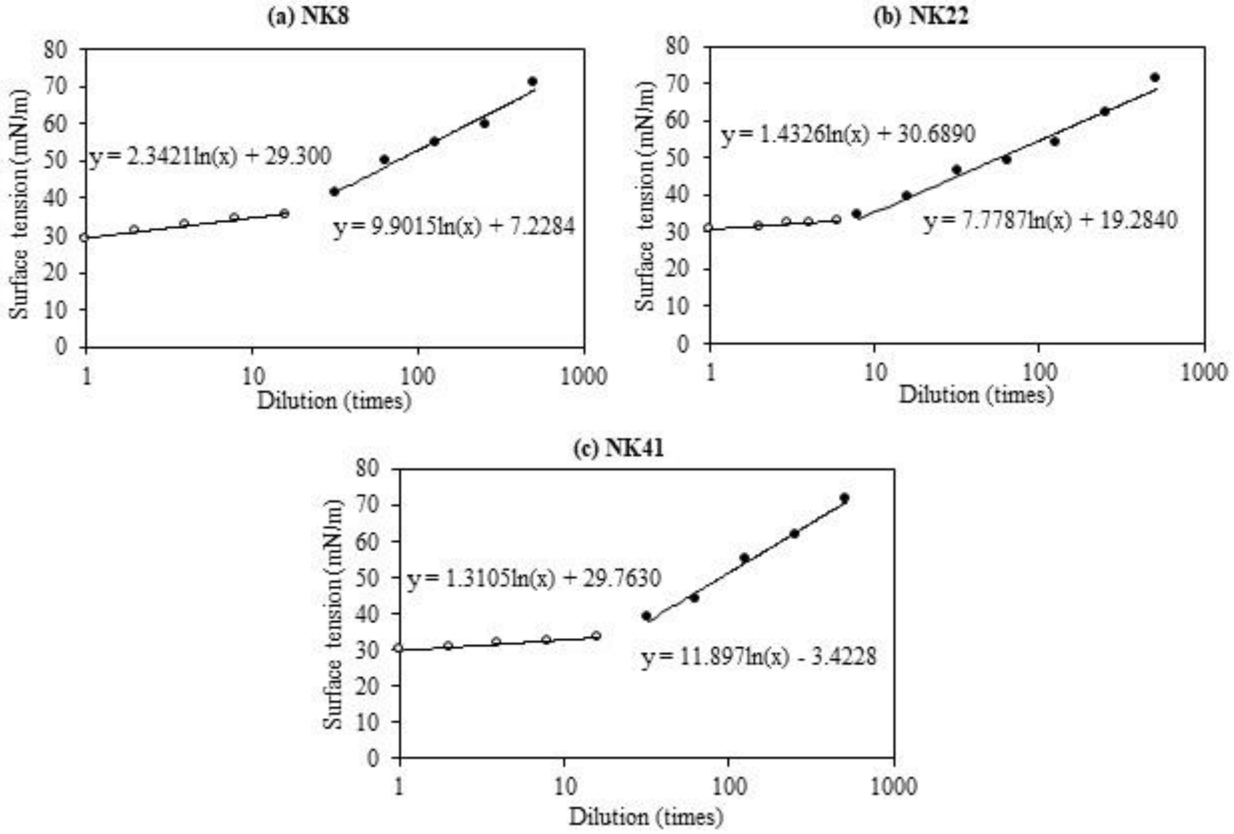

Figure 4. Surface tension of cell-free broth dilutions of (a) NK8, (b) NK22 and (c) NK41.

