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Fluxes and effects in terrestrial and aquatic ecosystems

Scientific Program 1

Oral

Heavy metal contents in meadows and pedogeological diversity

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INTRODUCTION

The region of Auvergne (in the center of France) presents a wide pedogeological diversity leaving her in a complex situation for the MTE mobility and bioavailability (acid pH, MTE high contents) in front of the regulations for organic wastes spreading (JO: decret n°97-1133 and arrete 8/1/98).

Relying on recent studies made in France about the soil (1), the aim of this work is to build up a typology of the meadows from the region in relation with soils and plants MTE contents.

MATERIAL AND METHODS

Five sites with geological and soil diversity, characteristic of the Auvergne are kept for the study of MTE bioavailability (Cu, Zn, Ni, Cr, Hg, Pb, Cd, Se, As).

The soils and plants (grassland) sampling is carried out in a geographical determined surface of 177 m² on which it is possible to work later. In this surface, 16 samplings of soils and 4 samplings (2m²) of plants constitute the analysed soil and plant sampling.

A physico-chemical and agronomic study is led with the MTE analysis (according to french norms and INRA methods for the preparation and by inductively coupled plasma atomic emission spectrometry (ICP-AES) for the analyse). The statistics (matrice of correlations and AFM) are utilized Statgraphics and SPAD4 software.

RESULTS

Generally, the five soils (tab.1) present the next characteristics according to ASPITET programme (1) : Pb high contents, middle contents in Zn, Cd low contents, with two soils enriched in Ni and Cr (fig.1).

The correlations meaningful positives have been studied between soils MTE contents and :

-soil Fe total contents : Cu (+0.99) Ni (+0.98) Cr(+0.98) and Zn (+0.87)

-carbone Hg(+0.92) and Se(+0.81).

-argile Zn (+0.52) Cu (+0.56) Ni (+0.50) Cr (0.48).

The Cu, Ni and Cr are the best correlated elements.

The ratio MTE soil contents/MTE plant contents varies according to the element of metal trace and to the meadow. The meadow 3 presents the highest ratio (minimal availability).

For Ni, Cr, Cu and Zn, the total content of elements in the soil enables to tell the content of this element in the plant unlike As, Cd, Pb and Se.

The ratio Ni soil contents/Ni plants contents on the soils naturally rich in Ni and Cr (M4 and M5) is the same that on the classic soils (M1, M2, M3).

CONCLUSIONS

The meadows 1 and 5 have a great interest because of that they represent the extreme situation of the Auvergne. The meadows 4 and 5 enriched in Ni and Cr but at different pH are interesting for understanding the Ni and Cr availability.

The characterization of meadows according to the MTE content in the soils and plants linked with more general parameters (geological deposit, pH, texture, organic matter, floristic composition, crop management) must be widened to integrate many meadows at this program. This study is a base of the better understanding on this cultural system about agro-environmental impact of the sewage sludge utilization.

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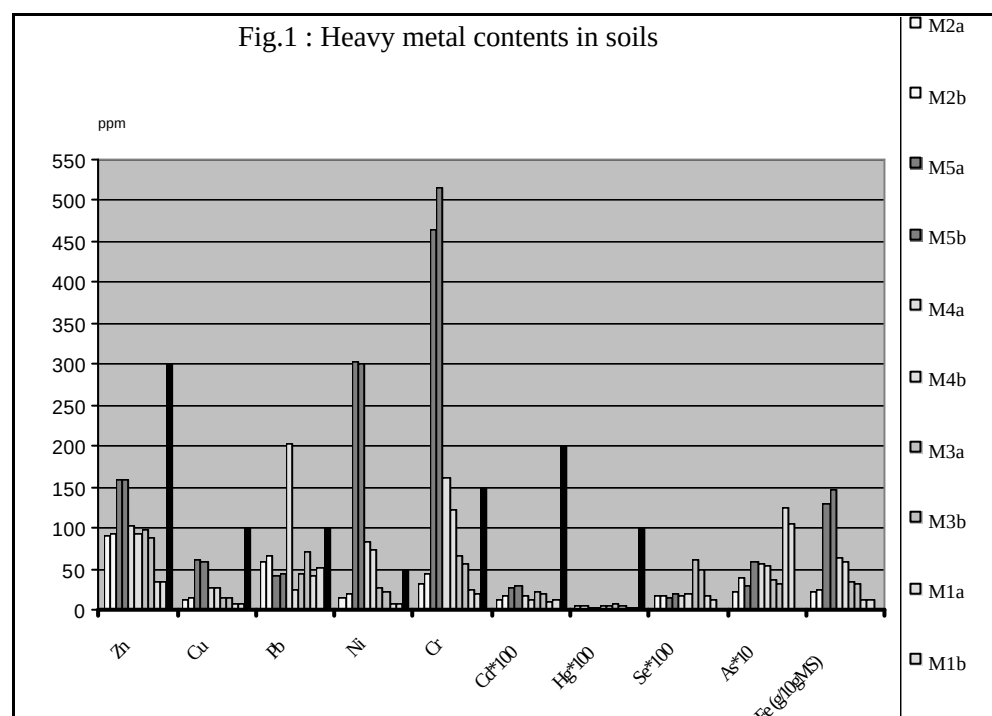
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ACKNOWLEDGEMENTS

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Table 1 : Description of soils

	Meadow 1 : M1a and M1b	Meadow 2 : M2a and M2b	Meadow3 : M3a and M3b	Meadow 4 : M4a and M4b	Meadow 5 : M5a and M5b
Géological material	sedimentary	crystalline	crystalline	volcanic	volcanic
pH	6.5	5.2	5.4	5.3	6.5
Organic matter %	2.94	5.84	8.51	5.54	4.16



Fluxes and effects in terrestrial and aquatic ecosystems

Scientific Program 1

Poster

SORPTION OF COPPER IN SOILS AS INFLUENCED BY SOIL pH AND ORGANIC AMENDMENTS

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INTRODUCTION

Copper (Cu) is generally added to agricultural soils either as an ingredient in fungicides or in soil amendments, i.e. sewage sludge or manures. Swine manure contains Cu levels up to 750 mg

kg⁻¹. High Cu levels in soils can antagonize the uptake of other micro-elements, most notably iron, zinc, and molybdenum. Copper content in most soils varies from 2 to 100 mg kg⁻¹. Copper is strongly bound to soil organic matter and clay content, thus, its mobility and transport in soils is slow. Soil properties and organic amendments influence the metal sorption. The objectives of this experiment were to investigate the affects of soil pH, anion enrichment, and application of incinerated sewage sludge (ISS) on sorption of Cu.

MATERIALS AND METHODS

In this experiment, three surface (0-15 cm) soil samples were used, i.e. Palouse silt loam (pH=6.2), Kennewick silt loam (pH=7.88), and Watum silt loam (pH=9.90). Sorption of Cu was evaluated under either Cl⁻ or NO₃⁻ enriched systems. Two grams soil were equilibrated with 20 mL of solution containing either 0, 5, 10, 20, 50, or 100 mg L⁻¹ Cu. To study the anion effects, the equilibrium solution was prepared using either CaCl₂ or Ca(NO₃)₂ at 0.01M concentration. The suspension was shaken for 30 minutes in a end-over-end shaker, centrifuged, and the supernatant was filtered. The concentrations of Cu and Ca were measured in the supernatant in the respective equilibrium solution to calculate the amount of Cu that was sorbed by the soil in each treatment. Three replications were used for each treatment. In a parallel experiment, Cu sorption was evaluated, at various Cu concentrations in the equilibrium solution as shown above, using the Palouse silt loam soil which received ISS amendments at either 0, 25, 50, or 100 mg g⁻¹ soil rates.

RESULTS AND DISCUSSION

The sorption of Cu was greater under NO₃⁻ enrichment as compared to that under Cl⁻ enrichment (Fig. 1). Greater than 95 percent of Cu applied (in the range of 0 to 100 mg L⁻¹ nominal Cu concentration or 0 to 98 mg L⁻¹ actual measured Cu concentration) was adsorbed by the soils (Fig. 2). The adsorption was greater by the soil which has the high pH. Sorption of Cu increased with an increase in the rate of ISS applied (Fig. 3). This coincided with a slight increase in soil pH with an increase in rate of ISS applied (Fig. 4). Furthermore, competitive sorption of Cu and Zn and the pattern of metal desorption from a soil amended (incubation for 150 d) with various rates (20 to 320 g kg⁻¹) of either manure, sewage sludge, or incinerated sewage sludge.

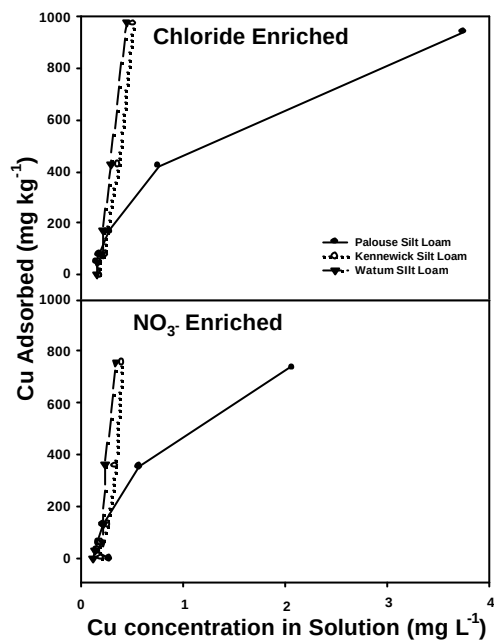


Fig. 1. Copper adsorbed by three different soils as a function of Cu in solution under different anion based equilibrium solutions.

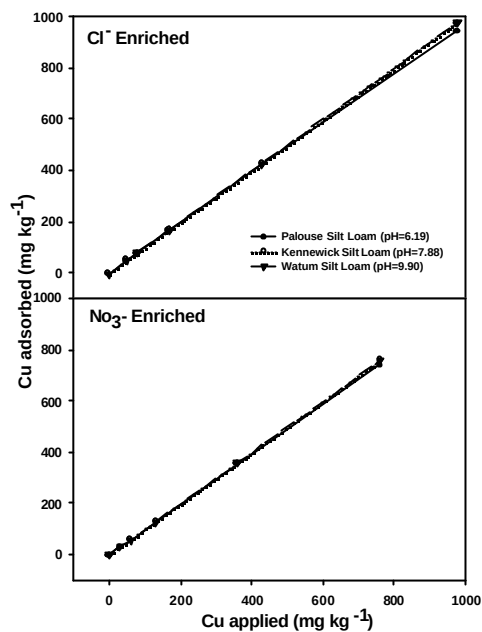


Fig. 2. Copper adsorbed by three different soils as a function of Cu applied under different anion based equilibrium solutions.

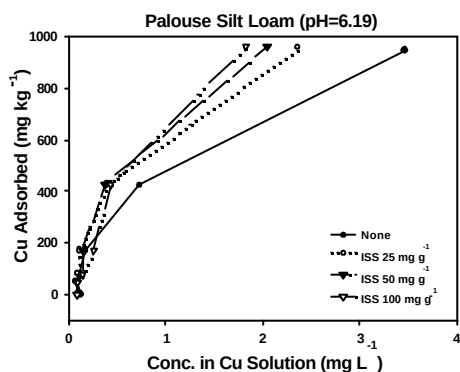


Fig. 3. Effects of various rates of incinerated sewage sludge (ISS) additions on Cu adsorption.

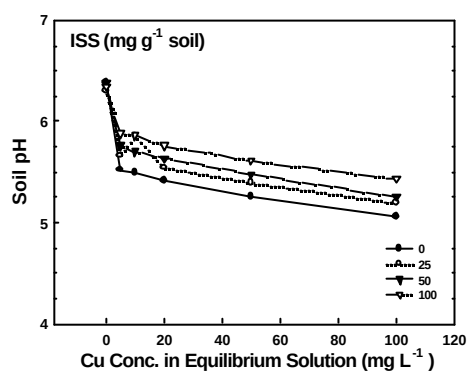


Fig. 4. Effects of various rates of incinerated sludge (ISS) additions on soil pH following equilibration with copper solution.

Speciation of Mercury in Surface and Deep Sea Waters in the Mediterranean Sea

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INTRODUCTION

An interesting feature of mercury biogeochemistry in the Mediterranean is that several fish species from the Mediterranean show higher concentrations of Hg in their tissues as compared to the same fish species in the Atlantic ocean although the concentrations of mercury in the open sea waters in both oceans are similar (Cossa et al. 1997; Mason and Sullivan, 1999; Mason et al., 1998; 2001). Although elevated Hg levels have been noted in environmental matrices from the Mediterranean regions adjacent to known mercury anomalies, the data do not clearly indicate that the effects of these anomalies have been transmitted to open waters or to lower trophic level species living in these waters. Despite these facts very little is known of the mercury distribution in the Mediterranean basin. The distribution and cycling of mercury and a mass balance was calculated for the western Mediterranean (Cossa et al. 1997). The present study was performed to improve our knowledge of and provide missing data on the mercury distribution in Mediterranean waters that have not yet been studied, including the eastern and part of the western Mediterranean.

METHODS

Sampling locations are presented in Figure 1. Samples in the Gulf of Trieste were collected several times in the period from 1995 to 1999 and the results are presented elsewhere (Horvat et al., 1999, Faganeli et al., 2001). Samples in the Eastern Adriatic Sea were collected during a regular oceanographic sampling campaign by the research vessel Bios operated by the Institute of Oceanography and Fisheries, Split, Croatia. The sampling period was between 29. September to 15 October, 1998. The route of research vessel Urania during the MED-OCEANOR campaign took place during a period between 14. July to 9. August, 2000. Ultraclean sampling protocols were employed. Detailed description of analytical methods is presented elsewhere (Horvat et al., 2003).

RESULTS AND DISCUSSION

A summary of the data obtained for mercury analysis and speciation (reactive Hg, total Hg and monomethylmercury (MeHg) in filtered and nonfiltered sea water samples, dissolved gaseous mercury (DGM) and dimethylmercury (DMM) in open and coastal waters of the Mediterranean Sea are presented in Table 1. The results are compared with those obtained in contaminated coastal environment of the Adriatic (The Gulf of Trieste and Kaštela Bay) and non-contaminated coastal waters of the eastern Adriatic coast obtained in 1998. Total mercury concentrations in surface ocean waters is relatively low with an average of 0.81 pM (0.49 – 1.91 pM). Reactive Hg represents a substantial part of Hg with an average of 57% of total Hg (15 – 97%). Most mercury in the open ocean waters was present in the dissolved form (32 – 95%, av. 70%), which is mainly due to the low abundance of particulate matter well known for the Mediterranean open ocean waters. In average the percentage of Hg as MeHg was about 20%, of which about 66% was present in the dissolved form. The percentage of DGM in the surface ocean waters represents about 9% of total Hg (2.5 to 24.5%) and may originate from photochemical, biologically mediated mechanisms or diffusion from deeper layer either due to biological and/or tectonic activity, which is typical for the Mediterranean region. The presence of DMM was only confirmed in waters below 20 m (up to 12

fM), while in the surface waters DMM was below the limit of detection ($< 0.1\text{fM}$). Concentrations of Hg in Eastern and Western part are comparable, except for DGM which shows significantly higher concentrations in Eastern part (mean value: 0.22 pM) as compared to the western Mediterranean (mean value: 0.09 pM). Distribution of Hg species with depth were only done at two stations, indicating variability comparing the eastern end and the western leg of the Urania cruise. Depth profile pattern confirm the importance of dynamic processes at the surface layers, while deep water continuously supply methylated Hg species and it's availability to marine life, as well in supply of DGM to the surface layer.

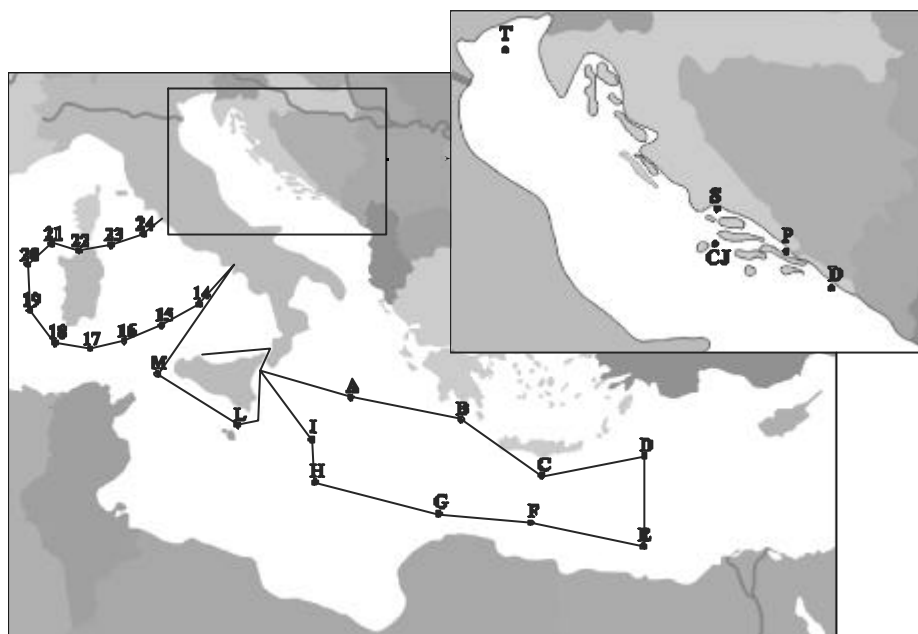


Fig. 1. Sampling locations

CONCLUSIONS

The data obtained in this study opened a number of questions that will be further addressed in a project approved and financed by the European Commission (MERCYMS – An Integrated Approach to assess the Mercury cycling in the Mediterranean basin) for a period between late 2002 and 2005. The overall aim of this study will be to develop an integrated modeling system in order to assess the relative contribution of different processes involved in the cycling of mercury in the Mediterranean region. This study provides basis for an improved and elaborated methodological scheme for mercury speciation and analysis in marine waters.

ACKNOWLEDGEMENT

The work conducted was financed by the Slovenian Ministry of Education, Science and Sport through a programme “Biological and Geochemical Cycles” P 531 and a research project Z2-3502-0106 and the EC funding through a project MERCYMS, EVK3-2001-00141.

Table 3. Comparison of the results for mercury speciation in surface ocean waters. All results are expressed in pM concentrations of Hg.

Study area/reference	Total Hg	Reactive Hg	DGM (Hg ⁰)	MMHg (nF)	MMHg _{diss}
URANIA 2000 Surface water (this work)	1.46 ± 0.41 (0.81-2.33)	0.81 ± 0.32 (0.45 – 1.91)	0.15 ± 0.12 (0.020 – 0.518)	0.28 ± 0.05 (0.19-0.39)	0.19 ± 0.03 (0.15 – 0.26)
Kaštela bay	0.17-3.25	0.17-7.70	<0.07-2.70	0.07-0.22	0.014-0.103
The Gulf of Trieste (Horvat et al. 1999. Faganelli et al 2001)	9.30±8.45 (1.4-25.5)	29.5±23.5 (2.55-63.0)	10.4±8.3 (0.9-24.5)	0.22±0.09 (0.12-0.43)	0.15 ±0.13 (0.03-0.41)
Western Mediterranean (Cossa et al. 1997)	± 1.25 (0.8 – 6.4)	0.38 (0.02 – 0.97)	<LOD – 0.39	<0.15	n.d.
North Atlantic (Mason et al.1998)	2.4 ± 1.6	0.80 ± 0.44	0.48± 0.31	1.04 ± 1.08	n.d.
South and Equatorial Atlantic (Mason and Sullivan 1999)	2.9 ± 1.2	1.7 ± 1.2	1.2 ±0.8	<0.05	n.d.
North Atlantic and Antartic bootom water Mediterranean outflow waters (Dalziel, 1995)	n.d.	0.48 - 1.34 0.67 - 1.25 0.86 - 1.06	n.d.	n.d.	n.d.
Equatorial Pacific (Mason and Fitzgerald, 1990, 1993)	n.d.	0.47-1.85	0.04-0.325	<LOD-0.28	n.d.

nF .. non filtered; n.d. not detected

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TRACE ELEMENTS AS TRACES OF HUMAN IMPACT IN SOILS OF ANCIENT SETTLEMENTS

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INTRODUCTION

Anthropogenically transformed soils of ancient settlements are a very special subject in soil science. Being formed on the same lithological substrate as a background undisturbed soil, they had experienced a high anthropogenic pressure in the past and inherited a big amount of biofile elements, major of which are C, P and trace elements (Limbrej, 1975; Griffit, 1981). In terms of archaeology these organically rich horizons are called cultural layers (CL). Soil metal assays in CL are treated as a tool to determine site boundaries and to identify the impact of human activity on soils and landscapes (Haslam, Tibbett, 2001). Being a component of bronze and brass archaeological artefacts, Cu and Zn are dominating trace elements in soils of archaeological sites, but the distribution of their chemical forms after hundreds of years passed after the cessation of direct human impact are not conclusively studied. The objective of this study was to investigate the distribution of Cu and Zn forms in landscapes of archaeological settlements and background soils using fractionation techniques.

MATERIALS AND METHODS

Soils were sampled on the territory of archaeological excavations of two ancient Russian settlements of IX-XII centuries called "Shestovitsa" (SAC) and Gnezdovo (GAC). In SAC we examined CL-samples from the sites, documented by archaeological findings as corresponding to different types of land use (dwelling house, tar-work, suburbs of the settlement) and located both on the river terrace and floodplain territories with Podzol and Fluvisol background soils correspondingly. Samples from GAC included CL from anthropogenically transformed soil and a background soil on the river terrace. The fractionation procedure included parallel extraction by 1 N HNO₃ (acid-soluble forms), NH₄-acetate buffer at pH 4.8 (AAB; available forms) and 1% solution of EDTA in AAB (available and organic bound forms). The concentrations of Cu and Zn in each fraction were determined by AAS.

RESULTS AND DISCUSSIONS

Profile distributions of Cu and Zn acid-soluble forms in background soils and in a number of CLs of SAC are presented at Fig 1. The same pattern was also observed for the landscapes of GAC. Background Fluvisol, situated in the accumulative part of the landscape originally contained more Cu and Zn, then Podzol, but currently all the CLs are in 5-10 times enriched in Cu and in 6-13 times in Zn, especially those ones from anthropogenically transformed soil located under the ancient dwelling house, which had experienced the highest peak of human activity. The pattern of acid-soluble forms distribution is different from those typical for contaminated soils with highest accumulation of metals not in the upper, but in the medium part of p profile.

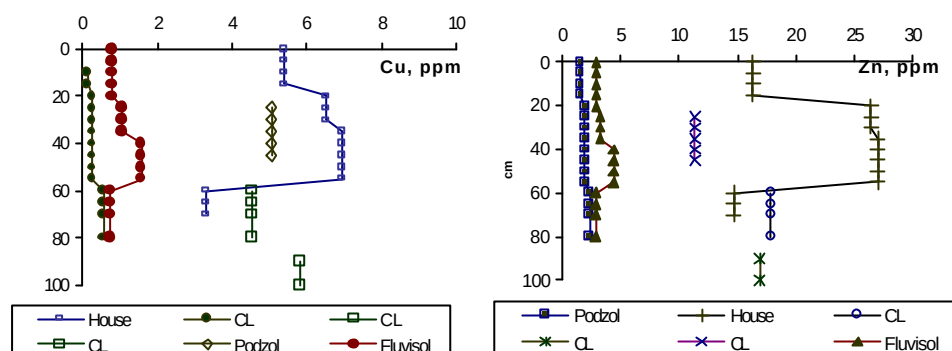


Figure 1. Profile distribution of Cu and Zn acid-soluble forms in some CLs and background soils of Shestovitsa archaeological complex (SAC).

Available forms of Zn and Cu demonstrate a similar tendency of distribution (Fig.2). Organic bound forms (treated by a difference between metal content in AAB+EDTA and AAB extracts) at average contributed about 20% for Cu, and for Zn varied between 6-22%. No expected correlation between total C content and organically bound forms of metals have been proved. This fact indirectly shows that in spite of a high accumulation of organic matter in CLs, only a small part of metals interacts resulting in the formation of organic forms. Probably it was either due to a special composition of OM in CL or to the chemical forms of input of Zn and Cu in archaeological sites which are different from ones typical in a case of regular soil contamination by metals.

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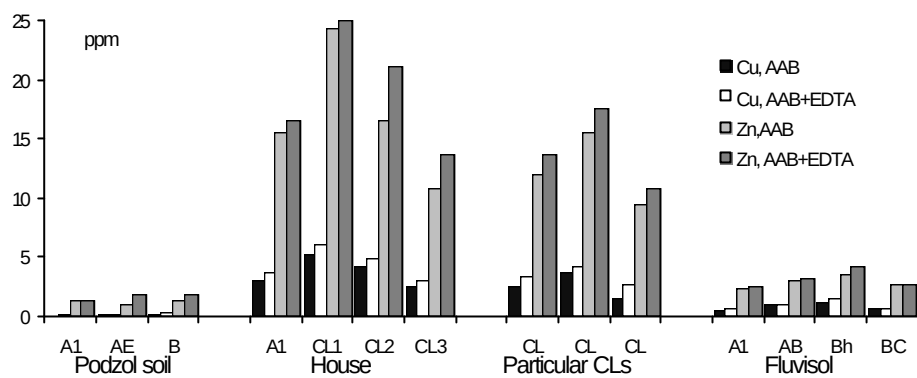


Fig.2. Content of available forms of Zn and Cu in soils and CLs in SAC

Study of the Heavy and Trace Metal Contents in Soils of Valladolid (Spain) and their Relationship with Clay Composition

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INTRODUCTION

Heavy and trace metals are a very hazardous group of pollutants because of their toxic and accumulative characteristics: they are non-biodegradable and undergo global eco-biological cycles, so their circulation amongst the different environmental compartments is a main concern. Because the soil can act as a temporary reservoir from which the trace and heavy elements can be released back to the environment, the mechanisms ruling their retention/releasing phenomena must be investigated. Amongst the parameters universally acknowledged as related to the mobility of trace and heavy metals in soils, the clay composition is one of the most relevant.

This communication presents the results obtained by our research team about this relationship in soils of Valladolid (Spain).

METHODS

The sampled zone is located around the town of Medina del Campo in Valladolid. Thirty two sampling points representative from the five main physiographic units of the area: *open country, bottom of valley, terrace, semi-endorreic* and *sandy* zones were chosen (A and B profiles) and the corresponding air-dried soil samples were characterized by means of standard edaphologic parameters (pH, conductivity, organic matter, carbonate, texture and exchangeable cations).

The heavy and trace metals studied were chosen in accordance with the priorities of the Junta de Castilla y León (autonomous government competent in environmental topics): Sb, As, Cd, Cr, Co, Cu, Pb, Ni, Sn and Zn. These elements were determined by using the EPA 3051 norm (microwave assisted digestion with HNO₃ acid) and standard ICP spectrometric methods (EPA 6010 and 7131 norms). 'Acid extractable' metals were determined as opposed to 'total' metals (available after total digestion of the soil) because that operationally defined category is more suitable for studies of environmental mobility.

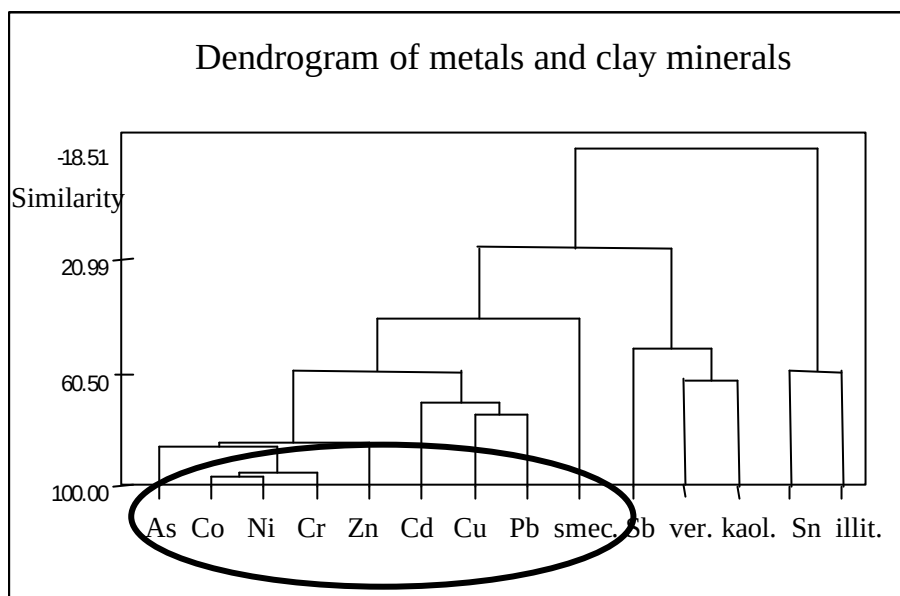
The semi-quantitative total crystalline soil composition was also determined by X-Ray Diffraction Spectroscopy (XRD), giving percentages of quartz, clay minerals, albite, orthose and calcite. This same technique was later used to elucidate the composition of the clay minerals fraction, extracted by the Boyoucos method, after the preparation of the corresponding Mg-oriented aggregates. When necessary, a post-treatment (by calefaction or equilibration with ethylene glycol) of the aggregates was carried out and new XRD diffractograms scanned to discriminate between the different clay minerals. In that way, smectite, illite, chlorite, vermiculite and kaolinite could be identified.

RESULTS AND DISCUSSION

The experimental data were studied by statistical uni and multivariate methods. From the univariate methods we have found statistically significant differences (at a p=0.05 confidence level) between the A and B profiles of the soils for: Organic matter (A>B), extractable sodium (B>A) and potassium (A>B), nickel (B>A), zinc (B>A) and quartz (A>B). Non-significant differences were found for the rest of parameters.

Multivariate methods allow for a best understanding of the relationship between the experimental variables. Principal Components Analysis (PCA) and Cluster Analysis (CA) have been used. PCA is a pre-visualization technique that concentrates the information of the data table into a set of abstract variables (factors or components) whose number is lesser than the original. These factors (scores) are lineal combinations of the original variables and the lineal coefficients (named loadings) indicate the way the original variables participate in the new ones. In our case, we have found seven significant factors explaining the 79.6 % of the information. A graphical plot of the factors with more information allows to visualize the differences between A and B profiles, but not between physiographic units. Varimax rotation has not cleared-up this picture.

On the contrary, CA techniques use all available information of the data table depicting it as a dendrogram, in which we can observe aggregations (clusters) between variables or samples. In our case, the best results were found when using agglomerative clustering by the Ward method. As can be seen in the enclosed figure, there is a strong association of heavy and trace metals (exception made from Sb and Sn) with smectite. As this clay possesses a great cationic exchange capacity, this association seems to confirm the importance given to the presence of clay minerals as a key parameter to explain, and to predict, the mobility of heavy and trace metals in soils.



ACKNOWLEDGEMENTS

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Atmospheric deposition of trace metals onto arable land in Austria

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INTRODUCTION

Atmospheric deposition of heavy metals may significantly contribute to positive soil balances and therefore lead to the accumulation of these elements in soils or to leaching into the ground water. Plant uptake of toxic heavy metals from the soil as well as airborne contamination of foodstuffs (with e.g. Pb and Cd) endanger the quality of these products and thus animal and human health.

Different management practices, as the use of fertiliser, manure, pesticides, potential sewage sludge and bio waste, may cause heavy metal input into soils, too. However, these can be better calculated quantitatively. Apart from these well-investigated sources of contamination diffuse atmospheric deposition occurs regionally and locally with considerable geographical variation. Therefore we considered it important to measure the amounts of heavy metals deposited onto agricultural land in Austria over a 36 month period (1999-2001).

SITES AND METHODS

A network of 10 sites for monitoring atmospheric deposition was installed, situated at experimental sites of Austrian agricultural research institutes, covering the most important areas of production.

The deposition values were determined with the bulk method "Bergerhoff" and according to Guideline VDI 2119 (determination of dust precipitation with collection pots made of glass). Sampling was done monthly (30 ± 2 days). Trace metals were analysed according to the Guidelines VDI 2267 and 2268. Cr, Ni, Cu, Zn, Pb and Cd were measured with ICP-MS.

RESULTS AND DISCUSSION

The medians of the investigated heavy metals deposited onto agricultural areas decreased in the order $Zn > Cu > Ni > Pb > Cr > Cd$ (see Figure 1). The 25%-75% percentiles of Zn, Cu and Ni showed a greater range either due to single high deposition events and/or to elevated depositions during a period of several months.

Compared to German investigations (Schulte et al., 1996 and Schulte and Gehrmann, 1996) median Cr and Ni depositions were up to 4 times higher in Austria, although they ranged in the same order of magnitude as reported for European low mountain range (Matschullat et al. 1997). The latter is also true for Cu and Zn. In Austria a tendency of higher Zn and Cu deposition on north-eastern sites in contrary to the most southern location was noticeable.

The Cd deposition was very similar to the values of the above-mentioned German investigations, Pb showed slightly lower values, however.

In the Austrian Ambient Air Quality Law thresholds for Cd ($2 \mu g m^{-2} d^{-1}$) and Pb ($100 \mu g m^{-2} d^{-1}$) have existed since 1997. The Cd depositions exceeded the threshold in 7.5% of the analysed cases. A tendency for higher atmospheric Cd deposition from November till February could be observed. This may be due to elevated consumption of fossil fuels. Annual averages were far below the threshold. This applies also to the threshold for lead, which was exceeded only two times in 2000 and 2001 respectively (1.1 %).

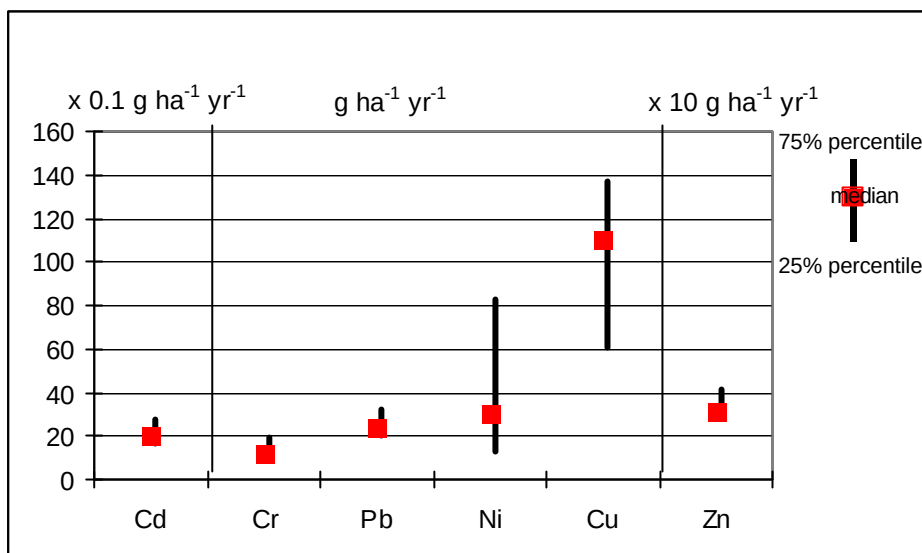


Fig. 1: Atmospheric deposition of Cd, Cr, Pb, Ni, Cu and Zn (25% and 75%-percentile, median) for 10 sites and over 3 years

Further statistical parameters as well as the influence of climatic parameters (precipitation, wind) on the deposition of heavy metals will have to be discussed.

CONCLUSIONS

Atmospheric deposition of selected trace metals in Austria are in the same range of those reported for the moderate climate area of Central Europe. Temporarily high depositions (Ni, Cu, Zn and Pb) occurred at the investigated site nearest to the city of Vienna, which may be due to emissions of this high density area in general and a landfill site close by in particular. However, similarly high immissions could be watched at more rural sites, too, which may be due to frontier crossing of heavy metals. Therefore it would be reasonable to extend the obligatory measurements for populous areas to intensively used agricultural areas, too.

ACKNOWLEDGEMENT

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Environmental contamination and ecotoxicity

Scientific Program 2

Poster

Geochemical Barriers and Distribution of Trace Elements in Soils in Different Landscapes of Tianshan Mountains, Xinjiang, China

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Geochemical barrier (GB) defined by Perel'man, a Russia geochemist, in 1965 referred the local area of the epigenetic zone where the conditions of elemental migration were distinctly alternated, resulting in a remarkable accumulation of element concentration. Since the concept was put forward, it had been concerned by many geochemists who studied the conditions of migration and accumulation of the elements related to metallogenesis. However, few soil scientists used the concept to study spatial differentiation of the elements in soils related to agricultural production and human health. Since 1980s, distribution of the elements in soils among climate zones of China has been clearly recognized. But, the information is limited to knowing the distribution of elements in soils of local area that is great important for environmental assessment and agricultural production. Therefore, more and more attention is being paid to study the migration and accumulation of elements in the soils of various local landscapes in term of the concept of GBs.

GBs in epigenetic zone can be divided into three types in terms of their soil-forming conditions, that is, mechanic GB, physio-chemical GB, and biological GB. Each type can be divided into different sub-types. Tianshan mountains are located in inland of China where the climate is arid and elevation of topography has very large difference (5000-6000m), resulting in very complicated micro-climate condition, soil types, and vegetation types. All types of GBs appear in the area. The objectives of this paper are (i) to discuss the formation and distribution of various GBs, (ii) to study distribution of trace elements and their availabilities within a GB. The results will be very benefit for sustainable farming and ecologically environmental protection.

We chose 4 sections within the landscapes as the targets studied, that is, warm temperate very arid desert landscape, warm temperate arid desert landscape, temperate arid desert landscape, and alpine-subalpine meadow and grassland landscape. Soil samples of each horizon were taken from 4-6 soil profiles along each section. Soil physical and chemical properties such as pH, organic matter, calcium carbonate (CaCO_3), gypsum, cation exchangeable content (CEC), particle composition, and compositions of water-soluble salts were determined. Total B, Ba, Co, Cr, Cu, F, Fe, K, Li, Mn, Sc, Sr, Ti, V, and Zn, and water-soluble B and F, DTPA-Cu, Fe, and Zn in soils were analyzed as well.

Mechanic GB is one of the main types in the area. It is characterized by the accumulation of clay in parent materials and can be divided into two sub-types in terms of transportation media while formation of parent materials. The first one was formed by change of water-flow velocity, which is distributed in exit of river or depress along piedmont of Tianshan Mountains. The second one is formed by a decrease of airflow velocity due to resistance of the maintains, which is distributed along margins of the Tianshan Mountains.

Transpirational GB, a physio-chemical GB, is another main type in this area. Various saline materials are accumulated in the GB. This GB can be further classified into three sub-types in terms of the composition of typical saline compounds, that is, CaCO_3 -, gypsum-, and soluble salt-GB. They are distributed from mountain to basin on the side of the mountain as a sequence of CaCO_3 -GB, gypsum-GB, and soluble salt-GB corresponding to the condition of microclimate from moisture to dry.

Biological GB is dominantly distributed in alpine-subalpine landscapes of the mountain where the climate is wet. It is characterized by the accumulation of organic matter in topsoil.

The trace elements can be divided into four groups based on the cluster analysis of concentrations of trace elements in the soils of arid desert landscapes: (I). Co, Cr, Cu, Fe, Mn, Sc, Ti, and V are

included; (II). Only B; (III). Ba, F, K, Li, and Zn are included; (IV). Only Sr. The elements within a group would have the same behavior in this region when they were migrating.

The relationships between the concentrations of elements and a few soil properties in arid desert landscapes were conducted. The elements of group I were usually hard to be accumulated due to their inactive and diluted in the transpirational GB due to the accumulation of various salts. B in the group II was strongly accumulated in gypsum, and soluble salt-GB. This is consistent to the condition of very dry climate. The accumulation of the elements in group III was related to the accumulation of clay in the mechanic GB and organic matter in surface soil, whereas the dilution of them has taken place in the soluble salt-GB. The accumulation of the element, Sr, in group IV was found in the gypsum GB.

The relationships between the concentrations of elements and a few soil properties in alpine-subalpine landscapes were also conducted. The accumulation of organic matter (C, N) in the topsoil was not resulted in the obvious accumulation of the elements we studied.

In the arid desert landscapes, the availabilities of Cu, Fe, Zn and B are only related to their total contents in the soils. The concentration of in soils besides total F also affects F availability, resulting in an increase of available F with increasing HCO_3^- . In biological GB of alpine-subalpine landscapes, the availabilities of these elements are mainly affected by accumulation of organic matter. An increase of organic matter in soils significantly increased available Cu, Fe, Zn, and B and decreased available F.

Porphyrins are potential early warning biomarkers of chronic arsenic exposure

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Arsenic is toxic and carcinogenic to both animals and humans (IPCS 2001; Ng *et al.*, 1999). Porphyrins are by-products of haem biosynthesis. The haem metabolism pathway is known to be highly susceptible to alterations induced by drugs and environmental chemicals and therefore offers a useful approach for monitoring exposure to arsenic prior to the onset of organ damage or clinical manifestations of toxicity. We aimed to investigate initially how various arsenicals impact on the excretion profile of porphyrins in rats followed by a field validation study on humans chronically exposed to arsenic in Guizhou Province of PR China.

A sensitive method using HPLC with fluorescence detection has been established for the measurement of porphyrins in biological materials (Ng *et al.*, 2002a). The assay recoveries ranged from 98.3±2.7% to 111.1±7.4% for various porphyrins in the urine. This method was employed to investigate the altered porphyrin profiles in rats after a single dose of various arsenicals including soluble sodium arsenate and sodium arsenite, and the relatively insoluble calcium arsenite and calcium arsenate. In rats administered 5 mg As(III)/kg body weight, protoporphyrin IX concentration elevated to 123% of the control values in rats, 24 h after the treatment. Higher increases were recorded in the urinary protoporphyrin IX (253% at 24 h; 397% on day 2), uroporphyrin (121% at 24 h; 208% on day 2) and coproporphyrin III (391% at 24 h; 304% on day 2), while there was no significant increase (109% on day 3) observed in the urinary coproporphyrin I excretion. In rats administered 5 mg As(V)/kg, urinary excretion of protoporphyrin IX, uroporphyrin, coproporphyrin III and coproporphyrin I elevated to the maximum levels by 48 h with the corresponding percentage values compared to the control being 177%, 158%, 224% and 143% respectively. Our results suggest that the metabolism of haem in rats can be altered by various arsenic species at sub-lethal exposure levels. In another study (Ng *et al.*, 2002b), harderoporphyrin concentrations of the harderian glands of mice chronically exposed to 500 µg As/L of sodium arsenate in drinking water for over two years were significantly elevated compared to those of the control mice (Very high concentrations harderoporphyrin are usually found only in the harderian glands (HG). HG is not present in humans). The concept of porphyrin profile as early warning bio-indicator of arsenic exposure was also investigated and validated in a human study.

Guizhou Province of PR China has a rich resource of coal. Unfortunately, the coal is often found to contain naturally high levels of arsenic in some areas. Coal is widely used by local villagers for cooking and drying crops over unventilated open stoves, and for indoor heating in winter and the cooler months. Deposition of arsenic on food items during burning and subsequent ingestion of the contaminated food is thought to be the major pathway of exposure. This has lead to serious arsenicosis in arsenic-exposed individuals. We collected urine samples from 113 people who live in Xing Ren of Guizhou Province, a coal-borne arsenicosis endemic area in southwest of PR China and from 30 people who live in Xing Yi (about 80km southwest of Xing Ren) where arsenicosis is not prevalent and analyzed for urinary porphyrin profiles and total arsenic as an estimation of internal dose (Table 1).

Table 1: Total coproporphyrin, total porphyrin (pmol/mg creatinine, mean±SEM) and total arsenic (µg/g creatinine, mean±SEM) in the urine samples collected from arsenic exposed and control groups of residents by gender and age groups in Guizhou PR China

Gender Treatment	Age group	n	Total arsenic (µg/g creatinine)	Total Coproporphyrin (pmol/mg creatinine)	Total Porphyrin (pmol/mg creatinine)
Male Exposed	<20y	29	212.52±43.85**	84.26±33.17	95.03±34.25
	20-40y	13	128.15±44.04	26.13±4.57	33.03±4.90
	>40y	13	74.18±14.16*	29.64±6.68	40.92±8.54
	Overall	55	160.47±26.68	57.61±17.86	67.58±18.48
	<20y	9	78.35±14.70	30.25±14.90	37.55±16.66
Male Control	20-40y	3	42.16±7.59	20.85±8.69	27.32±10.84
	>40y	4	37.09±7.53	34.61±10.22	41.25±11.73
	Overall	16	61.25±9.71	29.57±8.67	36.56±9.72
	<20y	31	236.08±43.39**	48.32±8.29*	60.66±8.60*
Female Exposed	20-40y	13	212.13±54.40	43.79±11.31	55.55±12.14
	>40y	14	208.16±102.23	46.30±9.89*	57.99±10.59*
	Overall	58	223.31±35.75**	46.82±5.55**	58.87±5.83**
	<20y	8	79.88±5.04	29.16±4.11	35.99±5.02
Female Control	20-40y	2	27.51	23.60±10.55	31.01±13.25
	>40y	4	49.21±2.25	11.33±3.98	16.38±4.07
	Overall	14	66.41±6.01	23.27±3.48	29.67±4.05

* significant difference between the exposed group and control group ($P < 0.05$); ** ($P < 0.01$).

Our study indicated that coproporphyrin and uroporphyrin concentrations (uroporphyrin data are not shown in Table 1) were higher in the arsenic exposed group compared to those in the control group. The correlation between the urinary arsenic levels and porphyrin concentrations demonstrated the effect of arsenic on the haem metabolism process resulting in the increased porphyrin excretion. Importantly, urinary porphyrins showed sensitive alteration in the excretion profile of the younger age (<20y) arsenic exposed group, suggesting that porphyrins could be used as early warning biomarkers for chronic exposure to arsenic in humans. This is a useful adjunct diagnostic tool for chronic arsenic poisoning prior to the clinical manifestation of arsenic-induced skin lesions. Higher increases of urinary arsenic and porphyrin profiles in women, children and older age people who spend much of their time indoor suggest that they might be at a higher risk. Intervention strategy should include the use of properly ventilated stoves and avoid the use of contaminated coal.

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EDXRF multi-elemental mapping of heavy metal containing pesticide residues on former horticultural soil

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INTRODUCTION

During the early 1900's considerable amount of heavy metal containing pesticides were used worldwide for pest control in horticulture. This has been leading to significant accumulations of heavy metals in the top soil of former horticultural soil. However only very few studies relates soil heavy metal levels to the former application of heavy metal containing pesticides. Hence the distribution of major-, minor-, and trace element were systematically investigated by use of Energy dispersive X-ray fluorescence spectrometry (EDXRF) on a former horticultural soil in Denmark suspected of previous use of heavy metal containing pesticides.

MATERIALS AND METHODS

The soil was sampled in a 10 m x 10 m grid-like pattern from the top 20 cm's of the soil surface on a 1 ha area at the former horticulture with a total of 100 soil samples. The soil content of most elements above atomic number 11 was determined by EDXRF. Based on the surface distribution of the soil elements 3 profiles were made. The samples were wet grounded to a particle size less than 2 µm and applied to a polypropylene film with a cationic detergent to obtain a dried up homogeneous thin sample layer (2.5 mg/cm²) (Laursen et al., 2001). To investigate the distribution and mobility of the elements in the soil profiles the soil solution was simulated by use of standardised aqueous extraction of the soil samples. The elemental composition of the soil extract was determined by Total reflection X-ray fluorescence spectrometry (TXRF).

RESULTS AND DISCUSSION

Chemometric analysis of element concentrations, pH, CEC, texture and organic matter revealed that all elements except As, Cu, Pb and Zn were found in almost constant concentration levels all over the investigated site. The concentrations of As, Cu, Pb and Zn follows the same spatial patterns throughout the investigated site, see Figure 1. The samples with significant accumulations of As have also have high amounts of Zn, Cu and Pb which indicate that heavy metal containing pesticides have been applied to the soil surface. The heavy metal containing pesticides applied were found to be lead arsenate and Bordeaux liquid (CuSO₄) while Zn has been used to minimize the leaf damaging effect during the treatments. The Pb/As mass relationship in the soil indicate that PbHAsO₃ has been the preferential lead arsenate for pest management at the former horticulture soil. Furthermore it was found that arsenic annually has been applied to the soil in the former greenhouses in doses up to 4 kg/ha while Pb annually has been applied in doses up to 12 kg/ha. The total concentrations of As and Pb exceeded the Danish human health criteria for soils of 20 mg As/kg and 40 mg Pb/kg in an area of former greenhouses. The enrichment of Zn, Cu, As and Pb are highest in the top 20 cm of the soil and no anthropogenic enrichment of these elements occur below a depth of 50 cm indicating that the heavy metals are rather immobile in this soil. Based on the TXRF measurements of soil extracts from the soil profiles only 0.06% of the total soil As is available in the simulated soil solution, while Pb and Cu are represented in even lower amounts.

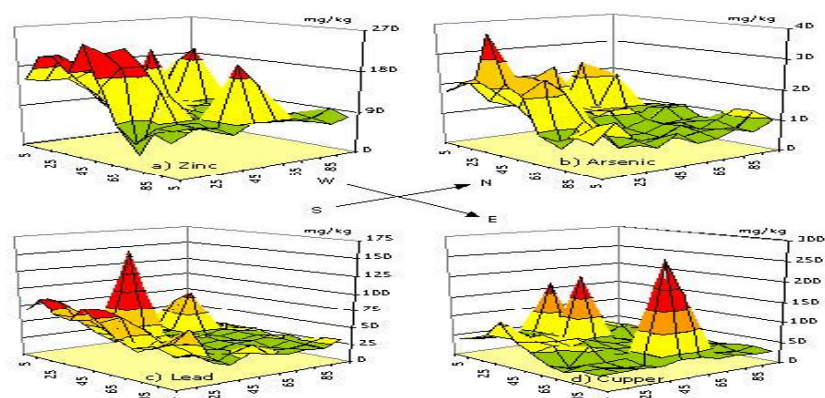


Figure 1. Surface distribution of a) Zinc, b) Arsenic, c) Lead, and d) Copper at the investigated area in Vestskoven, Denmark. The colours of the figures indicate the state of contamination at the study site, hence $\downarrow$ (green) equals the local background level. Concentration enlargement in the interval 0 – 100% compared to the local background level are shown with $\downarrow$ (yellow), in the interval 100 – 200% with $\downarrow$ (orange), and a concentration enlargement of >200% indicated with $\downarrow$ (red).

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Mercury uptake for plant species from an Almadén test plot

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INTRODUCTION

Environmental restoration of an affected area implies the selection, optimisation and application of the most appropriate techniques focused on the recovery of contaminated areas. A set of these methodologies considers the use of plants due to the low environmental impact and their probed efficiency. In this framework, it is necessary to improve the knowledge on the behaviour of the contaminant in the environment and, in this specific case, to determine the mercury absorption capacity of the different species. Field and laboratory studies have been carried out to determine the properties and parameters that control the behaviour of mercury in the soil, and to quantify the mercury accumulation in different plant species of the flora. The result of this experimental work is selecting the plant species with the highest mercury absorption capacity for future implementation in phytoremediation techniques.

DESCRIPTION OF THE STUDY AREA

The Almadén area constitutes the largest and most unusual concentration of mercury in the world. It has been estimated that before mining began, the area contained about a third of the earth's total known mercury resources. In spite of the low solubility of cinnabar, mercury is mobilised and absorbed by plants whether it is derived from natural or anthropogenic sources. The experimental field is located near Almadén, in the proximity of the Almadenejos village (SW of Spain). The lithology is comprised of detritic rocks from the Palaeozoic Era and the climate of this region is semiarid. The soil of the experimental plot is classified as a Alfisol Xeralf Haploxeralf (USDA-Soil Taxonomy), with a loamy to clay loam texture, a near neutral pH and 2% OM content in the upper horizon.

From the botanical point of view, the Almadén area is classed as Mediterranean Region, (Luso-Extremadurensis Subprovince). The flora, in the Mesomediterranean belt, is characterised by dominant sclerophyllous formations (Mediterranean oaks and cork trees) on siliceous bedrock. In the study area, the climax community is defined as *Pyro bourgaeanae-Quercetum rotundifoliae*. The habitat degradation has led to the presence of an open scrubland with *Cistus monspeliensis*, *Crataegus monogyna*, and grassland formation of the alliance *Taeniathero-Aegilopion geniculatae*, that in humid soil conditions, includes the genus *Aegilops* and *Phalaris*. Floristic composition shows a main presence of hemicryptophytes and camephytes, typical of the Mediterranean environment with a certain Atlantic influence. Furthermore, there is a significant presence of several species of the families *Asteraceae*, *Fabaceae* and *Poaceae*.

MATERIAL AND METHODS

The vegetation and soil sampling was carried out in June 2002, within an extensive experimental plot. For each one of the selected species, a minimum of twelve plants was collected. The stem and leaves were selected as the samples to measure the mercury content. These samples were placed in individual beakers and rinsed several times with distilled water to remove external contamination, and then oven dried at 35°C for 24 hours. Soil samples were sieved, and the fine earth fraction (<2 mm) was oven dried and ground. Mercury content in the plant and the soil samples were measured using an Advanced Mercury Analyser (AMA-254). Quality control of mercury measurements was achieved by analysing two standard reference materials: CRM-281 (rye-grass, 0.0205±0.0019 ppm of Hg) and

BCR-62 (olive leaves, 0.28 ± 0.02 ppm of Hg), being the average value from ten measurements of 0.0210 ± 0.0003 ppm and 0.290 ± 0.002 ppm, respectively.

RESULTS AND DISCUSSION

The total mercury concentration in the soil ranges from 20 to 30 ppm in the upper organic horizon, and about 15 ppm in the subsurface clayey horizon. The predominant mercury forms are those associated to the humic acids and the sulforganic phase (70-80%). The soluble and the exchangeable phases of mercury, that indicate the more easily available mercury from the soil water, only represent 3% of the mercury forms. These results indicate that only low mercury content is bioavailable for plants.