3.3 Utilization of agricultural/agro-industrial wastes for biosurfactant production by alkaliphilic bacteria

This research considered on the using of agricultural/agro-industrial wastes instead of glucose and oil to enhance cost-effective biosurfactant production. Moreover, the using of oily-byproduct lead to the cleaning problem of pilot-scale fermenter. The lignocellulosic wastes were pretreated by alkali hydrolysis during media sterilization process. The majority of hemicellulose and some portion of lignin are dissolved away from cellulose into pre-hydrolysis liquid through depolymerization and degradation (Bujanovic et al., 2012). This pre-hydrolysis liquid contains carbon sources for bacterial growth. Various agricultural/agro-industrial wastes used in the research were rice husk, durian shell, corn husk and defatted rice bran. Defatted rice bran provides greater biosurfactant production than other wastes (Figure 5(a)). The lower amount of *Brevibacterium casei* NK8 in HM broth of rice husk was from the attachment on the remaining biomass, while pre-hydrolysis process (Figure 5(b)). Defatted rice bran contains starchy, cellulosic polysaccharide, lipid and soluble proteins remains unutilized (Lee et al., 2009; Jojima et al., 2010). Therefore, the higher biosurfactant production from the vegetable oil extracted waste might due to the using of sugar as substrate couple with residual oil as inducer. Jain et other wastes were almost degraded from al. (2013) produced biosurfactant from several lignocellulosic wastes by alkaliphilic *Klebsiella* sp. RJ-03. They also reported the higher biosurfactant yield obtained from de-oiled *Jatropha* cake (1.58 g/L) than rice husk (0.94 g/L). The vegetable oil extracted wastes were found to be the promising substrate with respect to yield of biosurfactant.

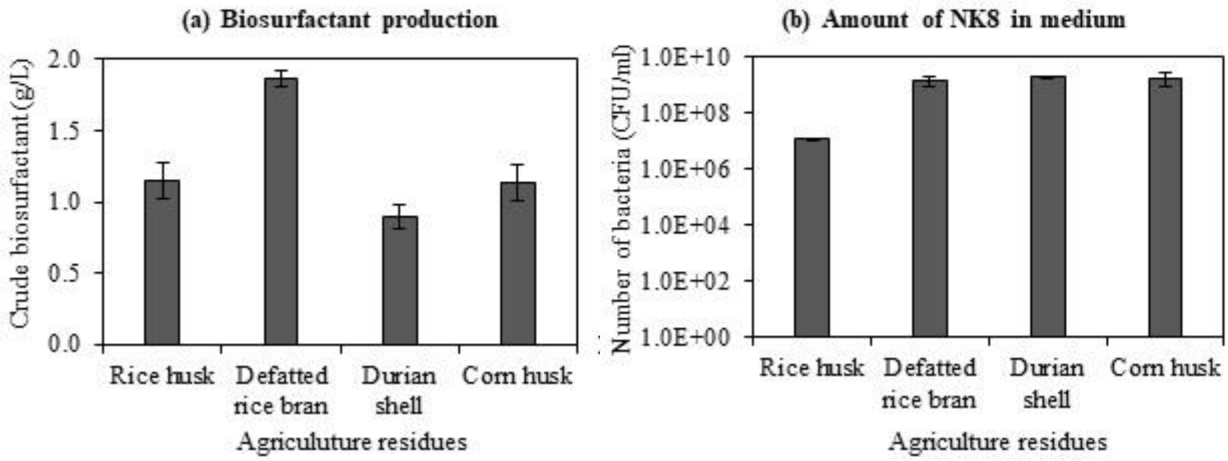

Figure 5. Amount of crude biosurfactant (a) and number of *Brevibacterium casei* NK8 in media (b) after culturing with agricultural/agro-industrial wastes in HM broth.

4. Conclusions

Biosurfactant producing bacteria were found from alkaline contaminated soils collected from rice bran oil refinery plant. Among 41 alkaliphilic bacteria, 14 strains could reduce surface tension of HM broth lower than 30 mN/m using oily-byproducts as substrate. Emulsification index and oil displacement values were varied which resulted from different chemical structure of the produced biosurfactant. *Brevibacterium casei* NK8 was the effective biosurfactant-producing bacteria that provide CMD values up to 18.5 times. The production of biosurfactant from *Brevibacterium casei* NK8 with pretreated defatted rice bran under alkaline condition proved to be a useful application of alkaliphilic bacteria. This benefit plays an important role in the assessment of the economic feasibility of agricultural/agro-industrial wastes as substrates for biosurfactant production. In addition, the optimization of media compositions and culturing conditions should be studied in future research.