The mercury content in the different plant species has been obtained as shown in the following table.

Plant	Hg content in aerial part (ppm)
<i>Filago lutescens</i>	3.117 ± 0.017
<i>Spergularia rubra</i>	2.159 ± 0.142
<i>Cistus monspeliensis</i>	2.073 ± 0.207
<i>Convolvulus arvensis</i>	1.250 ± 0.108
<i>Chrozophora tinctoria</i>	1.107 ± 0.095
<i>Asparagus acutifolius</i>	0.899 ± 0.070
<i>Cynara humilis</i>	0.880 ± 0.062
<i>Bromus sterilis</i>	0.646 ± 0.062
<i>Reseda luteola</i>	0.637 ± 0.062
<i>Diplotaxis virgata</i>	0.434 ± 0.030
<i>Eruca sativa</i>	0.390 ± 0.029
<i>Aegilops triuncialis</i>	0.160 ± 0.123
<i>Aegilops geniculata</i>	0.131 ± 0.011

The concentration in the plants range from 0.131 to 3.117 ppm where the different affinities can be appreciated. The plants that belong to the same botanical family (*Poaceae* and *Cruciferae*) show a similar behaviour of mercury content and fall within the minimum absorption values. These results enable a selection of the plants important for the absorption of mercury available in the study area, although in the studied species there is no hyper-accumulator of mercury. There is no doubt that the plant species is an important factor when applying phytoremediation techniques. A next step in the project is to consider a more extensive study area for sampling where the concentration of mercury for other plant species are included and further values for the same plant species can be compared between the different sampling points.

Mercury content in water bodies and *Eichhornia crassipes* Mart. Solms plants at Amritsar, India.

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High concentration of heavy metals adversely affects the behavioral, structural and functional activities of living organisms. On account of its harmful effects on living organisms, mercury has received a great deal of attention in environmental studies (Miettinen, 1994). In aquatic habitats, methylation of mercury causes severe damage to wetland flora and fauna (Guimaraes *et al.*, 2000). Keeping this in view, a study was carried out to determine the concentration of mercury in different aquatic sites at Amritsar (31°37'N, 74°52'E). *Eichhornia crassipes* (Mart. Solms) is reported to absorb and concentrate heavy metals such as cadmium, lead, nickel and mercury (Lenka *et al.*, 1992; Riddle *et al.*, 2002). Hence, *E. crassipes* plants growing in the same aquatic habitats were also analyzed for the concentration of mercury. The collected water samples were fine filtered and mercury content was determined using mercury analyzer (ECIL, model MA 5800/E). Roots, petioles and leaf laminae of *E. crassipes* growing in different habitats were digested and analyzed for the content of mercury. The observations (Table 1) indicated that the mercury content of water samples collected from different sites ranged from 0.0032 to 0.0180 mg l⁻¹. The mercury content in different parts of *E. crassipes* ranged as: 0.2125-0.7750 mg kg⁻¹ d.w. for roots, 0.1375-0.6375 mg kg⁻¹ d.w. for leaf laminae and 0.1250-0.6000 mg kg⁻¹ d.w. for petioles.

Table 1: Hg-content in water and plant parts of samples collected from different sites.

S. No.	SITE	Hg-content in water sample (mg l ⁻¹)	Hg-content in plant parts (mg kg ⁻¹) (dry weight)		
			Roots	Petioles	Leaf Laminae
1.	Malha-I	0.0180	0.7750	0.3750	0.4625
2.	Malha-II	0.0082	0.6250	0.2375	0.2500
3.	Malha-III	0.0042	0.6625	0.6000	0.6375
4.	Rajasansi-I	0.0045	0.2625	0.1875	0.2250
5.	Rajasansi-II	0.0047	0.2500	0.1250	0.1375
6.	Kharawad-I	0.0040	0.2500	0.1625	0.2125
7.	Kharawad-II	0.0035	0.2125	0.1375	0.1750
8.	Ghamtala	0.0047	0.2375	0.1500	0.2000
9.	Kot Khalsa	0.0052	0.6000	0.3125	0.4150
10.	FC Road	0.0032	0.3750	0.2125	0.2625
11.	AAF Cantt.	0.0060	0.6000	0.2500	0.4125

The bioconcentration factor (BCF) for different parts of *E. crassipes* ranged as: 43.05-157.73 for roots, 20.83-142.85 for petioles and 25.69-151.78 for leaf laminae (Table 2).

$$BCF = \frac{\text{Mercury concentration in plant part (mg kg}^{-1}\text{)}}{\text{Mercury concentration in pond water (mg l}^{-1}\text{)}}$$

Table 2: Bioconcentration factors for roots, petioles and leaf laminae of *E. crassipes* found in mercury contaminated habitats.

S. No.	SITE	Bioconcentration Factor		
		Roots	Petioles	Leaf Laminae
1.	Malha-I	43.05	20.83	25.69
2.	Malha-II	76.21	28.96	30.48
3.	Malha-III	157.73	142.85	151.78
4.	Rajasansi-I	58.33	41.66	50.00
5.	Rajasansi-II	53.19	26.59	29.25
6.	Kharawad-I	62.50	40.62	53.12
7.	Kharawad-II	60.71	39.28	50.00
8.	Ghamtala	50.53	31.91	42.55
9.	Kot Khalsa	115.38	60.09	79.80
10.	FC Road	117.18	66.40	82.03
11.	AAF Cantt.	100.00	41.66	68.75

The water of the ponds analyzed is being used by cattle for drinking purpose. The maximum allowable concentration of mercury in drinking water as given by Indian Standard Institution is 0.001 ppm, though for cattle it is not defined. The mercury content of water samples is thus higher than the permissible limit for human drinking. Hence, it can harm the cattle drinking that water, as well as humans feeding on the cattle milk. The data of mercury content of *E. crassipes* plants reveal that this plant is a hyperaccumulator of mercury. Moreover, the roots are shown to have higher capacity of accumulating mercury followed by leaf laminae and petioles. This has also been reported in earlier works done by Muramoto and Oki (1983) and Riddle *et. al.* (2002). It is considered that in roots, methylation of mercury occurs in the root-associated solids and thus, appear to be an essential link of the methylmercury cycle in aquatic systems (Guimaraes *et. al.*, 2000).

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The effect of metal pollutants leaching from the road constructed with bottom ash

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INTRODUCTION

Environmental impact assessment of pollutants (heavy metals) leached from the road constructed with bottom ash from municipal solid waste incineration may give relevant information for national regulation in road construction with residues. High concentrations of copper, chromium, zinc, and lead were observed in the leachate collected from the experimental road in Umeå, made within Swedish project BARC (bottom ash in road construction). Therefore, these metals were included in this study.

Toxicity assessment of some metal pollutants and leachate of bottom ash contaminated with copper and chromium was performed by toxicity tests, which are based on the reactions of microorganisms, for example, bacterial growth. Most short term toxicity tests are based on inhibition of a specific enzyme activity in bacteria, fungi, algae and protozoa (Bitton and Koopman, 1992). The microbial toxicity tests are rapid, simple and inexpensive, and only small amount of samples is needed to perform the tests (Blaise, 1991). The low cost of microbial tests has resulted in their ever-increasing use in environmental control (Blaise et al., 1988). Microbial tests can be performed using a pure culture of a well-defined single species or a mixture of microorganisms.

The aim of this work was to study toxic effects of heavy metal pollutants on different bacteria and to determine the suitable toxicity assessment method for metal pollutants leached from the road constructed with bottom ash from a municipal solid waste incinerator.

METHODS

Microbial tests were performed using four different bacteria. The growth of bacteria were investigated and compared by monitoring turbidity in the tubes and cell growth on agar plates in the presence and absence of model compounds, such as copper, chromium, zinc, cobalt, arsenic, nickel, lead and leachate of bottom ash contaminated with copper and chromium. Cell growth on agar plates were used to make sure that the turbidity in tubes was caused by bacterial growth. The agar diffusion method was also used to study the effect of inorganic ions on the bacteria. pH were measured after bioassay in the tubes.

Microorganisms and Growth Conditions

Strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* used in this study were maintained in nutrient broth (Merck). Inocula were grown aerobically overnight in 100 ml flasks in 15-20 ml in nutrient broth at 37°C.

Model Compounds

The inorganic substances tested were heavy metal salts or oxides. Stock solutions were prepared in deionised water in various concentrations according to the metal tested, and kept at 4°C.

Leachate used in this experiment was generated from the municipal solid waste incineration bottom ash according to the standart leaching test (NT ENVIR 002) at liquid to solid ratio (L/S) of 1 L/kg, and subsequently contaminated with chromium and copper.

Assessment of Metal Toxicity

In order to assess the effects of heavy metals, a bioassay in tubes, streak plate method and agar diffusion method were tested.

A bioassay in tubes

Bioassay in tubes were initiated by adding 0,1 ml of overnight bacterial cultures and 0,1 ml of inorganic salt solutions to 4,8 ml nutrient broth. The tubes were incubated at 37°C for 18-24 hours, and turbidity was determined.

Streak plate method

The assessment the bacterial growth was performed on nutrient agar plates. The bacteria were streaked on the plates and incubated at 37°C for 18-24 hours.

Agar diffusion method

It was performed on nutrient agar plates. Plates were inoculated with 0,1 ml overnight bacterial cultures by spreading the medium surface. Filter-paper discs with diameter 13 mm (Whatman®, England) were dipped in the heavy metal solutions with different concentrations, and then placed on the agar plates. The plates were incubated at 37°C for 18-24 hours. The inhibition zones around the discs were measured.

RESULTS AND DISCUSSION

The metal ions and bacterial strains used are shown in table 1.

Table 1. Effect of metal ions on bacteria

Metal ions	Concentration (mg/L)	Turbidity*	Bacterial growth*	Sensitive strains
Cr ⁶⁺	20	++	++	› <i>E.coli</i> , <i>P. aeruginosa</i>
	10	+++	+++	
Cu ²⁺	200	-	+ ⇒ -	› <i>E. coli</i> , <i>S. aureus</i>
	50 – 100	++ ⇒ -	+++ ⇒ 10 col.	› <i>E. coli</i> , <i>S. aureus</i>
Zn ²⁺	175 – 350	+++	7 col. ⇒ -	<i>E.coli</i> , <i>P.aeruginosa</i>
	35	++ ⇒ -	+++ ⇒ ++	<i>S. aureus</i> , <i>B. subtilis</i>
Co ²⁺	250	-	10 col. ⇒ -	› all bacterial strains
	125	-	++ ⇒ 2 col.	› <i>E. coli</i>
	35	-	+++	
As ⁵⁺	35 – 115	+++	+++	
Ni ²⁺	200	-	+++ ⇒ 5 col.	› <i>E. coli</i> , <i>B. subtilis</i>
	100	-	+++ ⇒ 6 col.	› <i>E. coli</i> , <i>B. subtilis</i>
	40	++ ⇒ +	+++ ⇒ ++	
Pb ²⁺	8 – 300	+++ ⇒ +	+++	

+++ very good, ++ good, + low turbidity or a little growth, - no turbidity or growth, "col." - number of colonies

All bacteria species grew very well at the concentration range 0.5 – 10 mg/L of chromium (VI), at the concentration range 8 – 150 mg/L of lead, and in the presence of arsenic (V). The decrease in turbidity for all bacteria was observed at the concentration 20 mg/L of chromium (VI), at the concentration range 50 – 10 mg/L of copper, at the concentration 300 mg/L of lead, and at the concentration 40 mg/L of nickel.

E. coli, *S. aureus* and *B. subtilis* were tolerant to copper at the concentration of 10 mg/L.

Cobalt and nickel were very inhibitory to all bacteria tested. The turbidity in the presence of high concentrations of zinc was due to possible interactions between metal and various components in nutrient broth, and not due to growth of the bacteria.

There is a strong association of low pH to toxic effects. A low pH is an important exposure factor. The toxic effects of metals are associated to the increased concentrations of these metals, which promote the toxicity and decrease in pH. Decreased pH increases the mobility of metals. In general, samples with a pH below 6 proved to induce toxic responses in the *in vitro* experiments. The turbidity and bacterial growth was affected when pH was below 5.5.

Leachate of bottom ash had no toxic effects despite high levels of leads, zinc, copper, and chromium. This could be explained by the existing high pH in the leachate.

CONCLUSIONS

- Studied bacterial strains are tolerant to the lowest concentrations of inorganic compounds tested.
- Some metals and components in nutrient broth could interact and be seen as turbidity.
- Copper, cobalt, nickel and zinc appeared as the most toxic metals to certain bacterial strains tested.
- Agar diffusion test showed good correlation with turbidity and bacterial growth tests in the presence of cobalt, copper, and zinc.

Low pH may be the determining factor on toxicity, due to its strong influence on the mobility of metal pollutants.

ACKNOWLEDGEMENTS

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Characterization of Contaminants at a Military Range in Alaska, USA

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The Washington Range at the Donnelly Training Area, Alaska is used mostly for mortar, artillery, and other similar indirect-fire weapon systems. The site is within the floodplain of the Delta River, and the soils are glaciated and include features such as glacial moraines, glaciofluvial sediments, and loess. We sampled the soil and vegetation on the Washington Range with the dual objectives of: (i) developing sampling methods and protocols to characterize explosive contaminants in plants, soil, and water at a military impact-area ecosystem and to help understand possible fate and transport mechanisms in a range ecosystem; and (ii) characterizing explosives and metals contamination on this range and determining if munition-related explosives and heavy metals are being transported in the soils or taken up by plants. We used a sampling grid to sample soils and plants. Soil analyses consisted of texture (% sand, silt, and clay), % organic matter, pH, cation exchange capacity, salinity/conductivity, plant-important nutrients (phosphorus, nitrogen, potassium, calcium, and magnesium), munitions, and total and extractable metals using standard methods for analysis. We sampled 34 different plant species and collected sufficient tissue for analysis from the most prominent 14 species.

The soil was a loamy sand consisting mostly of sand and gravel (74%) and lesser amounts of silt (23%) with very little clay (3%). Loamy sands are very porous soils that do not retain moisture or nutrients as well as finer grained soils. On this site, however, the near neutral soil pH of 7.61 helps contain to downward metal movement, and plant growth is assisted by what appears to be a high water table. Only three soil sampling locations were found to contain munition residues, and the levels were very low. The concentrations for the five extractable metals and six total metals analyzed have values that are considered low and do not exceed the recommended cumulative limits for metals applied from sewage sludge on cropland. The concentrations of Ba, Cd, Cr, Cu, Pb, and Zn were below values naturally occurring in Alaska soils. When the data was grouped to determine if geographic location (along river) or impact location (near center of range) was concentrating contaminants or nutrients important for plant growth, there appeared to be no movement of contaminants out of the impact areas or towards the river (Tables 1 and 2).

During sampling for plant composition and biomass, we found five shrubs, four grasses and sedges, five forbs, and moss. Three species, each from a different plant family, shared dominance on the range: *Eleagnus commutata* (buffaloberry, a shrub), *Poa glauca* (Arctic bluegrass, a grass), and *Potentilla multifida* (cinquefoil, a forb). *Eleagnus commutata*, a shrub, had the greatest weights or biomass of any of the plant species sampled and it had the greatest % soil cover for the vascular plants sampled. Concentrations of nutrients in plants across the entire range may be considered low and deficient for plant growth on an optimum growth, agricultural basis (Table 3). None of the plants sampled contained any munition compounds above the detection limit used.

In summary, the soils at the Washington Range are not significantly contaminated by elements analyzed and there is no apparent movement of contaminants in the soils or into the plants.

Table 1. Comparison of nutrients and extractable metals in soil at grid points near the center of the impact area and away from that area (rest of range).

	Impact area (ppm)	Rest of range (ppm)	Significant difference ¹
Soil Nutrient			
N	16.278	14.068	NS ²
P	6.8944	6.4525	NS
K	62.111	67.559	NS
Mg	110.78	131.86	NS
Total Metals			
Ca	1377.7	1557.5	0.0278
Cd	0.08	0.0863	NS
Cr	0.1072	0.0993	NS
Cu	5.4611	5.5542	NS
Pb	2.315	1.6712	NS
Zn	5.3778	6.6525	0.0204

¹ Significantly different at the 0.05 level of probability² NS = no significant difference

Table 2. Comparison of nutrients and extractable metals in soil at grid points away from (on the plain) or near the river.

	Plain (ppm)	River (ppm)	Significant difference ¹
Soil Nutrient			
N	13.21	6.2887	0.0033
P	6.4244	4.3687	0.0004
K	62.658	46.365	NS ²
Mg	122.49	90.789	0.0106
Total Metals			
Ca	1377.5	1379.6	NS
Cd	0.0698	0.0688	NS
Cr	0.0898	0.0895	NS
Cu	5.1311	4.8196	NS
Pb	1.5185	1.623	NS
Zn	5.6474	4.8265	NS

¹ Significantly different at the 0.05 level of probability² NS = no significant difference

Table 3. Mean concentrations of elements in three plant species at ten grid points on the Washington Range, Donnelly Training Area.

Species	N	P	K	Ca	Mg	Cu	Zn	Cd	Cr	Pb
	%	ppm								
<i>Eleagnus</i>										
<i>commutata</i>	3.46 ^a	0.18 ^a	0.81 ^b	0.67 ^b	0.27 ^a	8.1 ^b	24 ^b	0.03 ^a	0.26 ^b	0.62 ^c
<i>Poa glauca</i>	1.47 ^b	0.09 ^c	0.59 ^c	0.41 ^c	0.07 ^b	24.67 ^a	76.67 ^a	0.15 ^a	0.61 ^b	3.93 ^b
<i>Potentilla</i>						12.33 ^a	54.67 ^a			
<i>multifida</i>	1.81 ^b	0.11 ^b	0.97 ^a	1.6 ^a	0.24 ^a	^b	^b	0.18 ^a	1.41 ^a	7.28 ^a

Numbers followed by the same letter are not significantly different at the 0.05 level of probability

MERCURY IN BOTTOM SEDIMENTS OF THE Odra RIVER ESTUARY

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Mercury, like cadmium and lead is considered to be unambiguously toxic with only a negative effect on organisms. It was known in ancient times and its compounds were used for therapeutic purposes till our times. In 1914 fungicides containing mercury were introduced and applied in agriculture until the seventies of the previous century. Mercury was also used in industry. As a result there was local contamination of certain ecosystems. The Odra estuary, similarly to other parts of World, was especially exposed to contamination due to ship traffic, trans-shipment, discharge of municipal and industrial wastes, precipitation runoff and pollutants from other sources. Due to sorption capacity the suspended matter of surface water binds these elements, which leads to their deposition in bottom sediments from where they may be released in considerably limited amounts (Salomons and Förstner, 1984).

Turekian and Wedephol (1961) and Förstner and Müller (1974) state that geochemical content of mercury in river bottom sediments does not exceed $0.400 \mu\text{g}\cdot\text{g}^{-1}$ dry weight. Similar opinion may be found in other authors' publications. In our earlier papers it was proved that in deeper layers of Zalew Szczeciński (Szczecin Lagoon) sediments the values were often below $0.100 \mu\text{gHg}\cdot\text{g}^{-1}$ dry matter and there was a positive correlation between mercury content and the content of other metals and the level of organic matter (Protasowicki et al., 1993).

The aim of this study was to determine the degree of contamination of bottom sediments in the Odra River estuary.

Surface bottom sediments collected from port canals and basins, and cores of sediments from Dabie Lake and Dziwna (all in the Odra estuary) in the years 1995 – 2001 were examined. The sediments were sifted through a polyethylene sieve with openings 2 mm in diameter.

Mercury was determined by means of atomic absorption spectrometry using a cold vapour technique after digestion of sample in mixture nitric and perchloric acids according to Adrian method (1971). Accuracy of method was tested on the basis of mercury determination in reference material MESS-2, and at the same time precision of method was determined.

Organic matter content was estimated by losses on ignition at the temperature of 550°C .

As a result of 5 parallel analyses of the reference material MESS-2 (sea sediments) $0.091 \pm 0.004 \mu\text{gHg}\cdot\text{g}^{-1}$ dry weight was determined in relation to the reference value $0.094 \pm 0.009 \mu\text{gHg}\cdot\text{g}^{-1}$ dry weight. This result confirms that applied analytical method allows to obtain accurate and credible results. Precision of the method is very high, which is supported by a very small results variation ($V = 4.40\%$) in analysis replications.

The sediments under study consisted of both sands and mud. The losses on ignition, ranging from 0.07 to 25.83% show that there were considerable differences in their organic matter content. Generally, the sediments from Police and Szczecin were slimy with a considerable amount of organic matter whereas those from the remaining ports mostly sandy with a slight mud addition (Table 1), which determined the sediment mercury content. It was within range 0.000 to $3.706 \mu\text{gHg}\cdot\text{g}^{-1}$ dry weight ($0.646 \mu\text{g}\cdot\text{g}^{-1}\text{dw}$ on the average).

The analysis of the relationships between those parameters showed that they may be described by equation $y = 0.0839x$ and correlation coefficient $r = 0.820$.

According to decreasing content of mercury these bottom sediments may be ordered as follows: Szczecin > Police > Nowe Warpno = Swinoujście > Dziwnów > Wolin (Table 1).

This supports our formulated earlier conclusion that with the growing distance from Szczecin heavy metal content decreases (Protasowicki and Niedzwiecki, 1993). There were no great differences between the present results and those obtained before (Protasowicki and Niedzwiecki, 1993; Protasowicki et al., 1993, 1999).

Data presented in table 2 visibly indicate that concentration of mercury in surface (younger) layers of sediments were higher than in deeper ones (older). Based on this it can be assumed that within period of the sediment formation pollution with mercury highly increased.

Considering the results in the light of natural geochemical mercury content given by Turekian and Wedephol (1961) and Förstner and Müller (1974) it should be stated that ca. 60% of sediments from Odra estuary are not contaminated.

Contents of mercury were several times lower from those given by Förstner and Müller (1974) and Herms and Tent (1982) in the case of river estuaries in Germany, and even two orders of magnitude lower than mercury concentration found in river sediments in considerably polluted regions e.g. near goldmines or plastic works where the value was 150, and even $240 \mu\text{gHg}\cdot\text{g}^{-1}$ dw (Cocking et al., 1991; Malm et al., 1990).

Table1. Mercury content [$\mu\text{g}\cdot\text{g}^{-1}$ dw] and losses on ignition [% dw] in bottom sediments of port basins and canals in the Odra estuary.

Sampling area	Number of samples	Mercury content [$\mu\text{g}\cdot\text{g}^{-1}$ dw]		Losses on ignition [% dw]	
		min – max	mean $\pm$ SD	min – max	mean $\pm$ SD
Szczecin	71	0.000 - 3.706	1.346 ± 0.902	0.19 – 25.60	14.15 ± 7.11
Police	8	0.114 - 2.024	0.471 ± 0.687	0.11 – 19.60	11.46 ± 5.96
Swinoujście	77	0.000 – 0.728	0.189 ± 0.201	0.07 - 16.10	4.89 ± 4.40
Nowe Warpno	26	0.000 – 1.622	0.231 ± 0.434	0.07 – 23.60	5.33 ± 6.97
Dziwnów	3	0.006 – 0.136	0.060 ± 0.068	1.30 - 7.00	3.56 ± 3.03
Wolin	2	0.005 - 0.005	-	0.54 – 0.57	-

Table 2. Mercury content [$\mu\text{g}\cdot\text{g}^{-1}$ dw] and losses on ignition [% dw] in typical sediment cores from Dabie Lake and Dziwna.

Depth strata [cm]	Dabie Lake		Dziwna	
	Mercury	Losses on ignition	Mercury	Losses on ignition
0 – 10	2.830	13.30	0.573	25.83
10 – 20	1.505	16.13	0.412	12.18
20 – 30	0.328	11.98	0.177	11.91
30 – 40	0.157	22.77	0.079	21.06
40 – 50	0.183	15.28	0.010	22.15
50 - 60	0.177	15.58	-	-

Background levels of trace and ultra-trace elements in soils of Miyagi prefecture, northeastern Japan

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INTRODUCTION

In the previous paper (Yamasaki et al., 2001), we have reported the background levels of more than 60 elements in soils of Japan sampled in a nationwide scale. In this work, we have analyzed 42 trace and ultra-trace elements in around 900 soils sampled from arable lands in Miyagi Prefecture, northeastern Japan. The major purpose of this study is to clarify the differences of the background levels of elements between those obtained by the nationwide scale survey (380,000 km²) and those by much smaller areas (7,300 km²) but with a higher sampling density.

MATERIALS AND METHODS

Soil Samples: One hundred and eleven key sampling sites and four near by spots around each key sampling sites were chosen to cover the whole arable lands in the Miyagi Prefecture. The density of the key sampling site was about one per 100 ha. Sampling was conducted from 1979 to 1999 in such a way as to cover one fifth of the whole sampling sites each year. Accordingly, four samples were taken from the same site at an interval of five years. Among the large number of soil samples thus obtained, about 880 samples were used in this work.

Analytical Methods: A quadrupole type ICP-MS, HP-4500 (Hewlett Packard, USA) was used for most of trace elements ($> 0.1 \text{ mg kg}^{-1}$ soil). For several ultra-trace elements ($< 0.1 \text{ mg kg}^{-1}$) and/or trace elements susceptible to interferences due to spectral overlaps, however, it was necessary to use a high resolution ICP-MS, ELEMENT (Finnigan MAT, Germany).

Dissolution Techniques: The dissolution procedures were essentially similar to that used in the previous work (Yamasaki et al., 2001), but more efficient and less sample and acids consuming microwave digestion technique was adopted in this work. Briefly, 0.1 g of finely ground sample was first treated with 2 mL of HNO₃ in a tightly sealed Teflon container in a domestic microwave oven for 15 min, and then with 0.5 mL of HF for another 15 min, and finally with 0.5 mL of HClO₄ for 15 min. The digest was dried up after removing the sealed cup on a hot plate. The residue was heated with 2 mL of HNO₃ and dissolved by adding 10 - 20 mL of H₂O with gentle boiling and finally made up to 40 mL. Indium (In) was used as an internal standard element (Yamasaki et al., 1990). The validity of the proposed methods was examined by analyzing soil and rock Standard Reference Materials (SRM) provided by several different organizations.

RESULTS AND DISCUSSION

Histograms of the most elements in this work were strongly positively skewed. This result was quite similar to those obtained from the samples of the nationwide survey in the previous work. As can be seen in a box-and-whisker plot in Figure 1, however, the concentration ranges for Miyagi soils were around one order of magnitude narrower than those of nationwide soils for most of the elements, presumably because the variety of soil types in Miyagi samples was not so wide as those in the nationwide samples. In spite of these general tendency, the concentration ranges of such elements as Cu and Zn were wider in Miyagi soils than in the nationwide soils. These wider ranges of Miyagi soils were apparently due to their higher contents of these elements in the small numbers of higher outliers,

shown in Figure 1 as blank circles. These higher outliers were all from the surface soils of orchard, and therefore, possibly anthropogenic origins. Similar higher values of Cd due to mining and those of U, possibly caused by the long-term application of P fertilizers were also observed.