5. Acknowledgements

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Formulation of Lipopeptide-based Washing Agent for Oil-based Drill Cutting Treatment

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Abstract

The aim of this present work is to formulate a lipopeptide-based microemulsion to effectively remove synthetic oil from drill cutting. Lipopeptide biosurfactant was produced from *Bacillus* sp. GY19. The effect of microemulsion composition on phase behavior was studied. The mixture consisted of lipopeptide mixed with fatty alcohol ethoxylate surfactants (Dehydol LS series), isopropyl alcohol and sodium chloride. Winsor type III microemulsion was obtained from both Dehydol LS3TH and Dehydol LS5TH. Results showed the sorption of Dehydol LS3TH in drill cutting due to its high hydrophobicity. From washing experiment, the most effective formula (84% oil removal) comprised of 70% cell-free broth, 8% LS5TH, 8% isopropyl alcohol and 19% sodium chloride. This washing solution could reduce phytotoxicity of oil-based drill cutting to *Vigna radiata* as germination index of 72%, root elongation of 72% and seed germination of 100%. Therefore, lipopeptide-based washing solution have potential for the application on oil-based drill cutting treatment.

Keywords: Biosurfactant/ Lipopeptide/ Microemulsion/ Synthetic based fluid/ Drill cutting

1. Introduction

One of a major waste produced from petroleum extraction is drill cutting. The disposal of drill cutting unproperly causes the contamination to environment. Drill cuttings consist of excavated soil mixed with drilling fluid, which may include a fuel oil cut; drill cuttings can be separated from drilling fluid by using shale shakers, centrifuges, or other methods (Chaillan et al., 2006). However, drill cutting can contain significant amount of oil from residual drilling fluid as much as 17% oil by weight (Childs et al., 2004). The main functions of drilling fluids or mud are to transport rock cuttings in the wellbore annulus to the surface, to cool and lubricate the drill bit, and to provide a hydrostatic head to maintain the wellbore stability (Kania et al., 2018). Drilling process usually use synthetic-based fluids (SBF) since it is less toxic than other types. The synthetic oils in SBF include linear or isomerized C16/C18 alpha-olefins and/or esters derived from vegetable oils (Childs et al., 2004). During drilling process, there is further concern that acetic acid, an end-product of anaerobic biodegradation of oils, may mobilize heavy metals from both synthetic-based fluid and diesel-based fluid cuttings, resulting in groundwater contamination (Dow et al., 1990). Therefore, heavy metal and hydrocarbon are contaminated in oil-based drill cutting. This drilling waste are considered as hazardous waste and strongly regulated in many countries (Ball et al., 2012).

Several physicochemical treatment processes have been used to treat oil-based drill cutting such as reinjection, incineration, landfills, solidification/stabilization, thermal desorption, microwave treatment, supercritical CO_2 extraction, bioremediation, phytodegradation and surfactant enhanced washing (Chen et al., 2017).

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Surfactant washing is another potential treatment method in which interfacial tension reduction at the oil-surfactant interfaces takes place, displacing the trapped oil in soil due to capillary effect (Nazar et al., 2011). Microemulsion is the famous application of surfactant in many fields. Microemulsion consists of a surfactant, a water-immiscible oil and a cosurfactant in a proper ratio so that a single, optically transparent and thermodynamically stable phase is formed (Kahlweit, 1988). Microemulsion systems produce high solubilization capacity and ultralow interfacial tensions (IFT) of oil and water (Chai et al., 2007). The effective microemulsion formulas is basically determined by analyzing the phase behavior of the system comprised of surfactant, co-surfactant (alcohol), target oil and electrolyte (salt). The phase behavior of microemulsion is the transformation of microemulsion from Winsor type I to Winsor type II through Winsor type III at a particular pressure and temperature. The factors that affect the phase transition between different types of systems and physicochemical properties include salinity, temperature, molecular structure and nature of the surfactant and cosurfactant, nature of the oil and the water–oil ratio (Bera et al., 2014). Winsor type III system is the most desirable for ultralow IFT, in which the upper layer contains the excess oil phase, the middle phase consists of some solubilized oil, surfactant, co-surfactant mixture and the lower phase contains excess brine. When washing drill cutting with Winsor type III microemulsion, the obtained free oil layer can be removed and washing solution can be reused.

In recent years, much attention has been directed towards biosurfactants owing to their numerous advantages compared to chemical surfactants such as lower toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity, specific activity at extreme temperatures, pH and salinity, and the ability to be synthesized from renewable feed stocks (Bezza and Chirwa, 2017). Biosurfactants are structurally diverse and can have various chemical compositions mainly consisting of fatty acids, glycolipids, lipopeptides, lipopolysaccharides and lipoproteins depending on the producing microorganism, raw matter and process conditions (Makkar et al., 2011). In this research, lipopeptide was produced from *Bacillus* sp. GY19. According to Khondee et al. (2015), lipopeptide produced from *Bacillus* sp. GY19 had 100% oil displacement efficiency with diesel oil, followed by Arabian light oil and heavy oil. This result demonstrates that lipopeptide has potential in oil remediation application. The mixing of two surfactant can improve microemulsion formation and surface activity. He et al. (2017) added surfactin (one kind of lipopeptide) to enhance microemulsion formation of non-ionic surfactant (Tween80), sodium chloride, glycerol and docosahexaenoic acid single cell oil.

The aim of this work is to show the lab-scale feasibility of using microemulsion technique to formulate lipopeptide-based washing agent and thus remove oil from SBF drill cutting. The influence of mixed-surfactant system of lipopeptide and fatty alcohol ethoxylate on microemulsion formation against olefin oil was studied. The formulas that provided Winsor type III microemulsion were selected for washing experiment. Oil removal efficiency, phytotoxicity and cutting salinity were evaluated from SBF drill cutting before and after treated by lipopeptide-based washing agent.

2. Methodology

2.1 Materials and chemicals

2.1.1 Oil-based drill cutting

The SBF drill cutting was collected from offshore petroleum extraction site in Thailand. The synthetic-based oil in SBF was linear alpha olefins (C9-C21).

2.1.2 Microemulsion ingredients

Lipopeptide biosurfactant was produced from *Bacillus* sp. GY19 following Khondee et al. (2015) procedure using waste glycerol and palm oil as substrates. Bacterial cells was removed from culture broth by centrifuged at 8,000 rpm for 30 min. The residual fatty acids from fermentation was separated by adjusted pH of cell-free broth to 2.0 by 6.0 M HCl and stored at 4°C for 24 h. The floated fatty acids were skimmed. The partial purified cell-free broth was used to formulate microemulsion.

Fatty alcohol ethoxylates are non-ionic surfactant produced by adding ethylene oxide (EO) to linear fatty alcohols (C12-14) and sold under the trade name Dehydol LS. Dehydol LS series used in this work include Dehydol LS3TH and Dehydol LS5TH, where the number represents the number of EO groups in each molecule. They were supplied by the Thai Ethoxylate Co., Ltd., Thailand.

Sodium Chloride (NaCl) and isopropyl alcohol (IPA) were analytical grade and purchased from Sigma Aldrich. Olefin oil (C9-C21) obtained from an oil filed was used as model oil.

Figure 1. SBF drill cutting used in this study

Table 1. Lipopeptide properties

Form of lipopeptide	Lipopeptide concentration[1] (g/L)	CMD[2] (times)	Surface tension at CMD (mN/m)	Compatible solvent
Cell-free broth	4.8	10.2	30.061	Water (pH 7)

Note: [1] Lipopeptide concentration was analyzed following Khondee et al. (2015).
[2] CMD (Critical micelle dilution) value was analyzed following Andrade et al. (2016).