In addition, such information as correlation among trace and ultra-trace elements, differences between the surface and subsurface soils in their contents of elements, the effects of land use, and the spatial distribution of elements will also be presented.

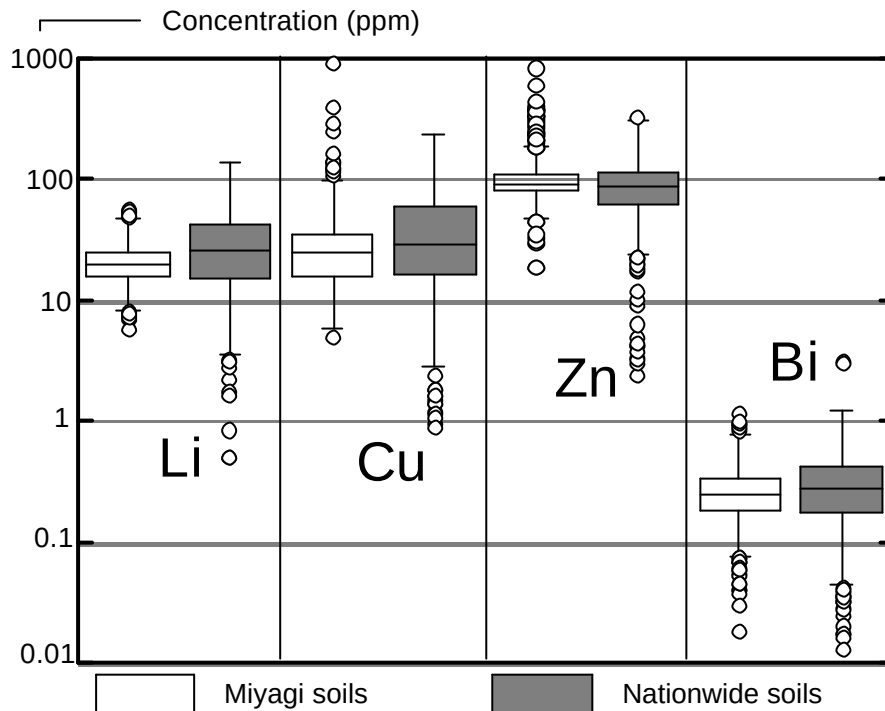


Figure 1. Concentration ranges of Li, Cu, Zn and Bi in Miyagi and nationwide soils

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Application of ISO 1269-1 root elongation test on 21 soils for the evaluation of soil health.

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In this paper the ISO 1269-1 root elongation bioassay was tested on 21 soils coming from agricultural and natural areas, in order to evaluate the role of native characteristics of soil on the test response. Experimental results showed a wide range of values indicating the high sensitivity of the bioassay to the soil environments. In this experiment soil pH is the parameter deeply influencing the results (Fig.1), differently to the indication of the ISO methodology that outlined the importance of texture in the choice of the control soil, that is defined as a “.. good quality soil of the same textural class as the soil under test” Therefore the criteria for choosing the reference soil need to be examined closely.

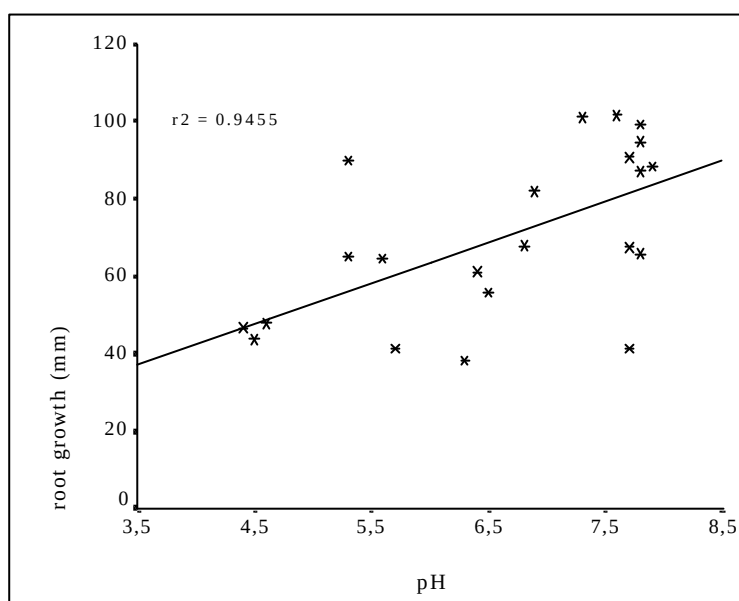


Fig. 1 – soil pH dependence of the ISO test

An alternative to a control was then explored, in order to distinguish between the effect of soil native characteristics and the effect of pollutants on soil flora. The test of inhibition of root growth was modified by substituting the control soil, defined by the method as a “good quality soil of the same textural class as the soil under test”, with the unknown soil examined weakly treated.

In this aim six soils with different physical and chemical characteristics were enriched with cadmium (CdCl_2) to reach the EC_{50} previously estimated on an artificial substrate. A similar inhibition

of root growth was observed in all soils and artificial substrate (Fig.2) suggesting a lack of interaction between soil native characteristics and metal toxicity.

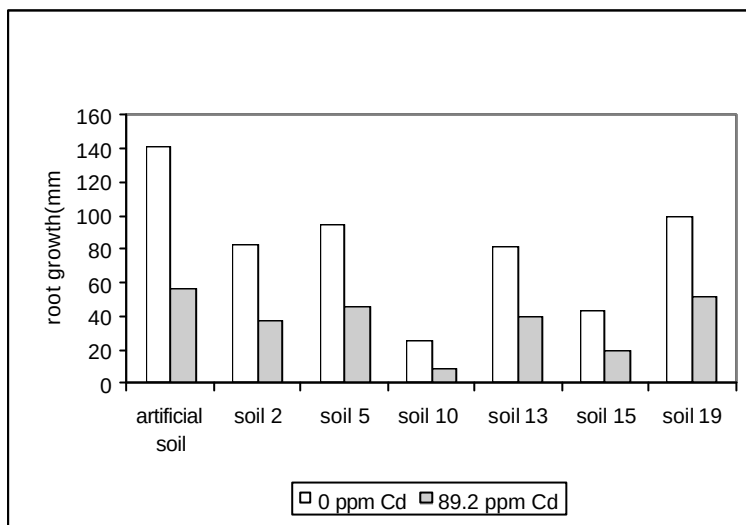


Fig. 2 - Effect of Cd addition to soils on the ISO test

Furthermore the test was applied to an unpolluted soil diluted with sand (0, 25% and 50% of sand); the same rate of root elongation was observed in all the trials (Fig.3a): the influence of soil characteristics was maintained in dilution treatment. Samples from the same soil were artificially contaminated with Cd and then diluted with sand obtaining at the highest dilution a significative increase of root elongation (Fig.3b). This experimental approach could be used to detect the presence of available pollutants in soils.

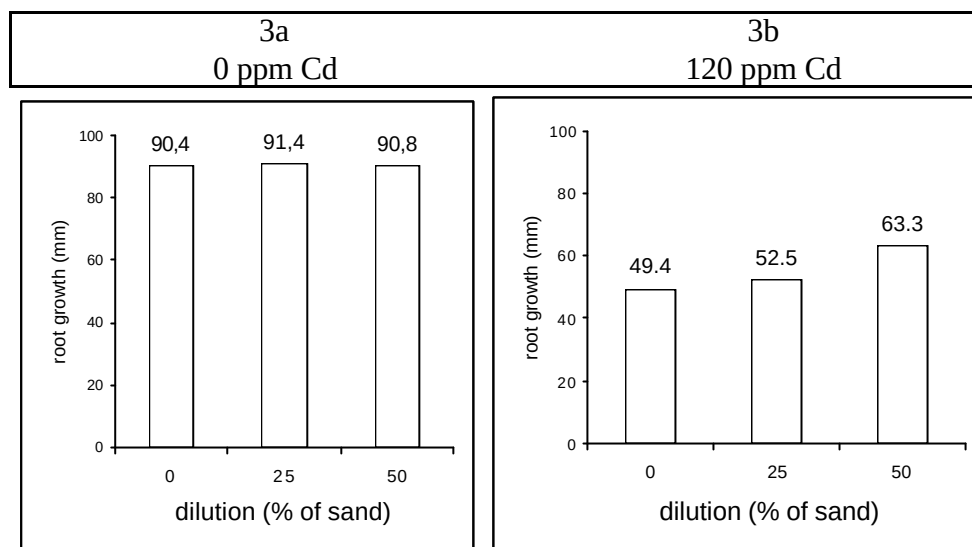


Fig.3: Effect of soil dilution on ISO test in healthy (3a) and artificially contaminated (3b) soil

ISO 11269-1:1993. Soil quality - determination of the effects of pollutants on soil flora Part 2: effects of chemical on the emergence and growth of higher plants.

Distance dependence of radionuclides concentration in the marine environment outside the Barsebäck nuclear power plant

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INTRODUCTION

The Barsebäck nuclear power plant (Barsebäck NPP) is one of the four nuclear power plants that provide Sweden with 44% of its annual electricity consumption. It consisted of two boiling water reactors till the end of the year 1999: the first reactor (Barsebäck 1) was in operation since early 1975 till the end of the year 1999, while the second reactor (Barsebäck 2) is in operation since March 1977. Barsebäck NPP is located on the Swedish coast at the Öresund 20 km east of Copenhagen. The location is controversial since there are around two millions inhabitants living in the vicinity. During normal operation of the nuclear power plant, small releases of activation (corrosion) products are released to the marine environment. These releases are regularly monitored by the Swedish Radiation Protection Authority (SSI). If an accident occurs to the power plant, the amount of activity released will drastically be increased.

Since the early 1960, the brown seaweed (also called the bladder-wrack) *Fucus vesiculosus* has been widely used as a bioindicator for monitoring the radioactivity in the marine environment. In recent years, there seems to have been decline of the population of *Fucus vesiculosus*. However, the *Fucus vesiculosus* still exists in sufficient amount in the marine environment in the region to carry out dispersion studies of radionuclide releases.

METHODS

Samples of *Fucus vesiculosus* were collected from different distances and directions from the Barsebäck NPP, and were used as bioindicators to measure the concentration levels of activation products released from the cooling water discharge pipe during the plant operation. The main goal of this study is to see whether there is a distance dependence in the concentration levels of certain radionuclides (C-137, Zn-65, Co-60, Co-58, Mn-54) released from the Barsebäck NPP. Furthermore, the study also aims to determine whether there is any relationship between the concentrations of the various radionuclides found in the samples.

The *Fucus* was sampled in August 2, 2002 at a water depth of around 0.5 m, at calm sea and at a water temperature of about 20°C. Each sample was dried separately at room temperature for a period ranging from 4-5 days, and was then grounded and put in 150 ml beakers for gamma spectrometry analysis. The activity concentrations of Cs-137, I-131, Zn-65, Co-60, Co-58, Mn-54 and K-40 were determined with acquisition times between 100,000 and 150,000 s. The measurements were performed using a HPGe (EG&G Ortec) detector of 36% efficiency, and energy resolution of 1.8 keV (FWHM) at 1.33 MeV.

RESULTS AND DISCUSSION

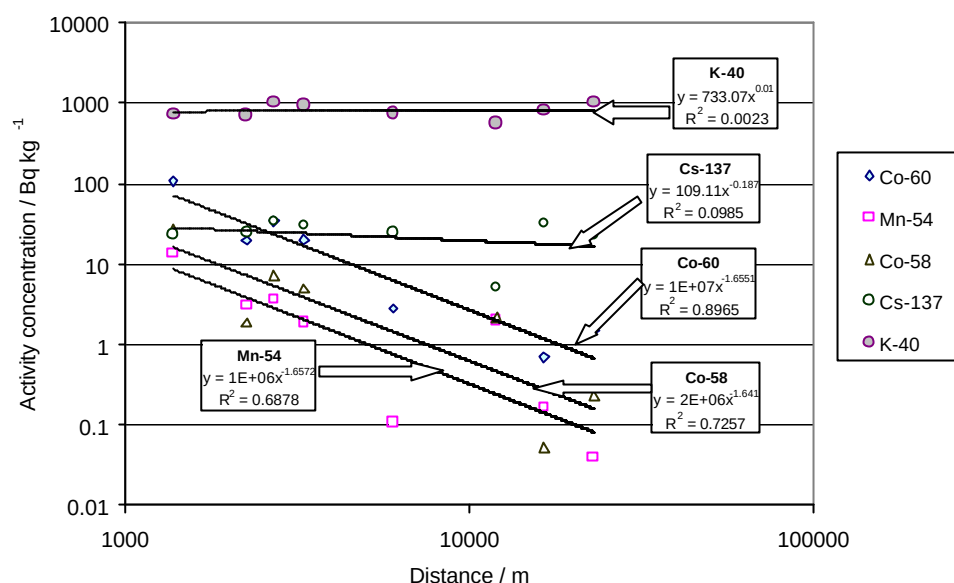


Fig. 1. Activity concentration in *Fucus vesiculosus* in relation to the distance from the outlet of Barsebäck NPP in August 2002. Power functions were fitted to the observed data.

The strongest correlation between the concentration levels of radionuclides and the distance from the discharge pipe of the Barsebäck NPP is found for Co-60, Co-58 and Mn-54 respectively. The distance dependence also appears to be similar for the three radionuclides, the slope of the log-log plots ranging from -1.64 to -1.66 (Fig.1.) However, there is no similar distance dependence between the activity concentration and the distance for Cs-137. This indicates that the majority of the Cs-137 concentration found in the *Fucus* samples outside the Barsebäck NPP is residue of the releases from Chernobyl accidents and from Western European reprocessing plants.

CONCLUSION

The activity concentration of gamma emitting radionuclides in *Fucus* from the bay just north of the Barsebäck NPP is today dominated by (in order of decreasing concentration): natural K-40, Co-60 from the plant, Cs-137 mainly from Chernobyl releases in 1986, Mn-54 and Co-58 from the plant.

Chemical speciation and modeling

Scientific Program 5

Oral

A CP-MAS ^{13}C NMR Study on Complexation of Cations to Soil Organic Matter

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INTRODUCTION

About 4% of the total area of Poland is polluted by heavy metals (Cu, Pb, Cd) and around 60% of Polish soils is acidic. Notwithstanding the scale of the problem, little is understood about the effect of pollution, including acidification, on soil organic matter. Therefore we studied metal complexation to soil organic matter by measuring both changes in T_{CH} and T_1 in ^{13}C -CPMAS VCT experiments.

To study the effect of conformational structure on complexation, unextracted samples, from which humus fractions have been concentrated by physical means, will be subjected to NMR. We studied metal complexation to soil organic matter by measuring both changes in T_{CH} and T_1 in ^{13}C -CPMAS VCT experiments.

METHODS

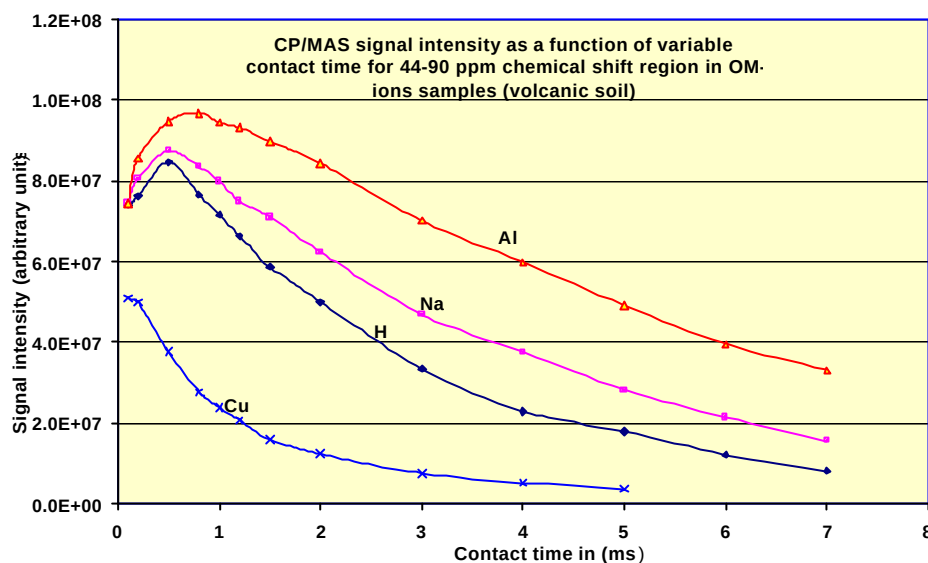
The experiment was carried out on NaOH-extracts of organic matter from two types of soil – two samples from a podzol and two samples from volcanic soils, which were widely different in chemical, physical and biological characteristics. All samples were mixed separately with H^+ , Na^+ , Cu^{2+} and Al^{3+} chlorides to obtain different organic matter-cations complexes. The protonated SOM (solid organic matter) were used as a reference; Al is a common counter-ion in soils, which is strongly complexed and not paramagnetic; Cu is both strongly complexed and paramagnetic; and Na is only loosely bound. The protonated and saturated samples were dialysed against demineralised water and freeze-dried.

The CP-MAS ^{13}C NMR spectra of prepared samples were obtained using a Bruker AMX 300 spectrometer operating at a frequency of 75.48 MHz. The samples were spinning at 5 kHz using room temperature air for drive and bearing pressure. The Hartmann-Hahn condition was determined using glycine as a standard. The samples were measured at 13 different contact times (VCT: 0.1; 0.2; 0.5; 0.8; 1–(three times); 1.2; 1.5; 2; 3; 4; 5; 6; 7 ms) to obtain full information on cross polarization times and observed carbon. A 1000 number of scans were used per experiment. The measurement of all protonated and saturated samples were performed three times in order to compare the obtained results.

The obtained spectra were sub-divided into regions: alkyl-C, O-alkyl-C, aromatic-C and carbonyl-C. A processing of the Free Induction Decay (FID) and the spectra was done using Bruker WINNMR software package version 6. A backward linear prediction of six points was used to reconstruct the start of the FID. Prior to Fourier transformation, the FID was multiplied with an exponential function producing a line broadening of 50 Hz. Spectra were phased by adjusting the zero order phase correction and a fixed first order phase factor. A sixth order baseline polynomial correction was applied [van Lagen and de Jager 2002]. For the curve fitting Win Sigma Plot by Jandel Scientific was used.

RESULTS

Figure 1 show signal intensity as a function of contact time for four investigate cations in one chosen chemical shift.



It was also established $T_{1\rho H}$ and I_0 values in (%) in several chemical shifts regions in organic matter extracted from Bhm1 layer of a well-drained podzol and from 2Bw2 layer of volcanic soil

CONCLUSIONS

No significant changes were found in TCH values between H-samples and Al-samplex. However, it seems, that cross-polarization was slower in Al-samples compared to H-samples. It was not possible to measure TCH values for Cu-samples.

Significant changes were found in $T_{1\rho H}$ values for Al-sampleas and Cu-samples and hardly changes in I_0 values for Al-samples, what could be explain as inter- and intra molecular spin diffusion.

They were found complexation sites for Cu on carbonylic, aromatic and O-alkyl carbon, but not on aliphatic carbon

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Colloid-mediated transport of heavy metals from a contaminated roadside soil

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INTRODUCTION

Due to trace metals content, highway runoff waters are a potential source of contamination of local groundwater resources. Since 1969, several studies have reported that a part of the heavy metals emitted is adsorbed in roadside soils or in retention pond sediments (Pagotto, 1999). But the use of NaCl as de-icing salt may change the behaviour of the accumulated contaminants in roadside soils, when the soil exposed to high Na concentrations is then supplied with lower salinity water. Soil column leaching experiments with NaCl and de-ionised water have provided evidence of colloid-assisted transport of heavy metals by the mobilisation of organics and Fe-oxides (Amrhein *et al.*, 1993). An increased concentration of heavy metals with soil depth in a infiltration pond showed that downward transport had occurred, supported by the fact that Pb and Zn occurred mostly in association with the oxide bound fraction, whereas Cu was mainly associated with the organic fraction of the soil (Norrström and Jacks, 1998). The objective of the present complementary study was to investigate the effect of salinity variations of runoff waters on the release of heavy metals from a roadside soil, in saturated chromatographic column experiments to simulate the input of salty waters followed by rainfall.

MATERIALS AND METHODS

The contaminated roadside soil was sampled at 0.50 m from the asphalt edge on the national road RN 12, built in 1971 at 50 km far from Paris (25,000 vehicles/day). The soil texture is loamy sand. The fine fraction (below 200 μm) used in the experiments contains about 1% oxides, 1% carbonates and 14% organic matter. The cationic exchange capacity of the soil is equal to 0.16 meq/g. The average total concentration of Fe is found to be 24000; Mn, 43000; Pb, 2600; Zn, 720 and Cd, 2.9 mg/kg of soil. Sodium chloride is used to model de-icing salts (generally 70% of the composition of de-icing salts). The column, packed with 60 g of soil, was initially saturated with NaCl ($5 \cdot 10^{-3}$ mol/L) at a low flow rate (0.1 mL/min) and then pre-flushed to equilibrate the soil with NaCl (10^{-2} mol/L) at a flow rate equal to 0.6 mL/min. A minimum of absorbance of the effluent was observed under this flow rate value. When equilibrium occurred in the soil, a decreasing step of NaCl concentration at $5 \cdot 10^{-4}$ mol/L was injected in the column and pH, conductivity and absorbance of the effluent were continuously registered. The collected fractions were filtered through a 0.45 μm pore size membrane and analysed for ions constitutive of oxides and heavy metals by Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) for Fe, Mn and Zn and by atomic absorption spectrometry for Pb and Cd.

RESULTS AND DISCUSSION

Delmas *et al.* (2002) showed that a small part of Pb and Zn (max. 0.5%) was released by an increase of the salinity by mixing in batch reactors this contaminated soil and a NaCl solution with a concentration of 0.1 or 1 mol/L (equal or higher than those of the initial interstitial solution of the soil). Therefore, in comparison with the release by ionic exchange, column experiments have been conducted to study the colloidal-mediated transport of heavy metals. The characterisation of the yellow effluent, obtained during the transient phase after saturation of the column, showed that this effluent had a very high but decreasing conductivity during leaching. It was composed mainly of fulvic acids and Fe- and Mn-oxides. Humic acids are not detected by acidification of the effluent. The results of the

step experiment pointed out that elution of colloids was connected to the decrease of salinity, as seen in literature on colloids, but also with Fe-oxides and not with Mn-oxides as the relationship between absorbance and Fe suggested (Fig.1). Increase of heavy metals (Pb, Zn and Cd) concentrations was detected as the concentration of Fe and absorbance increased. The concentrations of Pb and Cd remained above the concentrations for groundwater proposed by the Dutch regulation. The release of colloids was also correlated to an increase of pH from 6.95 to 7.4.

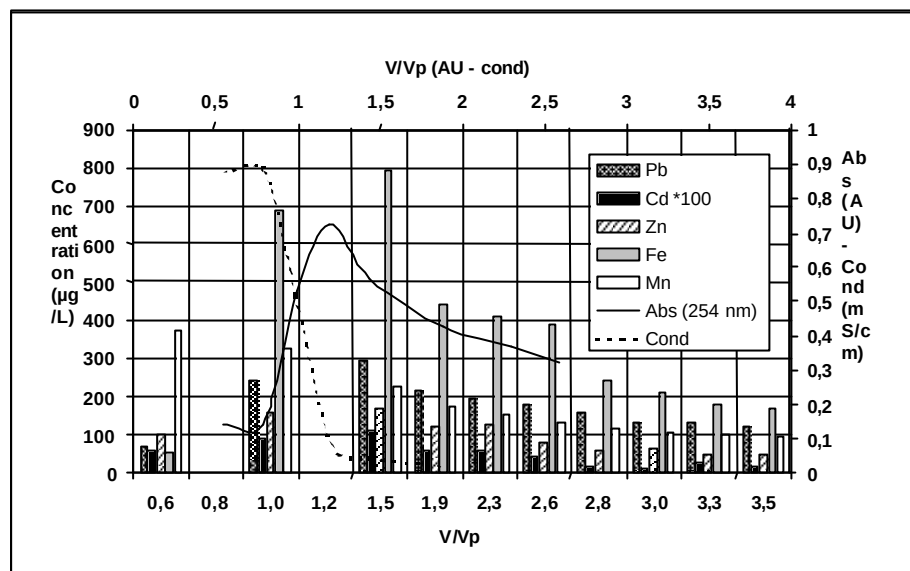


Fig.1 Effect of a decreasing salinity step on the absorbance and concentrations of heavy metals of the effluent from a roadside soil column ($V_p = 34 \text{ cm}^3$; $Q = 0.6 \text{ mL/min}$)

CONCLUSIONS

These results on a non calcareous contaminated roadside soil emphasise the contribution of fulvic acids and Fe-oxides to the transport of heavy metals. The impact of succession of salt flow and rain during winter season has been highlighted, even if the quantity of heavy metals transported by colloidal way was small (less than 0.5%). The continuous recording of physical parameters contributes to the investigation of the physico-chemical processes of colloidal transport.

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Kinetic Fractionation of Soil Trace Metals ; Interest, Principles and Relation with Bioavailability.

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INTRODUCTION

Regardless of their origins and the reasons for the increase in their concentration in soils, trace metals are liable to contaminate food chains by migrating towards groundwater or by accumulating in plants. The hazard due to the presence of large amounts of trace metals in some soils is the result of their reactivity in soils; in other words, it depends on their localization in different soil components i.e. is strongly related to what we call their speciation. This so-called speciation of trace elements in soils may be performed using chemical methods. Chemical methods consist of using different chemical reagents for extraction of trace elements from given soil compartments, terminating with their quantification in the extraction phase (normally when equilibrium is reached). Several reagents are generally used in what are called sequential extraction protocols.

However, everyone now agrees that these protocols cannot supply a reliable estimate of the speciation of trace elements in soils, particularly for thermodynamic reasons (measurements made at equilibrium) [2] so that a sequential extraction procedure is now only considered as operationally defined and gives limited informations on associations of trace metals with soil constituents. This is why it appears necessary to consider other methods of determining these associations of trace metals in soils in more details, particularly considering kinetic aspects that also characterize the stability of the various trace metal - soil constituent associations. In a previous work [3], we showed the feasibility of performing an operationally defined fractionation based on kinetics which could distinguish "labile" (quickly extracted) and "non labile or slowly labile *i.e.* slowly extracted) cations, using the extraction kinetics of trace metals by Edta and we will

PRINCIPLES OF A KINETIC FRACTIONATION

The principles of a kinetic fractionation is to fit of an experimental extraction curve with a sum of exponential, corresponding to two first-order reactions, according to the equation 1.

$$Q_{\text{tot}} - Q(t) = Q_L(1 - e^{-k_L t}) + Q_{NL}(1 - e^{-k_{NL} t}) \quad (1)$$

where Q_{tot} denotes the total quantity of a given trace element extracted by EDTA (labile + slowly-labile) and Q_L and Q_{NL} correspond respectively to the « labile » or « non labile » cations

RESULTS AND DISCUSSION

For this study, samples were issued from not contaminated areas. Copper and cadmium results obtained when using the kinetic method above will be presented and discussed according to the following points :

some aspects of the accuracy of the kinetic fractionation

comparison of cadmium and copper from the point of view of their labile and slowly labile fraction ;

relationship of copper and cadmium (labile and slowly labile fraction) to soil properties using regression methods and discussion in term of soil compartments ;

relationship of labile copper or cadmium fraction to their bioavailability; interest of kinetic fractionation (as shown for cadmium on Figure 1).