Table 2. Plant-based surfactant properties*

Surfactant	Formula	Hydrophilic-Lipophilic Balance (HLB)	Molecular weight (g/mole)	Cloud point (°C)
Fatty alcohol C12-14 (3) ethoxylate (Dehydol LS3TH)	$C_{18\text{-}20}H_{36}O_4$	7.9	318	51–53
Fatty alcohol C12-14 (5) ethoxylate (Dehydol LS5TH)	$C_{22\text{-}24}H_{46}O_6$	10.3	406	68–73

Note: * Data obtained from manufacturer.

Table 3. Additive properties

Additive	Property
Electrolyte: NaCl	The addition of electrolyte increases hydrophobicity of system.
Cosurfactant: IPA	The addition of cosurfactant increases interfacial area resulting to enhance the solubilization of oil into surfactant micelle and reduce IFT.

2.2 Microemulsion phase behavior study

The concentration of cell-free broth and retentate solution was adjusted to 10 times above its CMD. The influence of NaCl and type of Dehydol LS on microemulsion formation were studied. The washing solution consist of 70% lipopeptide solution, 8% Dehydol LS (Dehydol LS3TH and Dehydol LS5TH), 1-25% NaCl and 8% IPA by weight. The experiment was performed by mixed an equal volume (0.6 ml each) of olefin oil and washing solution. The mixture was hand-shaken for two minutes and left to equilibrate for 3 weeks. Microemulsions were visually identified by passing a laser light through the phase (Rongsayamanont et al., 2017). The mixtures produced Winsor type III microemulsion were selected for SBF drill cutting washing experiment.

2.3 Washing procedure

The batch washing studies were conducted by adding 4 g of SBF drill cuttings to 8 mL of the lipopeptide-based washing solution in 20 mL vials at 25°C. The vials were vortexed for 2 min and then centrifuged at 2500 rpm for 10 min. The liquid phase was separated from vial. The washed drill cutting was rinsed by distilled water for twice to remove the remaining oil and washing solution, and to prevent any emulsion formation during extraction

(Arpornpong et al., 2018). The oil remaining in treated drill cutting was extracted by chloroform and analyzed by thin layer chromatography and flame ionization detection (TLC-FID) according to Maruyama et al. (2003).

2.4 Phytotoxicity assay

The phytotoxicity of the treated and untreated SBF drill cutting was developed from Luna et al. (2013). The study based on seed germination and root elongation of the vegetables *Vigna radiata* and *Oryza sativa*. The experiment was performed in sterilized Petri dishes containing 20 g drill cutting mixed with 5 ml of distilled water. Ten seeds were placed on each Petri dish and incubated in the dark at 25 °C for 5 days. After five days of incubation in the dark, seed germination, root elongation (≥5 mm) and the germination index (a factor of relative seed germination and relative root elongation) were determined as follows ():

Relative seed germination (%) = (number of seeds germinated in the extract/number of seeds germinated in the control) × 100.

Relative root length (%) = (mean root length in the extract/mean root length in the control) × 100.

Germination index = [(% of seed germination) × (% of root growth)]/100.

2.5 Salinity measurement

Cutting salinity was measured as the electrical conductivity at 25°C from an unfiltered 1:5 of soil/distilled water suspension (Hardie and Doyle, 2012). Twenty grams of dried cutting was mixed with 100 ml deionized water by shaking at 200 rpm at 25°C for 30 min to dissolve soluble salts. The suspended cutting was precipitated by centrifugation at 2,500 rpm for 10 min. The clear solution was separated to measure the conductivity. The cutting conductivity was used to identified soil salinity class and plant growth ability following Abrol et al. (1988).