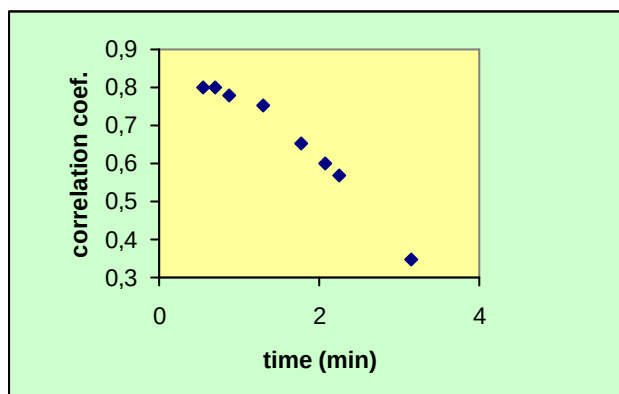


Figure 1 : Relationship [Cd] in plants vs [Cd] in soil (Cd is EDTA extracted at different time)

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Organotin speciation in plants: uptake, accumulation, and biotransformation

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INTRODUCTION

The extensive use of organotins in domestic and industrial products leads to the presence of these toxic species in sewage sludges, waters from plastic tubing, and freshwaters. Butyltins are the main contaminants, including the well-known tri-substituted tin, TBT. Sewage sludges input into agricultural soils and waters used in irrigation can increase cultivated plant species exposure to organotin compounds (OTC). A lot of pesticides (acaricides, fungicides...) contain organotins such as triphenyltin (TPhT) (Fent, 1996). Foliar treatment with these products directly exposes plants as well. Considering the toxicity of some OTC, especially the trisubstituted ones, knowledge of their transfer and behaviour into the plants is fundamental for agricultural production. However, references in literature are scarce. Simon (2002) reported plants can widely accumulate OTC after contact with contaminated water. The organotin transfer from pesticides to fruits and vegetables has been previously established (Miliadis *et al.*, 1998). Concentrations in vegetables, such as carrots and potatoes, and in apples ranged over 1 – 60 µg (Sn or organotin)/kg. Many mechanisms involved in the phytoavailability and uptake, partitioning, storage and the biotransformation of tin chemical species in plants remain unknown. Accordingly, this work focuses on the most toxic OTC: TBT and TPhT, and their uptake and accumulation in edible plant parts such as French beans and potatoes.

MATERIALS & METHODS

Both plants were cultivated in potted soils placed in a glasshouse. Plants were exposed to either a contaminated soil (Podzol durique, Gironde, France) or pesticide solutions. The speciation was performed in the plant parts (leaves, pods or tubers) at several growth steps. Plant samples were weighed, frozen in liquid nitrogen, lyophilised and ground. The OTC were extracted in HCl and ethyl acetate, following a method previously optimised in our laboratory (Simon *et al.*, 2002). All analyses were performed by direct aqueous ethylating / Liquid-Liquid Extraction (LLE)/ Gas Chromatography (GC) / specific detector. The main detectors used were Pulsed Flame Photometer (PFPD) or Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). Strong matrix effects occurred because chlorophyll and starch were co-extracted during LLE. Therefore, the headspace Solid Phase MicroExtraction (SPME) has alternately been used.

RESULTS & DISCUSSION

SPME is a powerful and promising extraction tool, able to improve the detection limits.

The analysis confirms a significant OTC transfer from soil to leaves and fruits, some µg (Sn)/kg being found in plant parts. The uptake is more important in young plants. The speciation in vegetables shows a degradation of TBT to less toxic tin species which does not seem very quantitative in French beans, while TPhT degradation appears significant. Other processes such as bioconversions could also occur since unphenylated species were found after TPhT spiking. However, the behaviour of the different Sn species obviously depends on the organotin type and plant species. These first results also show the particular behaviour of plants facing to two different organotin exposure pathways.

Consequently, questions rise about OTC toxicity in plants and the long-term consequences at once for the agriculture and human health.

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Effect of NaCl salinity and Zn-application on species of Cd and Zn in soil solution

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INTRODUCTION

Cadmium uptake by plant is a concern because Cd is potentially toxic when consumed (Norvell et al., 2000). Soil is the principal source of Cd accumulated by plants. Zinc, on the other hand, is an essential micronutrient that its deficiency is a major problem in salt-affected soils of central Iran. Bioavailability of Cd and Zn to plants may depend not only on the total concentration, but also on the species of these metals in solution (Bingham et al., 1984). Soil solution contains ions, which may exist as free, hydrated ions and/or as dissolved species complexed with organic or inorganic ligands (Helmke and Naidu, 1996). Computer predictions of metal species have been successful in well-defined solutions with known total concentration of metal and ligand (McGrath et al., 1986). Use of a speciation model is a relatively easy and fast method for estimating chemical species in solution. The experimental methods available to determine dissolved heavy metal species include the use of ion-exchange resins, chromatographic methods, ultra filtration, and dialysis (Batley, 1989). The objective of this study was to investigate the effect of salinity and Zn application on distribution of Cd and Zn species in soil solution.

MATERIALS AND METHODS

A Cd-polluted (3.2 mg kg⁻¹) surface (0-30 cm) soils were collected from Qom province in central Iran. Cadmium accumulation in this area is attributed to the overusing P-fertilizers contained Cd as impurity. A greenhouse experiment with two rates of Zn (0 and 1.5 mg Zn kg⁻¹ as ZnSO₄), four salinity levels of irrigation water (0, 60, 120 and 180 mM NaCl) and three replicates was conducted. The salt levels are typical of those found in irrigation waters in wheat fields of central Iran. Soil samples (<5-mm diameter) were transferred into pots (3kg soil in each pot). At planting, a uniform application of N-K fertilizers (100 mg kg⁻¹ urea and K₂SO₄) was applied to each soil. Wheat seeds were sown in each pot and thinned to five plants per pot after 10 days, and grown for 45 days.

After harvest, soil sub-sample of each pot, was air-dried, crushed to pass through a 2-mm sieve, and stored for further chemical analysis. Soil saturation extract was obtained for each sub-sample (US salinity laboratory staff, 1954). Electrical conductivity (EC), pH and total concentrations of Ca, Mg, Na, K, SO₄, HCO₃, P, NO₃, as well as Cd, Fe, Cu, Mn, and Zn were measured in the extracts.

Two methods used to evaluate chemical species of trace metals in soil solution: a thermodynamical model and an experimental test. To estimate chemical species experimentally, we employed a method developed by Holm et al. (1995) based on the calculation of partition coefficient of free ions between a Ca-saturated ion-exchange resin (Amberlite) and solutions. Also we used a geochemical speciation program, MINTEQA2 (Allison et al., 1991) to estimate activity of chemical species in solution. Concentration of major cations, anions, trace metals, and pH were used as input database to calculate the concentration of free hydrated trace metals (M²⁺) and ligand species with MINTEQA2.

RESULTS

Cadmium concentration in soil solution increased significantly (p<0.01) with increasing NaCl concentration both with and without ZnSO₄ treatment (Table 1). Free Cd²⁺ ion concentration was

decreased by the NaCl treatment for both soils with and without ZnSO_4 , whereas CdCl^+ complex increased significantly by increase of salinity (NaCl) rate. With increasing soil salinity, the ratio of Cd^{2+} /total-Cd decreased, significantly. In 180 mM NaCl treatment, more than 60% of the total Cd was found as chloride complexes, whereas in control treatment, free Cd^{2+} was the dominant species, with remaining being CdCl^+ , CdCl_2^0 , CdSO_4^0 , and organic complexes (Table 1).

Treatment with ZnSO_4 increased concentration of CdSO_4^0 in soil solution, but it had no significant effect on concentrations of CdCl^+ and Cd^{2+} (Table 1).

In all salinity levels, Zn was mostly found as Zn^{2+} and to a lesser degree as ZnHCO_3^+ , ZnSO_4^0 , and ZnCl^+ in the soil solution. The ratio of Zn^{2+} /total-Zn decreased, significantly with increasing NaCl concentration, free Zn^{2+} was, however, the dominant species in all treatments (Table 1).

Treatment with ZnSO_4 increased concentrations of total-Zn, ZnSO_4^0 , and Zn^{2+} in soil solution (Table 1).

Table 1- Effect of irrigation water salinity and ZnSO_4 treatments on major chemical species of cadmium and zinc, as percentages of total concentration or activity in solution. *

Salinity level	Cd (%)				Zn (%)			
	Cd^{2+}		CdCl^+	CdSO_4^0	Zn^{2+}		ZnSO_4^0	ZnCl^+
	[Exp.]	(Calc.)			[Exp.]	(Calc.)		
Plus Zn								
0	44.2	46.0	40.0	10.5	76.9	78.6	26.8	1.8
60	42.5	37.8	42.2	13.5	64.1	66.7	22.2	1.9
120	38.5	27.1	54.2	8.4	70.2	69.9	20.2	3.6
180	22.0	16.3	60.4	3.8	59.1	60.2	15.4	6.8
Minus Zn								
0	45.1	45.6	39.8	9.7	73.5	72.8	14.5	1.6
60	41.3	33.9	54.1	4.3	83.1	79.4	9.5	3.3
120	39.5	25.7	56.5	6.6	71.9	69.9	16.8	4.0
180	26.8	14.6	60.0	3.9	69.5	66.4	16.8	7.1

* Activities of free Cd^{2+} and Zn^{2+} predicted with a speciation model (Calc.) and their concentrations determined experimentally with a cation exchange resin (Exp.) in soil solution

CONCLUSION

Species of Zn and especially Cd in soil solution varied with soil salinity level. Total cadmium concentration increased with salinity level. In saturation extract of saline soils, Cd was mainly chelated by chloride (Cl^-) and the ratio of Cd^{2+} /total-Cd was small in the most saline extracts. Salinity also decreased the ratio of Zn^{2+} /total-Zn.

Treatment with ZnSO_4 increased total concentration of Zn in the soil solution, but it had no effect on total concentration of Cd.

Research on roadside soil pollution by heavy metals and soil remediation by natural zeolites

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INTRODUCTION

Since 1980 the main goal of geochemical investigations in Lithuania has been the estimation of technogenic changes of microelements in the Earth's surface sediments [1]. The transport system is the main source of pollution in Lithuania. Therefore the main goals of this research are to evaluate the environmental impact on roadside soil caused by motor transport sector and the recommendations for the soil remediation. The main objectives of this research are investigations of the environmental impact of pollution caused by the motor transport sector in Lithuania; evaluation of environmental impact of motor transport sector on soil; experiments with natural zeolites as a remedy for heavy metals immobilization in roadside soil; preparation of suggestions and recommendations for the reduction of impact on roadside soil.

METHODOLOGY

The whole territory of Lithuania was divided into the parts where heavy traffic is present causing the great pollution of soil. The topsoil samples were taken across the motorways and also across the roads that are not so very contaminated in the regional parks just for the purposes to compare the heavily polluted areas to not polluted ones. Nine top soil profiles were already taken next to the motorways. Each profile was composed of 12-14 topsoil samples accordingly to the topographical situation. Each sample was composed of 5 sub-samples according to the "envelope" principle, which were taken at depth of 0-10 cm [2]. The distances between the samples in profile were 1 m, 2 m, 5 m, 10 m, 25 m, 50 m, 75 m, 100 m depending on the relief.

RESULTS

The defined quantities of trace elements in the sample profiles that have been taken at different administrative districts, in comparison to background values are higher [2]. For example, concentration of lead in Klaipeda district profile, which was taken to evaluate pollution caused by the motor transport across the traffic circle, is very high (Fig. 1). The traffic is very intensive on this traffic circle. It is evaluated, 1056 motor vehicles pass per hour in average. Lead is concentrated mostly in sediments of ditches where it is related to mechanical deposition of translocated particles. The quantities of other trace elements in this profile are related mostly to pollution caused by the motor transport, but they don't reach very high concentrations. This may be related to the fact, that the traffic circle is located on the open area where translocations by airflows aren't restricted by forests or relief elevations. On the open place the pollutants are deconcentrated and are spread widely.

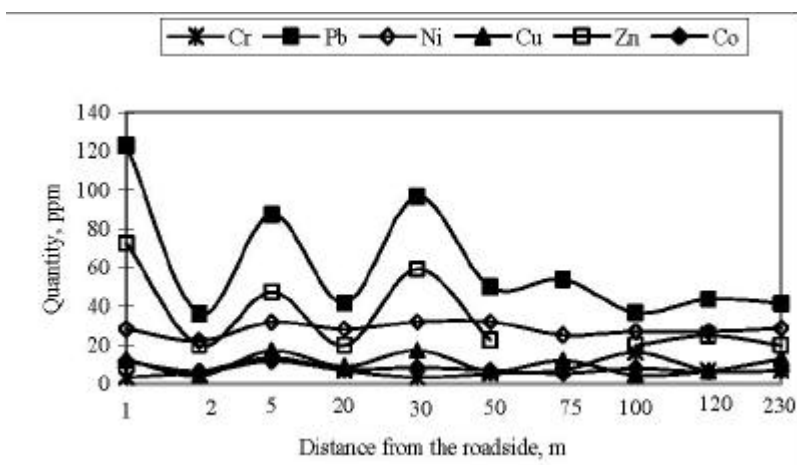


Figure 1. Distribution of trace elements in Klaipeda roadside topsoil (0-10 cm).

The second step in this research program, after the defining of quantities of trace elements in roadside soil, is to make some experiments in laboratory and in natural conditions with natural zeolites, as a remedy for the gentle roadside soil remediation.

CONCLUSIONS

Distribution and concentrations of trace elements on the other roads are variable and depends on the distance from the road, on the traffic intensiveness on different roads, on the topography of the contaminated sites. Substances emitted are exposed to various aerodynamic and gravitation forces resulting in their separation and, depending on their physical and chemical properties, they settle selectively on the soil surface at different distances from the road surface. The main amount of heavy metals is settling on the soil surface in the narrow limited zone of the highway itself, its slopes and ditches

ACKNOWLEDGEMENT

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Nickel sorption to goethite and Na-montmorillonite in presence of citrate

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INTRODUCTION

Nickel is a naturally occurring element found in the Earth's crust. Ni is released and spread in the terrestrial environment from Ni containing minerals and by extensive use in a variety of alloys and chemicals. The distribution of Ni between the soil solid phase and the soil solution is a key issue in assessing the environmental risk of Ni in terrestrial environments. In most aerobic soils, the solubility of Ni is controlled by sorption processes. Cation exchange and surface complexation are the two most important types of sorption processes affecting Ni. Among other factors these processes are affected by the type of sorbents, pH and the concentration and stability constants of Ni complexing ligands present in solution.

The objectives of this study were to understand and quantify sorption of Ni(II) in a well characterized model system in the presence of citrate as ligand.

MATERIALS AND METHODS

The Ni sorption experiments were conducted in 50 mL PE containers with an electrolyte of 10.0 mM NaNO₃, at 20 ± 2 °C, and with a liquid to sorbent ratio of 500:1 (g/g). Goethite (37 m² g⁻¹) synthesized according to Schwertmann and Cornell (1991), and Na-montmorillonite (SWy-2; Source Clay Minerals Repository; fraction < 2 μm; 29 m² g⁻¹) were used as sorbents with and without the presence of citrate (50 - 100 μM). Kinetic experiments revealed that an experimental period of 72 h was sufficient to obtain near equilibrium conditions. The pH in batch experiments was adjusted by adding small amounts of HNO₃ or NaOH, and pH measured at end of the sorption experiments. Sorption isotherms were determined at pH 6.0 according to the technique by (Tiller et al., 1979). Ni was determined by graphite furnace atomic absorption spectroscopy (GFAAS). Equilibrium concentrations of Ni in solution were in the concentration range 0-3.4 μM Ni (0-200 μg L⁻¹).

RESULTS AND DISCUSSION.

Ni sorption as function of pH. For goethite the sorption as function of pH (pH-edge) shows the typical sigmoid shape observed for surface complexation to hydrous oxide surfaces, with about 50 % of maximum sorption obtained at pH 5.92 in absence of citrate (Fig. 1). The sorption of nickel to montmorillonite shows little variation with pH up to pH 6.6 indicating cation exchange to be the principal sorption reaction. In presence of citrate sorption of Ni to goethite decreases insignificantly whereas sorption to montmorillonite decreases dramatically. Equilibrium computation shows that solution complexation of Ni²⁺ with citrate with formation of NiHcitrat⁰ and Nicitrat⁻ increases from about 15 % at pH 4 to about 100 % at pH 6. Hence, the low sorption of Ni to montmorillonite at the higher pH (Fig. 1) can be attributed to the transformation of Ni²⁺ aqua ions to neutral or anionic Ni complexes. In case of goethite it appears that Ni citrate complexes are sorbed as strongly as Ni²⁺, or that citrate cannot compete with surface bonding of Ni²⁺.

Ni sorption isotherms. Sorption isotherms at pH 6 are almost linear with distribution coefficients of 600 L kg⁻¹ and 1960 L kg⁻¹ for sorption to goethite and montmorillonite, respectively, in absence of citrate (Fig. 2).

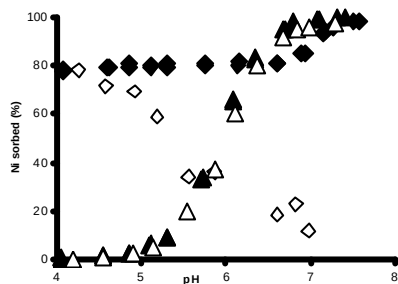


Figure 1. Sorption of Ni to goethite and montmorillonite in absence (,) and in presence of 50 μM citrate (, Δ). Initial concentrations were constant for each curve and varied from 2 to 9 μM between the four experiments.

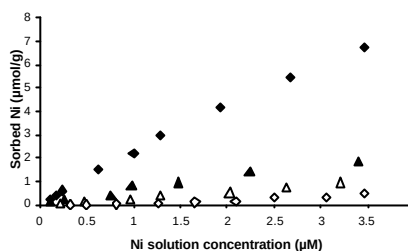


Figure 2. Sorption isotherms at pH 6 for Ni bonding to montmorillonite and goethite in absence (,) and in presence of 100 μM citrate (, Δ).

Again, the presence of citrate is found to strongly decrease sorption of Ni to montmorillonite whereas the effects of citrate on Ni sorption to goethite are smaller.

Sorption of Ni to montmorillonite was adequately described using a cation exchange model ($\text{XNa}^+ + \frac{1}{2} \text{Ni}^{2+} \rightleftharpoons \frac{1}{2} \text{X}_2\text{Ni} + \text{Na}^+$) with a Gaines-Thomas selectivity coefficient of $K_{\text{Na/Ni}}^{\text{GT}} = 0.605 \pm 0.038$. The model worked both in absence and presence of citrate; in the latter case the concentration of Ni^{2+} was found by equilibrium computation. Data for sorption of Ni to goethite in absence of citrate could be fitted by the diffuse double layer model (Dzombak and Morel, 1990) with the following constants: $\log K_{a1}^{\text{intr}} = -6.44$, $\log K_{a2}^{\text{intr}} = -10.1$ and $\log K_{\text{Ni}}^{\text{intr}} = -0.59$ ($\equiv \text{FeOH} + \text{Ni}^{2+} \rightleftharpoons \text{FeONi}^+ + \text{H}^+$). Sorption of Ni in presence of citrate is more complicated as both Ni^{2+} and Ni citrate complexes are sorbed. Work is being carried out to determine if sorption of Ni^{2+} and citrate to goethite is taken place at fixed stoichiometric ratios indicating the sorption of Ni citrate complexes, or if sorption of Ni^{2+} and citrate is independent of each other indicating that surface complexation to the goethite surface can successfully compete with in solution complexation for Ni.

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The organic carbon derived from sludge as a key parameter determining the fate of trace metals

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INTRODUCTION

The agricultural use of organic waste has been a common practice in waste disposal in the recent decades. Since they contain plant nutrients and organic matter, organic wastes may be beneficial to soils and their productivity. However, depending on its origin, they contain variable amounts of trace metal such as Cu, Pb and Zn, which tend to accumulate in soil surface. According to Shuman (1999), organic applications can reduce metal toxicity by redistributing metals to less available forms. On the contrary, the slow mineralization of organic matter in sludge could release metals into more soluble forms (McBride, 1995). The dynamics of sludge organic carbon appear therefore as a potential parameter driving the fate of trace metals in soil. Stable carbon isotope ^{13}C at natural abundance concentrations can be, in some cases, a good natural tracer of the dynamic of organic wastes in the soil. The principle of this method is based on the variations of ^{13}C natural isotope abundances induced by organic inputs on a soil. The use of the equations 1 and 2 allowed calculating the proportion of each source of organic matter (X_{native} , X_{maize} and X_{sludge}) at a given time. Therefore, we have chosen this method to follow the dynamic of the organic carbon derived from sludge and its influence on the behaviour of trace metals, during organic waste applications (1974-1993) and afterwards.

$$1) X_{\text{initial}} + Y_{\text{maize}} + Z_{\text{sludge}} = 100$$

$$2) \delta^{13}\text{C}_{\text{sol}} = X_{\text{native}} \cdot \delta^{13}\text{C}_{\text{native}} + Y_{\text{maize}} \cdot \delta^{13}\text{C}_{\text{maize}} + Z_{\text{sludge}} \cdot \delta^{13}\text{C}_{\text{sludge}}$$

MATERIAL AND METHODS

Soils were sampled in the 0-25 cm surface layer from the INRA field experiment of Couhins, near Bordeaux (France), on which sewage sludge was applied between 1974 and 1993 at two different rates, $10 \text{ t ha}^{-1} \text{ yr}^{-1}$ (S10) and 100 t ha^{-1} all 2 years (S100). Since 1993, no treatment was applied. Soil samples were collected from each plot in 1974, 1981, 1993 and 2000, air-dried and sieved through a 2mm screen. Sequential extraction (Shuman, 1985) and grain size fractionation (Bartoli *et al.*, 1991) were performed to follow the distribution of both, trace metals (Cu, Pb and Zn) and organic carbon between 1974 and 2000. The $\delta^{13}\text{C}$ value was measured using a continuous flow mass spectrometer (VG Isochrom-EA). Binocular and MEB-EDS were also used to observe the different grain size fractions.

Table 1 Sequential extraction procedure (Shuman, 1985)

Reagents	Targeted fractions
1M Mg(NO ₃) ₂ , pH 5	Exchangeable
0.7M NaOCl, pH 8.5	Organic matter
0.1M NH ₂ OH HCl, pH 3.5	Mn oxides
0.2M (NH ₄) ₂ C ₂ O ₄ + 0.2 M H ₂ C ₂ O ₄ , pH 3	Amorphous Fe oxides
0.2 M (NH ₄) ₂ C ₂ O ₄ + 0.2 M H ₂ C ₂ O ₄ + 0.1M C ₆ H ₈ O ₆ , pH 2.25	Crystalline Fe oxides
HNO ₃ + HF + HClO ₄	Residue

RESULTS AND DISCUSSION

Between 1974 and 1993, the repeated applications of sludge induced a strong accumulation of trace metals and organic carbon in the surface soil layer (0-25cm). Sequential extractions showed that trace metals were mainly located in both, iron oxides and organic matter fractions. Surprisingly, both, trace metals and organic carbon were mainly found in the coarse sand fraction (200-2000 μm). Binocular and microscope (MEB-EDS) observations showed that this fraction contained water-stable aggregates on which organic carbon, iron, manganese and zinc were detected. The $\delta^{13}\text{C}$ measurements gave additional information: organic carbon found in the coarse sand fraction was mainly derived from sludge. This source of organic carbon seemed to lead to the accumulation of trace metals in the coarse sand fraction, via the formation of water stable aggregates.

From 1993, when sludge applications were stopped, trace metals were still located in iron oxide and organic matter fractions. However, trace metals amounts strongly decreased in the coarse sand fraction and increased simultaneously in the silt-clay fraction except in the case of Cu. As for trace metals, organic carbon derived from sludge decrease in the coarse sand fraction and increase in the silt-clay fraction. The stop of sludge applications induced also changes as regards water-stable aggregates, which were mainly found in the silt clay fraction. This observation confirmed therefore the role of organic carbon derived from sludge in the formation of aggregates.

CONCLUSION

The repeated applications of sludge led to form water-stable aggregates in which accumulated trace metals. When sludge applications were stopped, the humification of the organic matter induced a change of distribution of organic carbon derived from sludge but also of trace metals from coarse fraction to fine fraction. Sludge appeared therefore as the main parameter determining the fate of trace metals.

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Influence of fly ash and sewage sludge application on wheat yield and soil heavy metal fractions

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INTRODUCTION

Fly ash (FA) is a by-product of combustion of lignite that is used as fuel by power stations of electric power production. Chemically it is consisted of elements essential to plant growth like Ca, Fe, Mg, K but also of others such as B, Se, Mo, and metals that can be toxic to the plants (Korcak, 1995). Most of the amount of FA produced, in practice is disposed while the rest is recycled in non or agricultural uses. In Greece there are several electric power plants using coal as a fuel producing considerable amounts of FA that needs sound management. Similarly, coal mining wastes management in China is a major problem because of the huge quantities produced from coal mining destined to energy production, 75% of which comes from coal (Wei et al., 2000). Recent research (Matsi and Keramidas, 1999) has shown that fly ash use as liming agent in acid soils may improve soil properties and increase crop yield. In addition, municipal sewage sludge (SS) has been found beneficial for agricultural use in unproductive Greek soils with no detrimental effects on soil quality when it is applied in the appropriate quantities (Tsadilas et al., 1995). The purpose of this study, was to investigate the possibility of using FA and SS to improve acid soils properties and their productivity.

MATERIALS AND METHODS

A field experiment was conducted in a strongly acid Typic Haploxeralf (pH 4.9) with winter wheat. The experimental design was complete randomized blocks with six treatments including a control (C) ; application of inorganic fertilizers only (IF); FA at two rates (22.5 and 67.5 ton/ha) plus IF (T1F, T2F respectively), and FA (22.5 ton/ha) plus SS at two rates (6.3 and 22.5 ton/ha) without IF (T1SS1, T1SS2 respectively). Fly ash came from a lignite-fired electric power in Northern Greece and it was alkaline, containing 15% CaCO₃ and about 116 mg/kg Zn, 30 mg/kg Cu, and 379 mg/kg Ni. Sewage sludge was from the waste water treatment plant of a city in Central Greece containing 32% organic matter, 1320 mg/kg Zn, 285 mg/kg Cu, and 45 mg/kg Ni. At the harvesting time soil samples were selected and analyzed for soil pH, organic matter content (O.M.), "available" Zn, Cu, Ni and for their fractions exchangeable, organic matter associated, carbonate bound and residual according to the procedure described by Emmerich et al. (1982).

RESULTS AND DISCUSSION

The influence of FA and SS application on wheat yield and on the soil properties, which were significantly affected, is presented in Table 1. Fly ash and SS significantly increased soil pH and in a lesser degree organic matter content. As a consequence of soil pH improvement, grain yield was significantly increased especially in the treatments including FA at the higher rate or FA plus SS. Total concentrations of the metals studied were not significantly influenced (data not presented). However, soil pH significantly affected "available" forms and some fractions of the metals studied. "Available" Ni and Zn concentrations were decreased in the treatment with the highest FA rate. The same

Table 1. Influence of FA and SS application on wheat yield soil pH, organic matter content and heavy metal forms

Treatments	Yield ton/ha	pH water 1:1	O.M. %	Ni _{DTPA} ^{**} mg/kg	Zn _{DTPA}	Ni _{KNO3}	Ni _{NaOH}	Ni _{EDTA}
C*	3.09ab	5.05ab	0.51a	1.12c	0.62a	0.82b	0.55a	1.49b
IF	3.21ab	4.72a	0.60a	0.89b	0.82ab	0.75b	0.56a	1.07a
T1IF	3.51ab	5.35b	0.66a	0.97bc	0.72a	0.42a	0.94b	1.55b
	c							
T2IF	3.76bc	7.22c	0.58a	0.52a	0.54a	0.26a	1.18c	2.76c
T1SS1	297a	7.10c	0.64a	0.43a	1.60b	0.20a	1.12bc	1.77b
T1SS2	414c	6.90c	0.90b	0.58a	2.44c	0.23a	1.24c	1.88b

* see text above, **Ni_{DTPA}, Zn_{DTPA} "available" forms. Ni_{KNO3}, Ni_{NaOH}, Ni_{EDTA} exchangeable, organic matter associated and carbonate bound fractions respectively.

was true for exchangeable Ni, while organic matter associated Ni as well as carbonate bound were increased in the treatments with the highest FA rate. Soil pH was strongly correlated positively with the unavailable to the plants metal fractions (organic matter associated, carbonate bound and residual) and negatively to available fractions like exchangeable Ni, and Ni and Cu extracted by DTPA (Table 2). Zinc extracted by DTPA was positively correlated with O.M. content. From the other hand Ni extracted by DTPA was negatively correlated with unavailable fractions and positively with the exchangeable fraction ($r=0.63^{**}$). The final conclusion is that FA in strongly acid soils at the rates used may improve soil pH without increase total heavy metal concentration while it decreases their available forms.

Table 2. Relationship between soil pH with metal forms

Metal form	r	Metal form	r
Ni _{DTPA}	-0.86***	Ni _{KNO3}	-0.64***
Cu _{DTPA}	-0.47*	Ni _{NaOH}	0.83***
Cu _{EDTA}	0.56**	Ni _{EDTA}	0.76***
Cu _{HNO3}	0.45*	Zn _{EDTA}	0.46*

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Chemical speciation and modeling

Scientific Program 5

Poster

Study of the Use of Two Chemical Reagents (NaOH and H₂O₂) in Order to Assess the Amount of Trace Metals Associated with Organic Matter in Soil Samples Submitted to a 100 Year Spreading of Urban Waste Water

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Keywords : pollution ; trace metal ; speciation ; organic matter, waste water

INTRODUCTION

In many developing countries water is scarce and urban waste water is recycled for irrigation and sewage farming in suburban market gardening. Such practices are often recommended by agronomists because they are a good way for recycling organic matter and nutrients. However they may present environmental hazards, depending on the nature and load of pollutants and soil types, with respect to contamination of ecosystems and human health. Such an example of a wastewater recycling scheme is given by the city of Paris (France) in an area of about 1200 ha (plain of Pierrelaye-Bessancourt), located less than 24 km northwest of Paris, in the heart of urban and industrial suburbs and which is here studied.

Since 1899, unfiltered and untreated waste waters have been pipelined to a sandy market-garden area at high rates with a twofold objective: flooding irrigation of food crops and discarding of an excess of wastewater. Soils were additionally amended with urban sludge and compost in the middle 60's, a period when metal pollutant contents were neither controlled, nor guided.

In 1992 a local ecologist association brought up the problem of waste water irrigation of vegetables and green salads which are often consumed crude. First studies were carried out in 1996 and 1998 (soil and plant analyses). High metal contents observed in reply to: *Thymus sp.* produced in this zone, pressed public authorities to prohibit selling of vegetables (June 1999) and to support a research project on environmental risks. Since then, grain maize is the only authorised crop in the irrigated area (with monitoring of grain composition).

SITE CHARACTERISATION ; LEVELS OF POLLUTION BY TRACE METALS

The first soil survey (279 soil samples including 110 topsoils) has evidenced a high metal pollution of uppermost horizons (**table 1**).

Table 1: Range of trace metal concentrations corresponding to more or less severe contamination - Topsoil samples - first survey. Comparison with local "usual agricultural concentrations" (UAC). Values are expressed as mg/kg.

Element	from	to	median	UAC
Cd (cadmium)	0.60	10.9	2.06	0.19 to 0.42
Cr (chromium)	37	126		15 to 29
Cu (copper)	50	308	95	8 to 19
Hg (mercury)	0.30	9.38	1.20	0.08 to 0.15
Ni (nickel)	30	51		6 to 20
Pb (lead)	80	668	200	18 to 43
Zn (zinc)	150	1293	366	34 to 63

USE OF CHEMICAL REAGENTS

A strong enough relationship has been observed between trace metal contents and organic matter but it does not really prove that trace elements are bound to the organic matter. In order to determine which part of trace elements is really bound to the soil organic matter, chemical reagents such as sodium hydroxide, hydrogen peroxide are commonly used [1, 2]. Extracted trace elements have to be determined in the solution after the reaction hydroxide or hydrogen peroxide with the soil sample.

A critical study of these extractions has been performed, using some samples from the previous site, in order to choose one chemical reagent and the experimental conditions, allowing an accurate determination of the trace element amount bound to organic matter. Some results will be presented and discussed :

Hydrogen peroxide has been shown, although it may nearly oxidize the soil organic matter, to be not a good reagent for the proposed aim : trace metal readsorption phenomena which are involved during the hydrogen peroxide attack do not allow the determination of trace metals bound to organic matter.

When using sodium hydroxide as an extractant of organic matter, it has been shown that one needs to perform successive extractions but the sum of the amount of trace metals determined in each extract, indicates that some trace metals (copper for instance) are probably bound with a great extent to organic matter. Moreover, for this reagent, kinetic measurements did not indicate readsorption phenomena.

As a conclusion from this study, hydroxide sodium seems to be the best reagent in order to determine the amount of trace metals bound to organic matter in these samples.

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The fluctuation of Zn fractions in incubated sewage sludge after lime and limestone additions

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INTRODUCTION

Sludge is a resource with both positive and negative attributes, containing large quantities of plant nutrients and also toxic elements. Solubility of heavy metals in sewage sludge is affected by many parameters and conditions - pH, redox potential, aeration, etc. (Chuan et al., 1996; Tlustos et al., 2001). The aim of this study was to determine by sequential extraction the changes in Zn fractionation in sludge with addition of lime and limestone after 8 months of incubation.

MATERIALS AND METHODS

Stabilised sewage sludge was obtained from three wastewater factories in the Czech Republic situated in towns of different population. The treatments were set up in three replications for each sludge as control treatment, treatment with addition of limestone (7 % of Ca w/w of sludge dry solid) and treatment with addition of lime (7 % of Ca w/w of sludge dry solid). All treatments were incubated under aerobic and anaerobic conditions in controlled room (20°C and 80% of Relative humidity). pH (1:20 w/v of 0.01 mol.l⁻¹ CaCl₂), carbon content, total nitrogen, and sequential analysis were determined at 0, 4 and 8 months. Zinc was divided into five fractions as water soluble, exchangeable (0.1 mol.l⁻¹ CH₃COOH), bound with Fe and Mn oxides (0.1 mol.l⁻¹ NH₂OH.HCl), organically bound (8.8 mol.l⁻¹ H₂O₂ + 1 mol.l⁻¹ CH₃COONH₄), and residual fraction. Zinc content in individual fractions was determined by AAS.

RESULTS AND DISCUSSION

The greatest changes in pH, N_{tot} and C_{ox} were observed at the beginning of incubation. Variations among replicates were caused mainly by substantial heterogeneity of analysed sludge. Aerobically incubated untreated sludge had a lower pH (6.3) than anaerobically incubated sludge (7.8). The lack of air may decrease microbial activity causing an increase in pH (Cresser et al., 1993). Carbon content decreased during the first half of incubation (in aerobic treatments by 41% and in anaerobic ones by 31%). Organic matter loss was caused by enhanced microbial activity. Aerobic sludge had a greater amount of N_{tot} compared to sludge incubated under anaerobic condition. Sludge treated with lime and limestone showed substantial N_{tot} decrease because higher pH caused ammonia volatilization.

Figure 1 illustrates Zn distribution in individual fractions of sludge incubated under aerobic and anaerobic conditions. Sufficiency of air (Figure 1a) in untreated sludge caused an increase in available Zn forms (water and exchangeable) and a decrease in organic fractions due to intensive organic matter decomposition (Lake et al., 1984). Portion of Zn bound in Fe-Mn fraction was twice increased in second half of incubation compare with the beginning of incubation. Lime and limestone addition decreased Zn content in exchangeable, oxide and residual fractions during the incubation.

Untreated sludge incubated under anaerobic condition (Figure 1b) led to stable Zn content in available fractions (5% of total content). Zn portion in oxide and organic fractions was increased in second half of incubation. Sludge treated by lime and limestone showed only little Zn portion in available and oxide fractions (7.5 % of total content). The most Zn was kept in organic and residual fractions. This occurrence can be explained by higher pH value (average 8.8) of sludge incubated under anaerobic condition treated by lime and limestone.

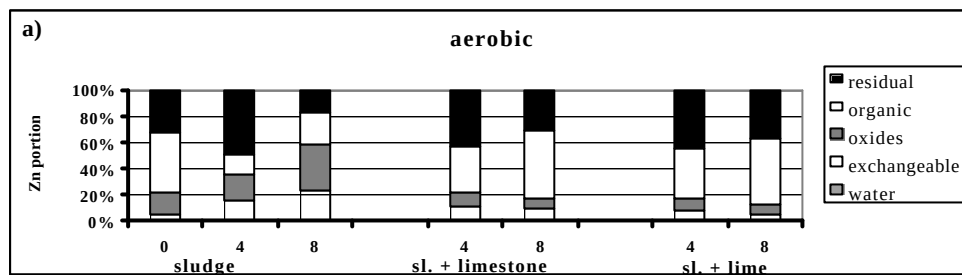


Figure 1 The fluctuation of Zn (%) among fractions in differently treated sludges incubated in aerobic (a) and anaerobic (b) conditions (0, 4 and 8 months)

CONCLUSIONS

The greatest differences in sewage sludge parameters were found at the beginning of incubation. Sludge incubated under anaerobic conditions showed higher pH compared to aerobic ones. The addition of lime and limestone into sludge showed a favourable effect on reduction of available Zn content. The better results were found after lime addition compared to limestone (because of higher pH). Most Zn was found in the organic and residual fractions.

ACKNOWLEDGEMENTS

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Research on heavy metals immobilization in roadside soil by Natural Zeolites

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INTRODUCTION

Since 1980 the main goal of geochemical investigations in Lithuania has been the estimation of technogenic changes of microelements in the Earth's surface sediments [1]. The transport system is the main source of pollution in Lithuania. Therefore the main goals of this research are to evaluate the environmental impact on roadside soil caused by motor transport sector and the recommendations for the soil remediation..

The object of this research program is the environmental impact of the motor transport system on soil. The main objectives of this program are investigations of the environmental impact of pollution caused by the motor transport sector in Lithuania; evaluation of environmental impact of motor transport sector on soil; experiments with natural zeolites as a remedy for heavy metals immobilization in roadside soil; preparation of suggestions and recommendations for the reduction of impact on roadside soil.

For some time in past the main source of lead pollution was the transport system in Lithuania [2]. Motor transport causes pollution not only with Pb, but also with other chemical elements as Cu, Co, Cd, Cr, Ni, Zn, etc. This transport sector impacts various ecosystems as air, water, soil and bio-system. It is also necessary to note that soil pollution by heavy metals is most dangerous, first of all it is not so obvious, and metals can be transferred to other environments (plants, water, animals, man) by ecological food circuits and have pernicious influence on organisms. When investigating pollutant dispersion in the environment, it is important to assess other factors as well. Pollutant dispersion is very dependant on atmosphere also, it is determined by some synoptic processes, e.g. in a case of powerful anticyclone, calm and sunny air is prevailing, temperature inversions are developed, and dispersion of pollutants is slower. Usually, conditions for pollutant dispersion are better at noon, when soil-surface inversion fully disappears, wind increases and turbulent mixing becomes more intensive, and at night pollutant concentrations go down because car traffic on the streets is considerably lower [3].

METHODOLOGY

The whole territory of Lithuania was divided into the parts where heavy traffic is present causing the great pollution of soil. The topsoil samples were taken across the motorways and also across the roads that are not so very contaminated in the regional parks just for the purposes to compare the heavily polluted areas to not polluted ones. Nine topsoil profiles were already taken next to the motorways. Each profile was composed of 12-14 topsoil samples accordingly to the topographical situation. Each sample was composed of 5 sub-samples, which were taken at depth of 0-20 cm. Some sample profiles in the sandy soils were taken at depth of 20-30 cm just for evaluation of vertical migration of chemical elements. The distances between the samples in profile were 1 m, 2 m, 5 m, 10 m, 25 m, 50 m, 75 m, 100 m depending on the relief. Topsoil samples were taken with a spade from the surface layer of 0-20 cm thick. The samples were composed of five sub-samples collected according to the "envelope" principle. The length of the "envelope" lines was 1-5 m. The samples were put into cotton bags or into "zip"-type bags [4].

RESULTS

The defined quantities of trace elements in the sample profiles that have been taken at different administrative districts, in comparison to background values are higher [4]. For example, concentration of lead in Klaipeda district profile, which was taken to evaluate pollution caused by the motor transport across the traffic circle, is very high (Fig. 1).

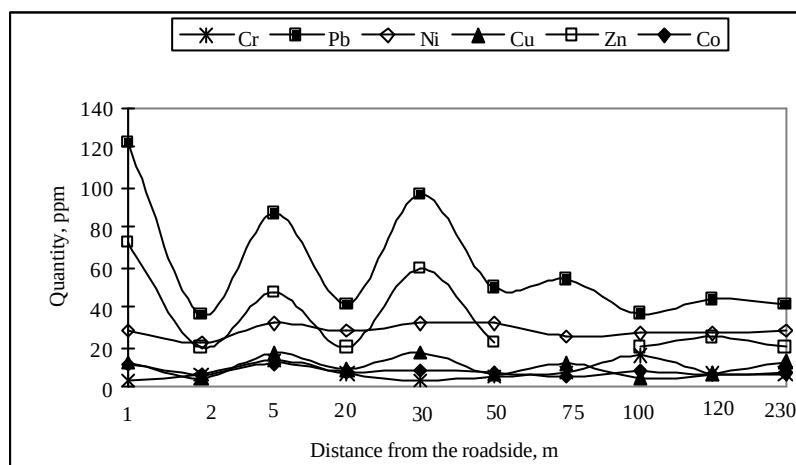


Figure 1. Distribution of trace elements in Klaipeda roadside topsoil (0-10 cm).

The traffic is very intensive on this traffic circle. It is evaluated, 1056 motor vehicles pass per hour in average. Lead is concentrated mostly in sediments of ditches where it is related to mechanical deposition of translocated particles. The quantities of other trace elements in this profile are related mostly to pollution caused by the motor transport, but they don't reach very high concentrations. This may be related to the fact, that the traffic circle is located on the open area where translocations by airflows aren't restricted by forests or relief elevations. On the open place the pollutants are deconcentrated and are spread widely.

CONCLUSIONS

Distribution and concentrations of trace elements on the other roads are variable and depends on the distance from the road, on the traffic intensiveness on different roads, on the topography of the contaminated sites. Substances emitted are exposed to various aerodynamic and gravitation forces resulting in their separation and, depending on their physical and chemical properties, they settle selectively on the soil surface at different distances from the road surface. The main amount of heavy metals is settling on the soil surface in the narrow limited zone of the highway itself, its slopes and ditches.

The second step in this research program, after the defining of quantities of trace elements in roadside soil, is to make some experiments in laboratory and in natural conditions with natural zeolites, as a remedy for the gentle roadside soil remediation. The purposes of these experiments are to find out if natural zeolites are suitable for the gentle roadside soil remediation or as the measure to protect the roadside soil for the continuing pollution caused by motor transport. Another thing is to define which fraction of clinoptilolite is more suitable for the immobilization of heavy metals in roadside soil.

ACKNOWLEDGEMENT

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Kinetics of Cadmium Release from a Soil Amended with Phosphate Fertilizers

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Phosphorus fertilizers contain a broad range of cadmium (Cd), ranging from less than 10 to over 100 mg Cd per kg, depending on the source of phosphate rock. Continuous applications of P fertilizers at high rates that consistently add more Cd to soils than is removed by the harvested plant parts can gradually increase Cd accumulation in the soil and in plants. Increased concerns over the entry of Cd into the food chain make it important to determine how available the Cd is in the fertilizers. The availability of Cd in the fertilizers is controlled by the lability and concentration of Cd in the fertilizers, as well as by the rate it is released from the fertilizers into the labile pool. The rate will be affected by chemical characteristics of the soil and solubility of the fertilizers.

The primary objective of this study was to determine the rate of the release of Cd from a triple superphosphate (TSP) (19.6%P) and a western phosphate rock (PR) (14.6%P) which contained 127 and 40 mg Cd kg⁻¹ and 1460 and 375 mg Zn kg⁻¹, respectively. Varying rates of the P fertilizers that added up to 1.70 mg Cd kg⁻¹ soil and 18.5 mg Zn kg⁻¹ soil depending on the P source were incubated with a Sultan silt loam soil (*Aquandic Xerochrept*) at a moisture content of 17 g kg⁻¹ in a constant temperature room (18°) up to 210 d. The amount of Cd and Zn extractable by diethylenetriaminepentaacetic acid (DTPA) (pH=7.3), the amount of P extractable by NaHCO₃ (pH=8.5), as well as pH level, were determined periodically during the incubation. In addition, the P fertilizers were sequentially extracted with H₂O, 1M HCl (dilute acid), and concentrated HNO₃ (strong acid) to partition the total Cd and Zn in the fertilizer into soluble, less soluble, and insoluble fractions that correspond to decreasing solubility of the metals.

Of the total Cd in the TSP 34.1% was water-soluble and 55.3% dilute acid-soluble, which were much higher compared to the amount of Cd in water-soluble (0.0013%) and in dilute-acid soluble (29.8%) forms in the PR. The low solubility of Cd in the PR was also reflected by close to 70.1% of the total Cd soluble only in concentrated acid. While the water-soluble fraction of Zn in the PR was very low (0.006%) as compared to TSP (50.7%), the dilute acid-soluble Zn in the PR was rather high (71.3% as opposed to 45.5% in the TSP). The low solubility of Cd and Zn in the PR than in the TSP is to be expected as, in the production of TSP, phosphoric acid is added to PR to enrich P and increase P solubility. The results also showed that Zn was comparatively more labile than Cd in the PR.

The initial amounts of Cd and Zn extractable by DTPA in the soil treated with TSP averaged 35.1 and 45.3 % of the total amounts of Cd and Zn added, respectively, which were close to the amounts of Cd (34.1%) and Zn (50.7%) the TSP had in the water soluble fractions. Given the very small amount of Cd the PR had in the water-soluble fraction (0.0013%), the small amount of Cd added extractable by DTPA (6.7%) is not surprising. However, despite the amount of Zn in the water-soluble fraction in the PR being equally small (0.006%), a high percentage of added Zn in the PR was extracted by DTPA initially (43.7%). Reaction of the PR with the soil had enhanced the extractability of Zn in the PR. The ratio of Cd to Zn in the DTPA extract for the soil treated with TSP (0.080) was essentially the same as that in the fertilizer (0.086), implying that the added Cd and Zn from TSP were equally accessible to DTPA. The ratio was much lower in the DTPA extract for the soil treated with the PR (0.015) than in the fertilizer (0.108) to suggest a greater accessibility of its Zn to DTPA than its Cd at least initially.

The amount of Cd and Zn added from TSP extracted by DTPA increased with increasing incubation time, reaching a maximum about 20 d of incubation before declining gradually at a rate depending on the amount of TSP added. A zero-order rate equation described well the amount of Cd extracted with time. The zero-order rate equation also described well the increase in the amount of Cd added from the PR extracted by DTPA with time up to 80 d. However, further increase in the incubation time beyond 80 d increased the amount of Cd extracted at a very slow rate rather than declined as was found for the soil treated with TSP, while the amount of Zn extracted by DTPA remained unchanged. The result indicates that the soil was not responsible for the decline in the amount of Cd and Zn extracted by DTPA with time. This argument is supported by the fact that no similar decline was found for the soil treated with the PR and the rate of the decline in the amount of Cd or Zn extracted in the soil treated with TSP increased with increasing amounts of the P fertilizer applied. It is speculated that a recrystallization involving certain constituents in the TSP proceeded to sequester the released Cd and Zn, thereby lowering the amount of Cd and Zn available for extraction by DTPA. Further research is needed to validate this.

Table 1. Zero-order rate equations describing the rate of Cd extracted by DTPA (y) (mg Cd kg⁻¹) with time (x) (day) for the soil amended with varying rates of TSP or PR.

P source	Rate	First stage	r ²	Second stage	r ²
TSP	0	y = 0.0021x + 0.107	0.99	y = -0.00003x + 0.145	0.13
	16X	y = 0.0053x + 0.300	0.97	y = -0.00003x + 0.402	0.02
	32X	y = 0.0051x + 0.483	0.99	y = -0.0002x + 0.588	0.56
	48X	y = 0.0061x + 0.618	0.76	y = -0.0006x + 0.803	0.62
PR	0	y = 0.0003x + 0.123	0.94	y = -0.00001x + 0.164	0.65
	16X	y = 0.0019x + 0.159	0.46	y = 0.00005x + 0.271	0.11
	32X	y = 0.0032x + 0.183	0.95	y = 0.0004x + 0.337	0.62
	48X	y = 0.0035x + 0.217	0.96	y = 0.0002x + 0.478	0.35

X = 35.3 µg Cd kg⁻¹ soil for PR and 33.5 µg kg⁻¹ for TSP.

The amount of Cd and Zn initially extracted by DTPA in the soil was influenced by the amount of labile or water soluble Cd and Zn added particularly from TSP. Dissolution of water-insoluble component in TSP and PR could be expected to be the process responsible for the increase in the amount of Cd extracted by DTPA with time. If it was, more P would have been released as well from the water-insoluble component in the fertilizers and increase in the amount of P extractable by NaHCO₃ paralleling that of Cd should be expected. This, however, is not the case. The amount of NaHCO₃-P in the soil treated with either P fertilizer changed little with time, and it even declined with increasing additions of PR, contrary to what was found for the amount of Cd extracted by DTPA. It appears that particularly for the PR, mechanism(s) other than dissolution controlled the release of Cd. What it is is unclear and should be investigated to further the understanding of the availability of Cd in this P fertilizer.

Comparison anion effect on zinc and lead adsorption by chernozem and its p^H value

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INTRODUCTION

The soil solution composition significantly effects the way in which heavy metals are absorbed by soil. The aim of this work was to study the effect of the solution anionic composition on Zn and Pb adsorption by chernozem.

MATERIALS AND METHODS

The samples of calcareous chernozem bolted through a 1mm mesh sieve, were added by Pb and Zn acetate and nitrate solutions of 0.001; 0.025; 0.05 and 0.1M/L concentrations. The soil-to-solution ratio made 1:2.5. The suspensions were shaken during 5 minutes, after which they were filtered; the heavy metal contents in filtrates was analysed.

RESULTS AND DISCUSSION

It has been established that heavy metals are most intensely adsorbed over acetate anion against nitrates, Zn being sorbed by an unlimited surface type (see Fig.1-2). The principal reasons of the heavy metal significant adsorption may be the following specific sorption mechanisms: 1) binding of those hydrolysed cation forms which have been produced in the solution, of a charge lesser than the initial ones; 2) direct metal binding with an organic surface-adsorbed ligand; 3) formation of a mixed complex where an adsorbed ligand and an adjoining surface functional group participate. Over a nitrate ion, the metals adsorption is characterised by a limited sorption isotherm (see Fig.1-2). Nitrates suppress specific sorption, therefore the maximum metal sorption is in this case (almost twice) lower than over acetates. The difference in Pb and Zn sorption against nitrate and acetate salt solutions is also stipulated by the peculiarities of metal forms in those solutions. The share of associated Pb and Zn ions in acetate salt solutions is higher than in nitrate solutions due to greater fastness constants of $\text{ZnCH}_3\text{COO}^+$ and $\text{PbCH}_3\text{COO}^+$ ion pairs as compared to ZnNO_3^+ and PbNO_3^+ pairs. Hence, in case of acetate solutions, more Zn and Pb cations are adsorbed as $\text{ZnCH}_3\text{COO}^+$ and $\text{PbCH}_3\text{COO}^+$ ion pairs than in case of nitrate solutions.

Such acetates may produce polymeric complexes like $\text{Me}^{2+}\text{-CH}_3\text{COO}^-\dots\text{OH-Me}^{2+}\text{-OH}^-$ adsorbed on the solid phase surface. Another probable cause is linked with stronger acetate hydrolysis resulting in the formation, within the solution, of a greater number of $\text{Me}(\text{OH})^+$ type associates.

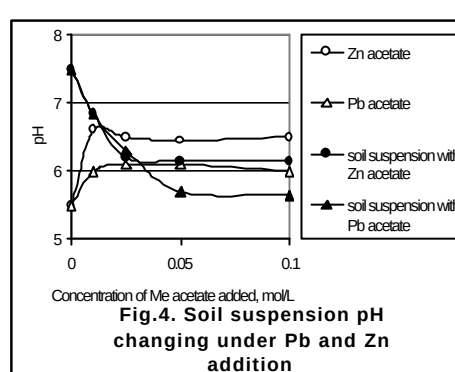
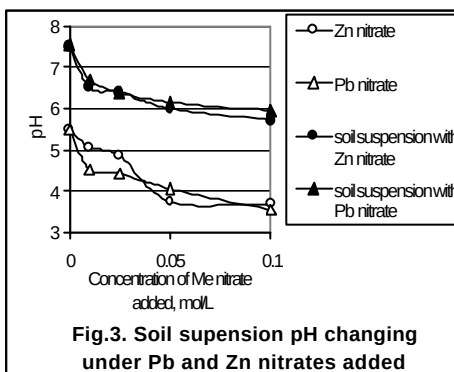
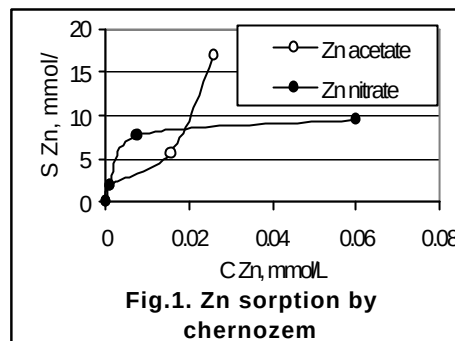
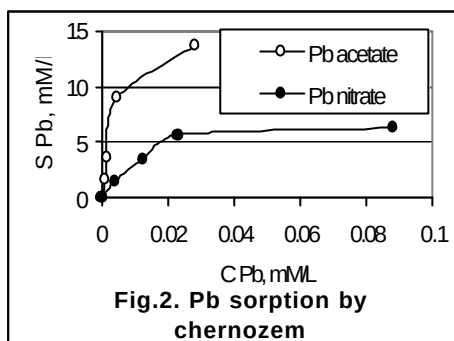
Simultaneous sorption of metals as free ions and singly charged associates is also among the reasons of a compound form of heavy metal isotherm adsorption from acetate solutions.