3. Results

3.1 Formulation of lipopeptide-based micro-emulsion

The mixtures of lipopeptide-based micro-emulsions at two lipopeptide solution forms (cell-free broth and retentate), two Dehydol LS series (LS3TH and LS5TH) and several amount of NaCl (1% - 25% w/v) were studied to evaluate the change in phase behavior of IPM-based microemulsions with these parameters. The amount of IPA was fixed at 8% (w/v). The mixtures of cell-free broth with Dehydol LS3TH did not require NaCl addition (Figure 2(a)), while the mixtures of cell-free broth with Dehydol LS5 TH must added NaCl to produce microemulsion (Figure 2(b)). The microemulsion transition is governed by the hydrophilicity and lipophilicity of the system (Bourrel and Schechter, 1998). The high hydrophobicity of Dehydol LS3TH could induce the phase transition of microemulsion without NaCl supplementation. When using Dehydol LS5Th, the phase behavior changed from Winsor type I to type III and Winsor type III to type II with increasing NaCl as observed in Figure 2(b). As the salt concentration is increased, some of the water molecules are attracted by the salt ions, which decreases the number of water molecules available to interact with the charged part of the surfactant. (Bera et al., 2014).

3.2 Removal of oil from SBF drill cutting

The influence of using Dehydol LS series with different hydrophobicity on the oil removal efficiency of lipopeptide-based washing solution was observed. Dehydol LS3TH (HLB = 7.9) has higher hydrophobic than Dehydol LS5TH (HLB = 10.3). Lipopeptide-based washing compositions were selected from formulas that provided Winsor type III microemulsion as shown in Table 4. The residual oil content after washing by solution containing Dehydol LS3TH was higher than the unwashed cutting (Figure 3). The high hydrophobic of Dehydol LS3TH might cause the sorption of this surfactant to drill cutting particles.

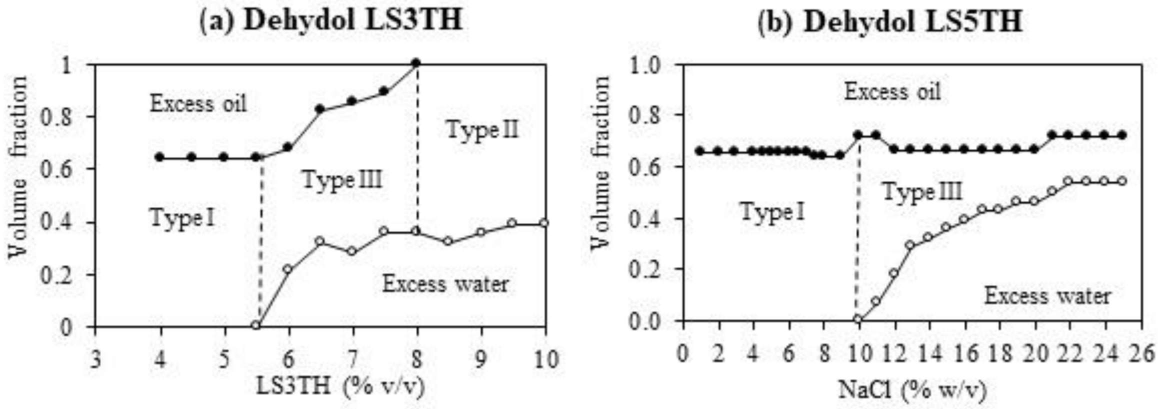

Figure 2. Phase behavior diagram of the system of 70% lipopeptide cell-free broth, 8% IPA and 8% Dehydol LS series with NaCl and surfactant scan for olefin oil.

As described by Yang et al. (2010), the mechanisms responsible for surfactant sorption to soil consist of ion exchange, hydrophobic bonding and π-electron polarization. Soil organic matter contributes significantly to surfactant sorption and soil minerals provide the sorption sites (Schwarzenbach et al., 2016). The highest efficiency was found at 84% oil removal from the mixture of 70% cell-free broth, 8% Dehydol LS5TH, 8% IPA and 19% NaCl. Childs et al. (2004) developed the non-microemulsion based washing solution (0.1% C14-C15 propoxylated sulfate surfactant, 1% octyl sulfobetaine and 13% Na_2SiO_3) which could remove 41% oil from SBF drill cutting. Therefore, the development of washing solution based on microemulsion technique is very useful for the remediation of hydrocarbon from drill cutting.