It has been established that heavy metal cation activity was noticeably lower in acetate suspension than in metal nitrate suspension. This proves the fact that a significant share of metal in acetate solutions is associated into outer-sphere complexes which are better sorbed on the soil particle surface as compared to nitrates and free metal cations.

The heavy metal adsorption mechanism is in many respects determined by p^H values. As shown in Fig.3-4, Pb and Zn introduction into soil (as nitrates) significantly reduces the soil p^H level. Nevertheless, one may speak of chernozem's significant buffering, since the p^H value of the initial solutions of the heavy metal nitrates was lower by about 2 points than prior to its reaction with soil. In this case heavy metals do not produce a noticeable effect upon the character of p^H alteration in the system.

A reverse situation can be observed during heavy metal acetate interaction with soil (Fig.3-4). Regardless of the fact that the initial solutions are characterised by a steady pH level, there occurred medium acidation after their interaction with chernozem. Taking into account the above-described mechanisms, such ?? changes are to take place simultaneously with significant heavy metal adsorption, which is confirmed by the data of metal sorption by chernozem. Zn and Pb acetate sorption is over 15 mM/kg, which exceeds the values of metal sorption over a nitrate anion (see Fig.1-2). Neutral and +1 charged hydroxocomplexes and the metal associates may be adsorbed in the volumes as significant as that. Possible also is direct exchange of non-associated cations to the protons normally inaccessible for any exchange. This is especially characteristic for Pb^{2+} being an element of high intrusion energy. Nitrates block specific sorption, hence they produce a lesser effect on ?? changes.

Thus, there is certain interdependence between heavy metal sorption by soil, soil solution composition and medium ?? .



Ni, Cr Cu & Zn speciation and biodisponibility in grazed field from the Cantal volcanic mountains - France

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CONTEXT AND OBJECTIVES

In the Cantal region, basaltic flows present particularly rich contents in Ni, Cr, Cu and Zn compared to other types of rocks analysed. These flows cover large cattle grazed areas. The MTE (Metal Trace Elements) deriving from the basaltic rock can therefore naturally be found in soil horizons and in the overgrowing grass. Objectives of our study are :

- 1) To determine the proportion and the type of the phases naturally bearing the MTE in the soil horizons (**speciation**)
- 2) To quantify the natural MTE distribution from the parental rock to the soil and into the overgrowing grazed plants (**biodisponibility**)

STUDIED AREA

The pastured field is located at the top of the occidental part of the Mauriac basaltic flow. A wet mountain climate is found locally (altitude = 700m / annual averages over the last 6 years : rainfall = 1300 mm, air temperature = 10,4°C). The rock is a massive basalt Ni and Cr contents encompass the values obtained in 59 over basalts and basanites analysed all over the Cantal volcanic mountains (bulk rock MTE contents : Cr = 371 ppm, Ni = 214 ppm; Zn = 118 ppm; Cu = 49 ppm). The soil is a andic ranker, non allophanic, 24 cm deep and fairly differentiated (A1/A2/R). The grass roots density is important in the A1 horizon (0-8 cm). Roots still appear in great number at the profile bottom between the horizontal basaltic flagstones.

METHODOLOGY

Soil samples were taken along a pedological pit. Total MTE amounts were measured in bulk soil (with roots) and rock by ICP-MS after crushing and alkaline melting of dried samples (CRPG, Nancy). The MTE speciation was explored by optic and electronic microscopy (SEM & microprobe) and using selective extraction : water, cobalt hexamine chloride, sodium phosphate, ammonium oxalate. Grass samples were taken at the grass haymaking period (maturation stage) in tree 15 m-diameter circles located nearby the pedological pit. Composite topsoil samples (0-8 cm) were taken in each circle (5 sub-samples). The plant crushing and MTE contents measurements were determined by the LUPRAVE laboratory (INRA- Bordeaux). The soil bulk density and the plant dry matter yield required for calculating MTE stocks (in g.m⁻²) were measured at the time the soil and the plants were sampled.

RESULTS AND PERSPECTIVES

1. **Speciation in soil** : Referring to the fractions generally attributed to the extraction solution we have used, none of the studied MTE was found to be water soluble, except Zn (> 1%). Ni and Cu are the only exchangeable cations in small proportion (< 6% of their total amount in soil). Organic matter and amorphous oxi-hydroxides bear up to 30 % of the total amount of

Pb and Cu, and only 10% of the Ni and Cr contained in soil. The major parts of Ni and Cr are located in unweathered minerals (spinels, pyroxenes).

2. **Biodisponibility** : Similar bulk soil MTE and major elements contents are obtained along the pit and in the 3 plant sampling circles allowing the comparison of the MTE amounts calculated in the rock, the soil horizons and the grass. An important dilution of the total MTE amounts is found from the rock to the plants, except for Pb. The ratios of **1/4** (Cu et Zn) to **1/5** (Cr et Ni) measured between the stocks measured in the A2 horizon vs the rock shall indicate a dilution by OM accumulation in soil. The ratios of **1/40** (Zn) to **1/80** (Cr) reached from the plants vs the A1 horizon could be explain by the root activity.

Fraction accepted to be associated with the organic matter (Na pyrophosphate extracts) could plenty explain the low MTE quantities found in the grass compared with the topsoil. Further analysis will aim at assessing the environmental risk (for water and grazed plants) of the long-term weathering of mineral phases (spinels and pyroxenes) bearing up to 80 % of the total amount of Ni and Cr in soil.

Copper and Zinc Sorption onto Sod-podzolic Soil in Model Experiment

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INTRODUCTION

The study of soil sorptive peculiarity in environmental pollution gives the opportunity to prognosticate the soil status and to develop techniques for the rehabilitation of such suffered areas. Adsorption is seemed to be one of the main processes of the heavy metal distribution between liquid and solid phases. The Cu and Zn sorption is found to be presumably the ion exchange and adsorption mechanisms in according with the adsorption isotherms equations of Freundlich and Langmuir (Sposito, 1981). The dynamic equilibrium is consisted in the soil between the heavy metal species of soil solution, cation exchange complex and those soil components, which sorbed specifically (McLaren, Crawford, 1973). The aim of this study was to examine sorptive properties of sod-podzolic soil and to evaluate the competitive relationships between Cu and Zn in case of their common presence in soil solution.

OBJECTIVES AND METHODS

The sod-podzolic soil (H, E, B-horizons) was sampled at the distance of 30 km to copper plant smelter. Some soil properties were as follows: pH (water) - 5, Corg 5.4 (in H hor), cation exchange capacity (CEC) 20-40 $\text{meq}/100\text{ g}$; Cu total - 35-40 ppm, Zn total - 60-90 ppm. The samples placed into beakers and contaminated by industrial dust containing both Cu (17%) and Zn (16%) and then incubated during 70 d. The ratio between soil and dust were varied to approach the modelling of the technogenic pollution conditions. The total soil sorptive capacity after incubation has been studied in static conditions. Cu and Zn nitrate solutions were added to the samples (soil:solution as 1:10, concentrations used from 0.10 to 8.0 mg/l), stayed during 48 h, filtrated and analysed by the atomic absorption method. The study on the competitive relationships between Cu and Zn has been done on those solutions containing both elements studied in equal relations from 0.16+0.16 to 4.0+4.0 mg/l and prepared on both the deionised water and 0.05 M $\text{Ca}(\text{NO}_3)_2$. The specific sorption experiment was the same, but $\text{Ca}(\text{NO}_3)_2$ was used instead of the deionised water.

RESULTS AND DISCUSSIONS

The Langmuir equation parameters were accounted for the evaluation of the total and specific Cu and Zn sorption onto the different soil horizons to characterise the metal ion stability and the sorptive centre bonds. The upper and intermediate horizons are seemed to have the most high sorption capacity for Cu both for the contaminated and background ones.

Table 1. The Langmuir equation parameters of total Cu sorption

Variance	$Q_{\max}$, mg/g				K , L/g	
	H	E	B	H	E	B
1	101	52	110	4.1	4.4	2.0
2	100	49	109	2.9	4.1	1.9
3	97	49	105	2.5	4.0	1.8
4	96	45	102	2.1	4.1	1.6
5	94	43	98	1.9	3.8	1.4
6	93	41	79	1.8	3.1	1.5
7	90	39	63	1.3	2.7	1.5

During the increasing of contamination level the decrease of sorption capacity took place. The specific sorption process was characterised by the same tendencies as in case of total sorption. The technogenic impact is influenced as the decrease of sorption Cu capacity and the stability of soil-Cu bonding species. The character of the Zn total isotherm curve is greatly differed from the Cu total one. The first one is characterised by the increase of the quantity of the element sorbed in according with the increase of zinc equilibrium concentration in solution. On the other hand, the second one is characterised by the decrease of the quantity of the element sorbed. Such decrease is found to be the much higher than the level of contamination is became bigger. The ability of the sod-podzolic soil to sorb Zn is supposed to be less in comparison with Cu. Also the ionic solution strength is influenced much more intensively on Zn sorption than Cu bonding. Those findings have been confirmed the different metal bonding hypothesises due to diverse mechanisms and various type sorbtive centres.

Table 2. The Langmuir equation parameters of total Zn sorption

Variance	$Q_{max}, \text{mg/g}$			$K, \text{kg/l}$		
	H	E	B	H	E	B
1	21	12	24	11.0	37.5	6.1
2	19	6	20	10.8	15.6	7.3
3	18	3	18	10.0	9.1	7.7
4	17	3	16	7.4	8.3	7.0
5	17	2	13	4.8	8.8	7.3
6	12	1	13	4.4	4.7	3.4
7	11	1	10	3.6	2.1	3.6

The sod-podzolic soil is slightly sorbed Zn specifically. Zn and Cu being put together have been occupied the specific sorption centres and do not give the possibility of Zn ions to react with them afterwards. Zn is shown to be more mobile in comparison with Cu that is confirmed by its weak soil bonding by non-specific adsorption (or ion exchange) type. The Q_{Zn}/Q_{Cu} dependence (as total) was found to be a linear to suggest one mechanism of the ion competition only. In case of specific adsorption such dependence is shown to be not linear. It is characterised by decrease of the sorbed zinc species in addition of sorbed copper. Cu have to be occupied apparently the specific sorption positions, Zn and Ca opposite has been competed to the ion exchange positions. Ca ion is depressed the Zn sorption. The presence of Zn and Ca ions in the system is weakly influenced the Cu sorption onto soil whereas the presence both Cu and Ca is clearly depressed the Zn sorption.

CONCLUSIONS

Cu is specifically sorbed onto soil and is stable bonded by CEC: sorption capacity was more than 100 mg/100 g. Zn is sorbed by ion-exchange type bonding onto CEC, or non-specifically, being weak bounded and easily migrated in the soil - solution system. Its sorption capacity is consisted of 20 mg/100 g. The cations studied is ranged due to their sorption property as follows: **Cu > Ca > Zn**. This dependence is shown to be more evident in case of pollution in comparison with natural areas or slightly contaminated ones.

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Modeling Adsorption-Desorption Kinetics of As(V) in soils

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INTRODUCTION

Knowledge of adsorption and desorption of As on soil mineral surfaces is a prerequisite for predicting its fate in the soil environment. Several studies have demonstrated that arsenic adsorption is correlated with soil clay contents and also Al and Fe oxides. Inner-sphere complexation on various charge mineral surfaces (Fe, Al and Mn oxides and hydroxides; allophanes, imogolite) is a possible pathway of As sorption in soils (Violante and Pigna, 2002). Numerous examples are available which illustrated the use of the equilibrium approach for describing As adsorption-desorption in soils. Kinetic data, which are infrequently measured, have the advantage of taking into account possible time-dependent reactions for adsorption, release, or desorption. Nonequilibrium conditions may be due to heterogeneity of sorption sites and slow diffusion to sites within the soil matrix, i.e. slowly accessible sites with variable degrees of affinities to heavy metals. In this study our focus was (i) to quantify the kinetics of adsorption and desorption of As in two different soils: Sharkey clay and Olivier silt loam; and (ii) to assess equilibrium and multiple-reaction kinetic type models in their capability of describing the sorption as well as release behavior of As in these soils.

METHODS

Surface soils of Sharkey clay (Very-fine, montmorillonitic, nonacid, thermic Vertic) and Olivier silt loam (Fine-silty, mixed, thermic Aquic Fragiudalf) were used in this study. Batch experiments were conducted to determine adsorption and desorption isotherms for As (V) by our two soils. The technique used here is kinetic batch type. Six initial As(V) concentrations C_0 's (5, 10, 20, 40, 80, and 100 mg L⁻¹) of KH₂AsO₄ were prepared in 0.01M KNO₃ background solution. Batch experiments were initiated by mixing 3 g of air dry soil with 30 ml of As(V) solution in a 40-mL Teflon tube. Triplicates were used for each input concentration C_0 and the amount of As(v) adsorbed was taken as the average of the three replicates. The mixtures were kept shaking, and centrifuged at 500 × g for 10 minutes for each specific reaction time before sampling. A 1-mL aliquot was sampled from the supernatant at reaction times of 2, 6, 12, 24, 72, 168, 336, and 504 h. After sampling, the slurry was agitated using a vortex mixer and returned to the shaker. The collected samples were analyzed for As(V) using ICP-AES. The extent of release or desorption of As(V) was also quantified using the batch method described above. For each initial concentration (C_0), desorption commenced immediately after the last adsorption time step (504 h). Sequential or successive dilutions of the slurries were carried to induce heavy metal release or desorption. Each desorption step was conducted by replacing the supernatant with heavy metal free 0.01 M KNO₃ background solution and shaking for 48 hours. Six desorption steps were carried out with a total desorption time of 12 days. The amount of As(V) retained by the soil following the last desorption step was determined using sequential extraction methods (Keon et al., 2001). Three fractions were quantified; namely that strongly adsorbed (1M NaH₂PO₄), oxide bound (0.2 M ammonium oxalate), and residual (4N HNO₃). The first two phases were measured by mixing the soil with 20mL of the extractant solution, shaking for 16h, and centrifuging, whereas the residual As was determined by mixing with 4N HNO₃ solution and shaking for 2h in a water-bath maintained at 80°C.

RESULTS AND DISCUSSION

The extent of time-dependent retention for As(V) in Sharkey clay soil is illustrated by the adsorption isotherms for the different reaction times (see Fig. 1). Moreover, adsorption isotherms are commonly described by either an equilibrium type model of the Freundlich- or Langmuir-type. In this

study, adsorption results were described based on a Freundlich equilibrium model. For Olivier soil, the isotherms exhibited somewhat similar nonlinearity and kinetic behaviour except that lower retention maxima were observed.

Desorption results for As behavior in Sharkey clay soil are presented as isotherms in the traditional manner in Figs. 2. The hysteretic behavior resulting from discrepancy between adsorption and desorption isotherms was not surprising in view of the kinetic retention behavior of As(V) in our soils. Several studies indicated that observed hysteresis in batch experiments may be due to kinetic retention behavior and slow release and/or irreversible adsorption conditions. Adsorption-desorption isotherms indicate that the amount of irreversible or nondesorbable phases increased with time of reaction. As(V) may be retained by heterogeneous type sites having a wide range of binding energies. At low concentrations, binding may be irreversible. The irreversible amount almost always increased with time. In this study, we discuss our modeling efforts when a multireaction kinetic approach to describe As retention kinetics in soils was utilized. A major feature of the multireaction model is that it considers several interactions of a reactive solute with soil matrix surfaces (Selim and Amacher, 1997). Specifically, the model assumes that a fraction of the total sites is kinetic in nature whereas the remaining fractions interact rapidly or instantaneously with solute in the soil solution. The model accounts for reversible as well as irreversible retention reactions of the concurrent and consecutive type.

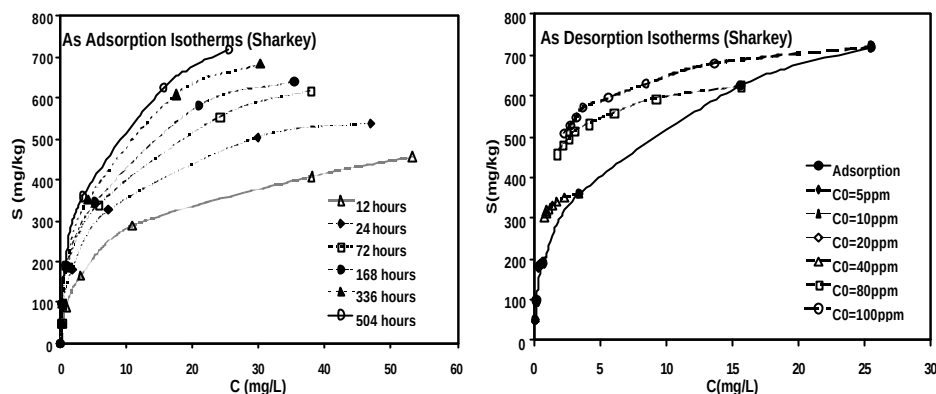


Fig. 1. As(V) adsorption isotherms for Sharkey clay soil at different reaction times.

Fig. 2. Traditional As(V) desorption isotherms for Sharkey clay soil based on seven successive desorptions after 504 h of adsorption.

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A comparison of sequential extraction procedures for fractionation of arsenic in soil

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INTRODUCTION

Sequential analytical schemes belong to the reasonable analytical procedures leading to precise evaluation of element distribution within individual soil fractions as well as to elucidation element fluctuation in soil. The number of different extraction procedures developed in this context leads to limited comparison of results from individual laboratories. Standardization studies performed by Standards, Measurement and Testing program (SM&T) resulted in a three-step sequential extraction procedure recommended in laboratories within European Union^{1,2}. However, arsenic was not included among the elements investigated in the range of standardization tests. Because of different chemical properties of arsenic, single-purpose sequential extraction schemes reflecting different behavior of this element in soil have been recently published^{3,4}. This study compares results of arsenic fractionation using the SM&T scheme with two frequently applied sequential extraction procedures^{5,6} which have been developed for determination of a wide spectrum of elements, especially metals.

MATERIALS AND METHODS

Twelve soil samples differing in physical-chemical properties and ranging the total arsenic concentrations between 5.04 and 259 mg As.kg⁻¹ were extracted by three sequential extraction procedures (Table 1), and arsenic was determined in individual extracts by hydride generation AAS (Varian SpectrAA-300). Wilcoxon's non-parametric test and linear regression model were applied for the evaluation of the analytical data.

RESULTS AND DISCUSSION

Compared to SM&T extraction scheme, the method B and C divided the first fraction into two parts to describe in more details the most mobile portion of elements. The method C was concentrated on detailed characterization of element bindings into clay minerals resulting in resolution of element portions in three soil oxide fractions. For the comparison of analytical data with SM&T method, sum of mobile fractions (fraction 1+2) was calculated for methods B and C and sum of oxide fractions (fractions 3+4+5) for method C.

The arsenic portion released as sum of mobile fractions varied in range from 0.85 % (method B) to 4.2 % (method A) of total arsenic content in soil, and the differences among the methods were significant at $\alpha = 0.05$. However, the correlation coefficients varying in range 0.64 – 0.91 suggested similar trend in arsenic extraction ability from experimental set of samples. For sum of oxide fractions, medians of released arsenic portion represented between 2.9 % (method B) and 17.6 % (method C) of total arsenic content and differed significantly, as well. Correlation coefficients varied in range 0.59 – 0.96. Similar pattern of individual extracting procedures was observed in the case of determination of organically bound arsenic fraction where arsenic portions varied between 2.2 % (method C) and 7.4 % (method B) differing significantly at $\alpha = 0.05$ while the data tightly correlated ($r = 0.70 - 0.94$). Concerning residual fraction representing the most stabile and non-extractable element fraction ranging between 71 % (method C) and 87 % (method B), the results confirmed low mobility of soil arsenic. Fairly good correlation of the data ($r = 0.75 - 0.97$) was expected regarding the previous arsenic fractions.

Table 1. Sequence of extractants and soil element fractions defined within individual sequential extraction schemes

Method A (SM&T) ¹			Method B ⁵			Method C ⁶		
1	exchang.	0.11 mol.l ⁻¹ acet. acid	1	exchang.	0.5 mol.l ⁻¹ MgCl ₂	1	mobile	1 mol.l ⁻¹ NH ₄ NO ₃
			2	carbonate	1 mol.l ⁻¹ CH ₃ COONa	2	weakly bound	1 mol.l ⁻¹ CH ₃ COONH ₄
2	Fe-Mn oxides	0.1 mol.l ⁻¹ NH ₂ OH.HCl	3	Fe-Mn oxides	0.04 mol.l ⁻¹ NH ₂ OH.HCl	3	Mn oxides	0.1 mol.l ⁻¹ NH ₂ OH.HCl
						4	Fe oxides amor-phous	0.2 mol.l ⁻¹ (COONH ₄) ₂
						5	Fe oxides crystalline	0.1 mol.l ⁻¹ ascorbic acid + 0.2 mol.l ⁻¹ (COONH ₄) ₂
3	organ. bound	8.8 mol.l ⁻¹ H ₂ O ₂ + 1 mol.l ⁻¹ CH ₃ COONH ₄	4	organ. bound	8.8 mol.l ⁻¹ H ₂ O ₂ + 3.2 mol.l ⁻¹ CH ₃ COONH	6	organ. bound	0.025 mol.l ⁻¹ NH ₄ EDTA
4								

CONCLUSIONS

The results confirmed that determination of arsenic portions bound in individual soil fractions is strongly dependent on extracting agent and/or procedure applied within individual extracting schemes. Even small modification of sequential extracting procedure can result in significant variability of results among laboratories⁷. Expectably, absolute values of arsenic released among individual extracting procedures are weakly comparable. However, significant correlations of analytical data suggested that trends in characterization of arsenic distribution into main soil fractions agreed regardless of extraction procedure applied.

ACKNOWLEDGEMENTS

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Does reverse osmosis affect metal binding properties of natural dissolved organic matter?

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INTRODUCTION

The speciation, mobility and toxicity of heavy metals in natural water is strongly affected by the presence of dissolved organic carbon (DOC) due to the formation of dissolved complexes between DOC and the metals. The distinction between free and organically complexed metals is of extreme importance for assessing the speciation and bioavailability of metals in the environment.

In order to study effects of organic matter on metal binding and toxicity, reverse osmosis (RO) has been widely used as a highly efficient method for rapid collection of dissolved organic matter (DOM) from natural surface waters. However, to our knowledge, no studies are available that investigated the possible effect of this isolation method on metal binding properties of DOM and its relation with metal toxicity.

In this study the differences and similarities were tested between organic matter present in a water source and organic matter concentrated from the same source using RO. To that end, in parallel, equilibrium dialysis experiments and toxicity tests with *Daphnia magna* were performed using the metals Cu and Zn. Equilibrium dialysis is a common technique used in binding studies of low molecular weight solutes to macromolecules.

MATERIALS AND METHODS

The source water for this study was 'Ruisseau de St. Martin', located in Bihain, Belgium. This is a small, slightly acidic (pH ~ 5.7) creek running through a highland peat area. DOM was concentrated in the field using a portable Reverse Osmosis system as described by Sun et al. (1995). Natural water was collected during the same isolation run after the micro-filter unit. Reconstituted water was obtained by diluting RO isolates with distilled and deionised water in the laboratory to natural DOM level. Both natural and reconstituted water were used in dialysis and toxicity experiments.

For the dialysis experiments, Spectra/Por® CE dialysis membrane tubing with a molecular weight cut-off (MWCO) of 100 Da was used. Poly-ethylene containers (PE) were filled with the spiked solution. In this outer solution a dialysis tube filled with metal free water was immersed. In all cases the pH and electrical conductivity (EC) of the metal free water were adjusted to match the EC and pH of the outer solution. The volume ratio of outside/inside solution was set at 10:1. During the experiment the closed containers were continuously shaken at a constant room temperature of 25°C. At day 4, samples were taken from both the inside and outside solutions and analysed for metals to allow calculation of the 'free' and 'bound' metal.

For the toxicity tests with juvenile *D. magna*, natural and reconstituted water were spiked with 5 metal concentrations. Toxicity tests were performed according to OECD (1984).

RESULTS AND DISCUSSION

Organic (DOC) and inorganic composition (Ca, Mg, Na, K, Cl, SO₄, Fe, Al, Cu and Zn) of natural and reconstituted water samples were similar (t-test for dependent samples, $\alpha=0.05$). The results from

equilibrium dialysis experiments indicated that within the tested concentration ranges of Cu and Zn no saturation of binding sites on organic matter was reached for both the natural and the reconstituted sample. Regression equations of 'bound metal' versus 'free metal' were not significantly different (Table 1). 'Free metal' as measured by dialysis equilibrium consisted of hydrated metal cations and inorganic metal complexes such as hydroxides and carbonates, while 'bound metal' refers to metal bound with organic matter (Jansen et al, 2001).

Table 1. Linear regression equations of $y = p(M_{\text{bound}}) = -\log(M_{\text{bound}})$ versus $x = p(M_{\text{free}}) = -\log(M_{\text{free}})$

	Natural	Reconstituted
Copper	$y = 0.895x - 1.065$ ($R^2=0.98$)	$y = 0.934x - 1.115$ ($R^2=0.98$)
Zinc	$y = 1.150x - 2.311$ ($R^2=0.96$)	$y = 1.200x - 2.676$ ($R^2=0.93$)

Similar conclusions can be drawn from results of the toxicity tests with *Daphnia magna* (Table 2). 48-hour EC50 values of copper and zinc were not significantly different for the natural water and the reconstituted water sample.

Table 2. 48-hour EC50 values of copper and zinc (in $\mu\text{g/L}$) to *Daphnia magna* in natural water and reconstituted water. Figures between brackets indicate 95% confidence intervals.

	Natural	Reconstituted
Copper	77.1 (70.0-84.9)	66.7 (59.5-74.7)
Zinc	335 (291-385)	340 (271-426)

CONCLUSION

Both dialysis experiments and toxicity experiments indicate that isolating organic matter using reverse osmosis does not significantly affect the metal binding and effects on toxicity of present organic matter. Consequently, the advantage of RO-concentration, i.e. yielding large quantities of organic matter, can safely be exploited in both metal binding and metal toxicity research of natural organic matter.