Figure 4(a) shows the example of Winsor type III microemulsion obtained from the best formula which includes three-phase systems in which the middle phase microemulsions are in equilibrium with both excess oil and excess aqueous phases. Figure 4(b) shows the majority of the oil was removed as a free oil layer activated by the best formula. The surfactant micelle tends to adsorb at the cutting and cutting-water interfaces, which increasing the contact angle between oil and cutting surface and decreasing capillary forces that trap the oil in the cutting.

Table 4. Formulas of lipopeptide-based washing agent for SBF drill cutting treatment.

Sample	Washing composition			
	Cell-free broth (%v/v)[1]	Dehydol (%v/v)	IPA (%v/v)	NaCl (%w/v)
1	70	8.0 (LS5TH)	8	13
2	70	8.0 (LS5TH)	8	14
3	70	8.0 (LS5TH)	8	15
4	70	8.0 (LS5TH)	8	16
5	70	8.0 (LS5TH)	8	17
6	70	8.0 (LS5TH)	8	18
7	70	8.0 (LS5TH)	8	19
8	70	8.0 (LS5TH)	8	20
9	70	5.5 (LS3TH)	6	-
10	70	6.0 (LS3TH)	6	-
11	70	7.0 (LS3TH)	6	-
12	70	8.0 (LS3TH)	6	-
Control (Synthetic based mud cutting)				

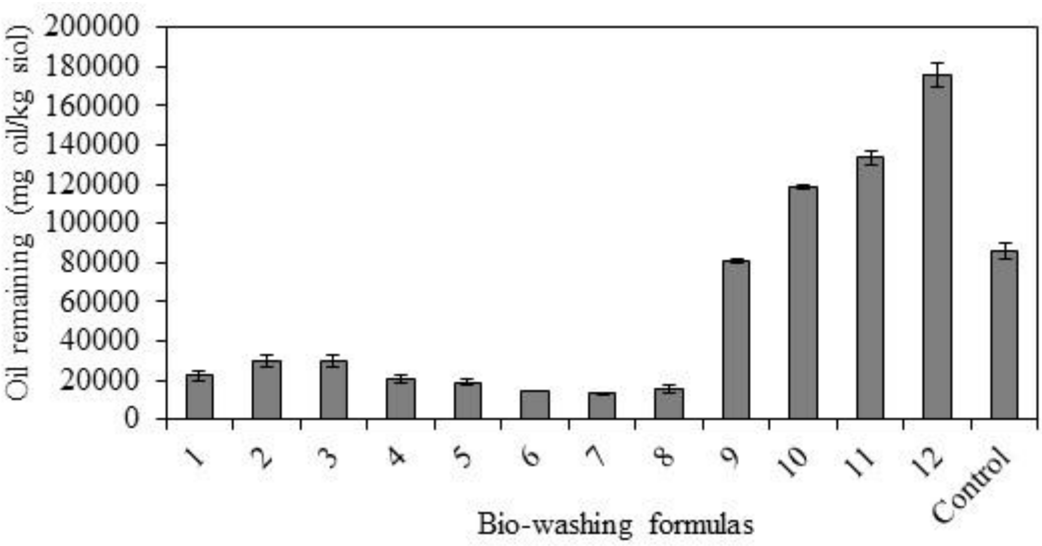

Figure 3. Amount of oil remaining in SBF drill cutting after treated by biosurfactant-based washing agents (mixture of cell-free broth, Dehydol LS5TH, IPA and NaCl) compared to untreated SBF drill cutting as control.

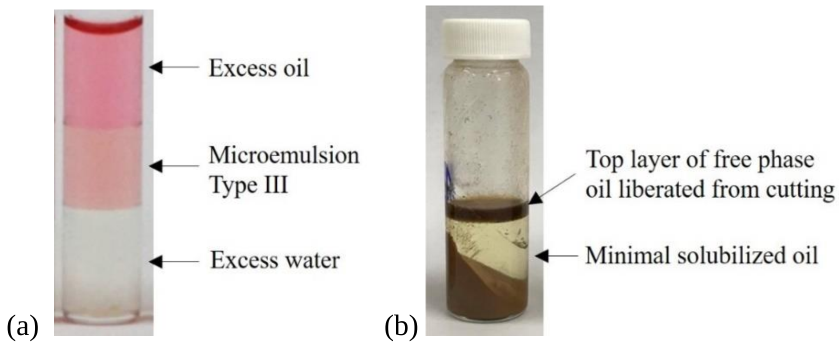

Figure 4. Examples of (a) Winsor type III microemulsion obtained from phase behavior study of lipopeptide-based solution against olefin oil and (b) physical characteristic of free oil layer liberated from cutting.

3.3 Phytotoxicity of SBF drill cutting

The germination index, which combines measures of relative seed germination and relative root elongation, was used to evaluate the phytotoxicity of SBF drill cutting before and after treated by lipopeptide-based washing solution. *Vigna radiata* and *Oryza sativa* were used in the experiments. The germination index value of 80% has been used as an indicator of the absence of phytotoxicity (Luna et al., 2013). Results indicated that the untreated SBF drill cutting have much higher inhibitory effect on seed germination or root elongation in plant analyzed (Table 5 and 6). Besides oil contaminated in cutting, cutting salinity cause adverse effects on plant growth. Soil salinity imposes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress on plants, and thus limits water uptake from soil (Shrivastava and Kumar, 2015).

Based on the soil salinity classification system of FAO, five salinity classes were established using the conductivity values as follows: (1) very strongly saline, >16 mS/cm; (2) strongly saline, 8-16 mS/cm; (3) moderately saline, 4-8 mS/cm; (4) slightly saline, 4-2 mS/cm; and (5) non-saline, 0-2 mS/cm (Abrol et al., 1988). The conductivity values indicated that the salt concentration of both SBF drill cutting before and after treatment was slightly saline (Table 7).

Table 5. Phytotoxicity of SBF drill cutting before and after treated by lipopeptide-based washing agent against *Vigna radiata*. Experiments were performed in triplicate and the results represent means ± standard deviations of the three independent experiments.

Sample	Seed Germination (%)	Root Elongation (%)	Germination Index (%)
Cutting before treatment	70±00	34±6	24±6
Cutting after treatment	100±00	72±1	72±1

Table 6. Phytotoxicity of SBF drill cutting before and after treated by lipopeptide-based washing agent against *Oryza sativa*. Experiments were performed in triplicate and the results represent means ± standard deviations of the three independent experiments.

Sample	Seed Germination (%)	Root Elongation (%)	Germination Index (%)
Cutting before treatment	0±00	0±00	0±00
Cutting after treatment	80±28	44±8	35±18

Table 7. Soil salinity classes and crop growth of SBF drill cutting before and after treatment by lipopeptide-based microemulsion.

Sample	Conductivity (mS/cm)	Soil salinity class[1]	Effects on crop plants[1]
Cutting before treatment	3.20	Slightly saline	Yields of sensitive crops may be restricted.
Cutting after treatment	3.36	Slightly saline	Yields of sensitive crops may be restricted.
Clean soil (control)	0.55	Non saline	Salinity effects negligible.

Note: [1] Data obtained from Abrol et al. (1988).

4. Conclusions

Microemulsion technique was applied to formulate lipopeptide-based washing agent for SBF drill cutting clean-up. The addition of Dehydol LS3TH to washing solution caused adverse effect to oil removal ability, whereas Dehydol LS5TH gave the great results. The treated SBF drill cutting showed low toxicity to *Vigna radiata* and *Oryza sativa*. Some inhibition on these plants might due to the slightly salinity of cutting. Thus, this paper shows the ability and robustness of lipopeptide biosurfactant in formulating microemulsions for their potential applications in petroleum industry. To improve the formulation and economic viability, the development of an alcohol-free washing solution should be studied in the future.

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