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Evolution of soil solution copper concentration as a function of Eh, pH and dissolved organic carbon

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INTRODUCTION

It is well known that the total soil content of any element is a poor estimation of its mobility and bioavailability. Water solubility is in many cases a better, if imperfect, tool to assess mobility. However soil chemistry is subject to strong spatial and temporal variation and so a single measurement is often inadequate to allow reliable predictions. We have chosen to investigate the evolution of water soluble copper over a period of 3 months as a function of incubation conditions. We have focussed on soil moisture content and the addition of an organic amendment. These factors are likely to induce changes in both redox potential and pH, thereby modifying the adsorption properties and solubility of various soil minerals, and of course the solubility of soil organic matter that plays important roles in the speciation of trace metals in both the solid and solution phases.

MATERIALS & METHODS

The soil studied was a sandy clay loam from the Batcombe Series (UK) with a pH of 6, a cation exchange capacity of 142 mmol_c kg⁻¹, an organic carbon content of 32 g kg⁻¹. Soil was airdried and sieved to 0.25 mm. Soil was wet to either 38 % (by weight) moisture content (60% field capacity) or flooded, amended with 0 or 2% (by weight) of dried, ground alfalfa straw and amended with 0 or 100 mg kg⁻¹ Cu as the chloride salt. The initial total Cu content of the soil was 16 mg kg⁻¹ and that of the alfalfa straw 9 mg kg⁻¹. Mixtures were mixed thoroughly, packed into polypropylene containers and loosely covered. All samples were incubated at 20°C and 85% relative humidity. Soils were sampled after 1, 8, 15, 30, 60 and 100 days. Water soluble Cu and dissolved organic carbon (DOC) were measured after suspending 2 g soil (on a dry weight basis) of moist soil in 15 ml water, shaking for 1 h at 20°C, centrifuging then filtering. Both water soluble Cu and DOC are expressed with respect to soil dry weight. For the same incubation periods, Eh was measured potentiometrically and the pH measured in soil suspension.

RESULTS & DISCUSSION

The redox potential of the moist soils was fairly constant, whereas that of the flooded soils fell rapidly. The rate of decrease was faster in the amended soil, but there was little difference between samples after 1 month. Similarly the pH of the flooded soils was greater than the moist soils, there was little effect of organic amendment and only small changes with time. DOC was somewhat greater in the flooded soils and unsurprisingly greater in the amended samples. In general a rapid increase in DOC was observed followed by a more gradual decrease.

Water soluble Cu followed similar trends in the Cu enriched and native-Cu samples. Added Cu was more water soluble than native soil Cu. There was a tendency for water soluble Cu content to increase then decrease with time. Despite the large changes in redox potential, that might be expected to induce changes in Cu speciation, no correlation between water soluble Cu and Eh was observed (data not shown). We have therefore investigated the possible effects of pH and DOC that are widely reported to strongly influence metal solubility in soils.

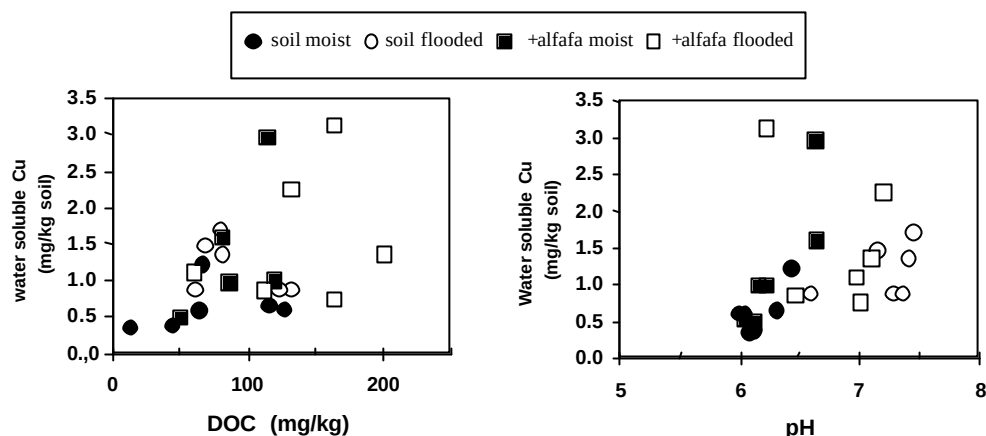


Figure 1 Water soluble Cu content of Cu-enriched samples as a function of DOC.

Figure 2 Water soluble Cu content of Cu-enriched samples as a function of pH.

Figures 1 and 2 show how water soluble Cu in the Cu-enriched samples varies with DOC content and pH for the 4 treatments and the 5 incubation periods. Qualitatively similar relations were observed for the native soil Cu. The positive correlation between water soluble Cu and DOC suggests the importance of the formation of organic complexes in solution. However, the positive correlation with pH is surprising. If the two points corresponding to a release of Cu one day after organic amendment are ignored, there appear to be two distinct correlations for moist and flooded soils. In the case of the flooded soils there is a strong inverse correlation between DOC and pH (not shown) and so the apparent link with DOC and soluble Cu may be driven by complexation organic matter in solution. This is not the case in the moist soil.

In conclusion, soil moisture content and organic amendment caused major changes in soil redox potential and DOC and lesser changes in soil pH over a 3-month period. These changes in soil chemistry have variable consequences on the solubility of both native and added Cu.

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Flow Analysis of Inorganic Species of Arsenic in Groundwater by Stripping Voltammetry at Tubular Gold Electrodes

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INTRODUCTION

Arsenic is widely distributed throughout the earth's crust and is used commercially, primarily in alloying agents. It is introduced into water through the dissolution of minerals and ores, industrial effluents, and atmospheric deposition; concentrations in ground water in some areas are sometimes elevated as a result of erosion from natural sources. The average daily intake of inorganic arsenic in water is estimated to be similar to that from food; intake from air is negligible.

Inorganic arsenic is a documented human carcinogen and has been classified by IARC in Group 1. A relatively high incidence of skin and possibly other cancers that increase with dose and age has been observed in populations ingesting water containing high concentrations of arsenic. Inorganic arsenic can occur in the environment in several forms but in natural waters, and thus in drinking water, it is mostly found as trivalent arsenite (As^{III}) or pentavalent arsenate (As^{V}). Organic arsenic species, abundant in seafood, are very much less harmful to health, and are readily eliminated by the body.

Drinking water poses the greatest threat to public health from arsenic. With a view to reducing the concentration of this carcinogenic contaminant in drinking water, the World Health Organization has established a provisional guideline value for arsenic in drinking water of 0.01 mg/L [1]. A similar value may be derived (assuming a 20% allocation to drinking water) on the basis of the provisional maximum tolerable daily intake for inorganic arsenic of 2 $\mu\text{g/kg}$ of body weight established by Joint of JECFA in 1983 (WHO, 1993).

Accurate measurement of inorganic arsenic species in drinking water at levels relevant to health requires very sensible methods. Stripping voltammetric detection in a flow system has demonstrated to be adequate for monitoring of low levels of toxic elements in water due to its inherent advantages: higher sampling rates, low detection limits, good selectivity, and improved precision and accuracy as a result of a lesser handling of the sample (Stulik and Pacakova, 1987). Moreover, the stripping step can be carried out in a different medium, which reduces the interferences caused by the analytical matrix thus rendering increased accuracy (Aldstadt et al, 1994).

METHODS

Groundwater samples were collected during summer 2001 from shallow dug wells, deep drilled wells and springs used for drinking and irrigation, all located in the region of Castilla y León (Spain).

Samples were filtered through 0.45 μm membrane filters into acid-washed polyethylene bottles, and acidified to pH 2 with hydrochloric acid. 2 mM hydrazine was added to each sample to prevent oxidation of As^{III} . Samples were kept refrigerated (4°C) until their determination. The performance of a flow-through electrochemical cell for speciation of inorganic arsenic (As^{III} and As^{V}) in water by anodic stripping voltammetry has been investigated. The tubular cell (see Figure) consisted of a tubular body of perspex carrying a tubular gold working electrode, a tubular glassy carbon auxiliary electrode and a standard Ag/AgCl reference electrode. The internal diameter of the tubular electrodes is 0.8 mm.

The speciation of inorganic arsenic consisted in two separate in-flow measurements: (i) As^{III} is plated onto the gold electrode from a solution slightly acidic (pH 1-2) and then anodically stripped in 2 M HCl. (ii) Total As is determined as As^{III} after in-flow reduction of As^{V} with iodide in a slightly acidic medium (pH 1-2). Stripping of accumulated As(0) was performed in the stopped-flow mode after medium exchange by square-wave anodic stripping voltammetry (SWASV).

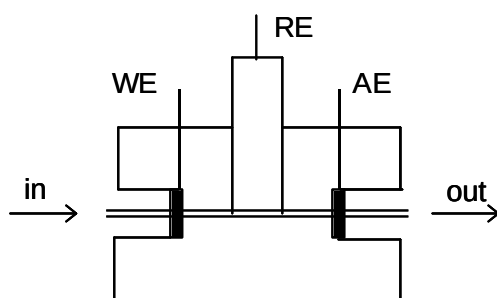


Fig. 1. Scheme of the tubular flow-through electrochemical cell designed for As speciation. WE, tubular gold working electrode; RE, Ag/AgCl, KCl reference electrode; AE, tubular glassy carbon electrode.

RESULTS AND DISCUSSION

The electrochemical cell was integrated in a flow system. A peristaltic pump and a commuting valve used for medium exchange propelled sample and reagents towards the cell.

The effect of several variables such as flow rate, pH, electrolyte concentration, iodide concentration, deposition potential, deposition time or scan rate, on the anodic stripping peak of arsenic was investigated. Optimal peaks were obtained in the following experimental conditions:

Accumulation step: deposition potential, -0.4 V; flow rate, 0.5 mL/min; pH, 1-2 (by addition of HCl); iodide concentration (for total As determination), 2 mM.

Stripping step: HCl concentration, 2 M; scan rate, 200 mV/s; frequency, 25 Hz; pulse amplitude, 25 mV.

The procedure was validated by comparison with a similar batch method optimised with this purpose, and with electrothermal atomic absorption spectrometry (ETAAS). Limits of detection of 0.09 µg/L and 1.22 µg/L were calculated for deposition times of 5 and 1 min, respectively.

Arsenic species in groundwater samples were quantified by the standard additions method. Concentrations ranged from 0.1 to 20 µg/L (mostly as As^V).

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Phytoremediation

Scientific Program 6

Oral

Ability of cadmium tolerance in transgenic tobacco plants

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INTRODUCTION

Natural hyperaccumulators of heavy metals grow slowly and have low biomass yield. Improvement of plants by genetic engineering opens new possibilities especially for phytoextraction. Genetic engineering is a technique that might be applied advantageously to the search for larger phytoremediation potential of plants combining high metal accumulating capacity and high above ground biomass yield (Kärenlampi et al. 2000). The introduction of an additional metal binding domain to the implemented protein should further enhance the metal binding capacity (Kotrba et al. 1999).

MATERIAL AND METHODS

Tobacco, *Nicotiana tabacum* L., var. Wisconsin 38 as the control (WSC), and genetically modified line of the same variety, bearing the transgene coding for the polyhistidine cluster, combined with yeast metallothionein (HisCUP) was tested in sand medium. The plants were genetically transformed as described by Macek et al. (2002).

The screening test was conducted on sand nutrient medium with addition of Knop's nutrient solution modified by addition of elevated cadmium doses (0.2 – 0.6 – 1.8 – 5.4 – 16.2 mg Cd .l⁻¹ of nutrient solution as Cd(NO₃)₂.4H₂O). The plants were inserted into medium, planted and harvested after six weeks of growth.

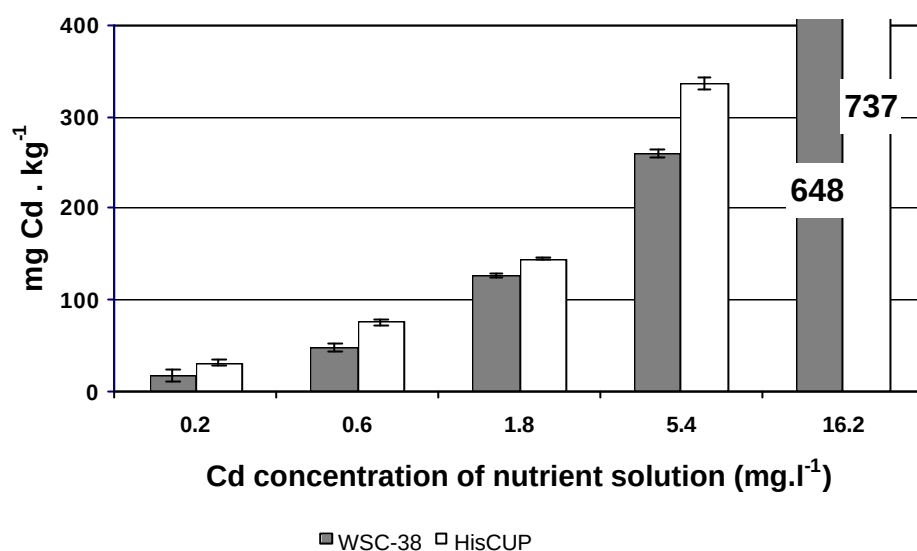
Plant material was decomposed by dry ashing procedure and Cd concentration in plants and in roots was determined by atomic absorption spectrometry (Varian SpectrAA-300).

RESULTS AND DISCUSSION

The HisCUP construct proved to have a positive effect on Cd accumulation. The Cd content in above ground biomass of tobacco growing in Cd concentration 0.2 mg Cd.l⁻¹ was increased by 80 % compare to the non-transformed control. These results confirmed our previous experiments that accumulation of cadmium significantly increased in HisCUP tobacco plants (Macek et al. 2002). Significantly increasing Cd accumulation was also determined in HisCUP tobacco growing in solution with Cd concentration 0.6 mg Cd.l⁻¹. Cadmium content in roots of control tobacco did not differ from transgenic plants and was lower compare to above ground biomass.

Next three doses 1.8, 5.4 and 16.2 mg Cd.l⁻¹ caused lower differences of Cd contents in plant above ground biomass and roots between control and HisCUP. Dose of 5.4 mg Cd.l⁻¹ induced cadmium toxicity symptoms in leaves of control tobacco described by Adriano (2001). Control plants exhibited Fe chlorosis, red coloration of young leaves and mainly reduction in growth. The highest dose of Cd (16.2 mg Cd.l⁻¹) depressed root and plant growth and was lethal for them at this treatment. HisCUP showed increasing plant resistance against stress response induced by cadmium. Both doses were not toxic for transgenic plants. Kärenlampi et al. (2000) suggested that metallothionein gene might be useful in improving metal tolerance of plants. It seems to have significant effect on Cd tolerance of HisCUP tobacco in our experiment. Increasing cadmium tolerance of transgenic tobacco confirmed a good chance to prepare improved transgenic plants for phytoremediation purposes.

Cd content in above ground biomass



CONCLUSION

Higher doses of cadmium (5.4 and 16.2 mg Cd.l⁻¹) induced Cd toxicity symptoms in control plants and depressed their root and plant growth. Transgenic tobacco showed significant increasing Cd accumulation and resistance against stress response induced by cadmium. Toxicity symptoms were not observed even at concentrations, which were detrimental to the control non-transgenic plants.

ACKNOWLEDGEMENTS

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Changes in bioavailability and Cd and Zn accumulation by *Salix* sp. in polluted soils

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INTRODUCTION

Willows meet main requirements for suitable phytoextraction, and could be used for reclamation of low to moderately contaminated soils by heavy metals^{1,2}. Willow plants have different capabilities to accumulate different heavy metals among the clones. The mechanisms behind the accumulation, transport, and tolerance are specific for each of the different metal, and uptake capacity of the clone is genetically stable. *Salix* sp. under environmental conditions of Czech Republic can reach yield of dry biomass about 10 t.ha⁻¹.yr⁻¹ ref. ³. *Salix* sp. was shown as cadmium and zinc shoot accumulator⁴ leading to high removal of the elements from the soil by harvest. Long-term *Salix* cropping decreased the plant-available Cd throughout the whole soil profile, thus total content of Cd in soil was only slightly influenced⁵. Greger and Landberg² found that 24% of the total and up to 35% of exchangeable fraction of Cd had been removed from the soil by willows in a pot experiment.

MATERIALS AND METHOD

Bioavailability of Cd and Zn and their accumulation by *Salix* sp. was investigated in a pot experiment. Heavily polluted Fluvisols Litavka, Cambisols Příbram with moderate contamination, and unpolluted Chernozems Suchdol as control were used. Mean total contents of Cd and Zn in Litavka soil were 30.5 and 3718 mg.kg⁻¹, in Příbram soil were 4.73 and 180 mg.kg⁻¹, and in Suchdol soil 0.416 and 87.1 mg.kg⁻¹, respectively. Seven clones of high biomass production willows (*S. x smithiana* S-218, *S. x smithiana* S-150, *S. viminalis* S-519, *S. alba* S-464, *S. alba* 'Pyramidalis' S-141, *S. dasyclados* S-406, *S. x rubens* S-391) were planted for five and half months. After harvest, the aboveground biomass was separated to leaves and twigs and plant samples were decomposed by modified dry ashing procedure in the mixture of oxidizing gases (O₂ + O₃ + NO_x) in APION Dry Mode Mineralizer. The soil samples collected at the beginning, in the middle and at the end of vegetation period were extracted by 0.01 mol.l⁻¹ CaCl₂ in ratio 1:10 (w/v). Element concentrations in the digests were determined by atomic absorption spectrometry (VARIAN SpectrAA-400, Varian, Australia).

RESULTS AND DISCUSSION

Tested *Salix* sp. clones confirmed high aboveground cadmium and zinc accumulation potential. Comparing individual parts of plants, Cd and Zn were more intensively accumulated in leaves than in twigs. Cd accumulation in leaves varied from 9.5 to 101 mg.kg⁻¹d.wt. at Litavka soil, from 38.3 to 89.3 mg.kg⁻¹d.wt. at Příbram soil, and from 0.9 to 3.7 mg.kg⁻¹d.wt. at Suchdol soil. Cadmium was mostly accumulated by leaves of *S. x rubens* S-391, but only at Příbram and Suchdol soils, and by *S. x smithiana* S-150 at Litavka soil. Zn accumulation in leaves varied from 1464 to 4509 (*S. x smithiana* S-150) mg.kg⁻¹d.wt. at Litavka soil, from 243 to 572 mg.kg⁻¹d.wt. at Příbram soil, and from 36 to 144 mg.kg⁻¹d.wt. at Suchdol soil. Zinc was predominantly accumulated by *S. x rubens* S-391 at Příbram and Suchdol soils. High pollution of Litavka soil significantly reduced yield of aboveground biomass in comparison with moderately contaminated Příbram soil and control soil. Thereby remediation factors of Cd and Zn in highly contaminated soil were negatively influenced by low yield of harvested biomass (table 1).

Table 1. Remediation factors Rf (% of element taken off by aboveground biomass from total content of element in soil) of seven clones of *Salix sp.* (see above), (four replications)

Clone no.	S-218	S-150	S-519	S-464	S-141	S-406	S-391
Rf Cd (%)							
Litavka	0.162	0.395	0.209	0.037	0.183	0.467	0.321
Příbram	10.3	12.5	8.1	7.6	9.5	11.5	10.9
Suchdol	2.83	1.70	0.584	0.627	0.845	1.60	2.36
Rf Zn (%)							
Litavka	0.112	0.139	0.117	0.032	0.099	0.183	0.093
Příbram	1.17	1.07	0.907	0.669	1.09	1.12	1.68
Suchdol	0.648	0.137	0.145	0.129	0.295	0.212	0.452

At the beginning of vegetation period the CaCl_2 extractable Cd and Zn contents represented 12.63, 6.56 and 0.92 % of total Cd and 5.87, 0.29 and 0.08 % of total Zn at Litavka, Příbram, and Suchdol soils, respectively. For Suchdol and Příbram soils, no differences were found in plant-availability of Cd and Zn during vegetation period. However, significant decrease of Zn (45%) and Cd (27%) plant availability during vegetation was reported in highly contaminated Litavka soil (Figure 1).

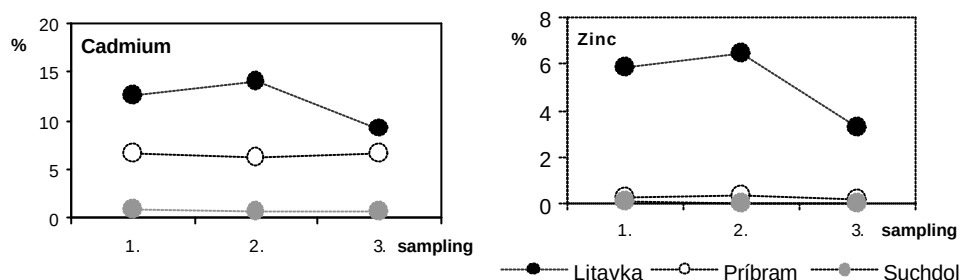


Figure 1. Medians of relative soil element portions released by $0.01 \text{ mol.l}^{-1} \text{ CaCl}_2$ (Sampling 1 before, sampling 2 in the middle and sampling 3 at the end of vegetation period)

CONCLUSIONS

Selected clones of *Salix sp.* (*S. x smithiana* and *S. x rubens*) were confirmed to be suitable phytoextractors of moderately Cd and Zn contaminated soil. *Salix* cropping decreased plant-available portions of Cd and Zn in the most polluted soil.

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Treatment and remediation technologies

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Oral

Radioactive caesium in a housing area in the Bryansk Region, Russia.

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INTRODUCTION

The Chernobyl accident led to a contamination of more than 8000 km² of Russian territory with ¹³⁷Cs levels exceeding 200 kBq m⁻². The greatest part of these affected areas lie in the Bryansk region, where prolonged heavy rain over the days following the accident in some areas resulted in ¹³⁷Cs contamination level as high as 4 MBq m⁻². In the Bryansk region as a whole, 15 settlements with a total population of 22,000 received a ¹³⁷Cs contamination exceeding 1.5 MBq m⁻². As a consequence of the contamination, large living areas were deserted over the following years. Due to the severe social and economical penalties of relocations, reclamation of the affected settlements remains a desirable option, and results obtained during a field campaign to the Bryansk region in 1995 (Fogh et al, 1999) showed that optimised implementation of simple countermeasures involving hand-tools could reduce the external dose rate considerably, even though nearly 10 years had passed since the accident. In the Autumn of 1997, the same research team consisting of Russian and Danish scientists made another decontamination effort in a housing area in the Bryansk region. The main objective was here to examine whether common contractor machinery (a 'Bobcat' mini-bulldozer) was suitable for reduction of the dose rate contribution from soil areas in the living environment. Compared to the use of hand-tools, this would be expected to greatly speed up the process of decontamination of the vast contaminated living areas.

RESULTS AND DISCUSSION

The methods that were used in the work were topsoil removal using shovels or a 'Bobcat', application of an uncontaminated layer of sand or gravel to restore the ground and at the same time shield against radiation from any residual contamination in the ground. Roofs were treated in different ways: cleaning by brushing, cleaning by high pressure driven rotating brush and by replacing the roof. Fig. 1 shows the results of the dose rate measurements made on a line between two houses in the treated area during the various stages of the operation. As can be seen, there is a distinct change in dose rate going from an untreated to a treated part of the area.

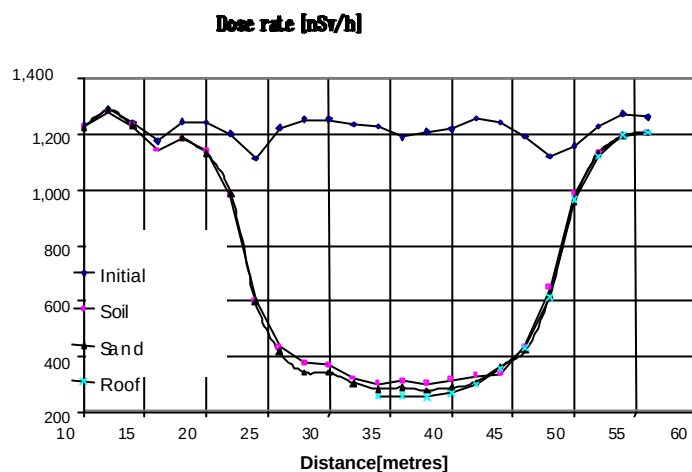


Fig. 1. Dose rate along the centreline between two houses in the area

Some examples of the results from outdoor and indoor measurements (at ground level and first floor level) of the reduction in dose rate in the area due to various parts of a 'clean up strategy' are shown in Figures 2, 3 and 4.

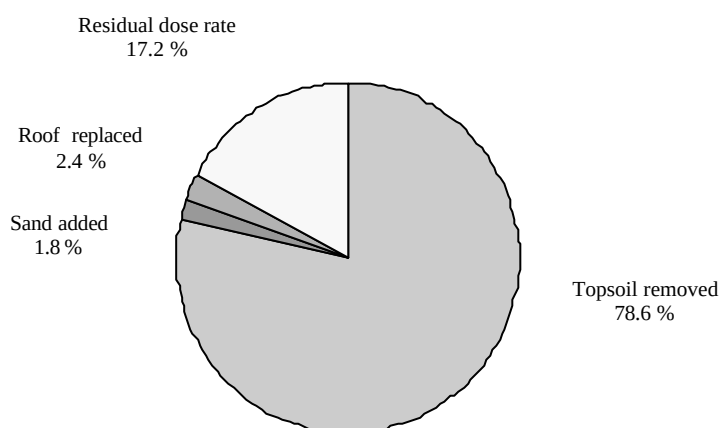


Fig. 2. Reduction in the contribution to dose rate from the contamination, measured outdoors between two houses.

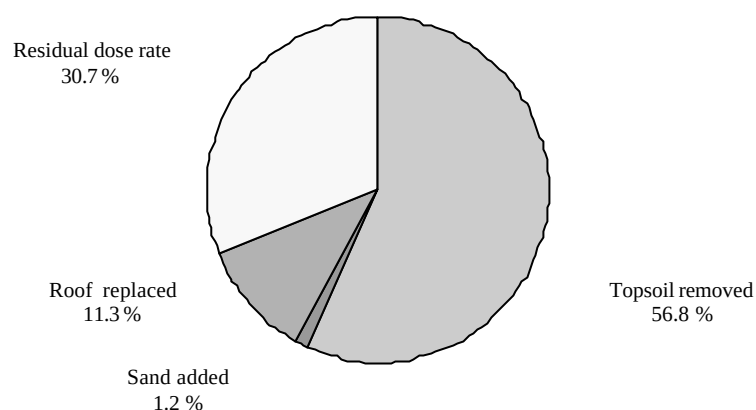


Fig. 3. Reduction in the contribution to dose rate from the contamination, measured on the ground floor of house 1.

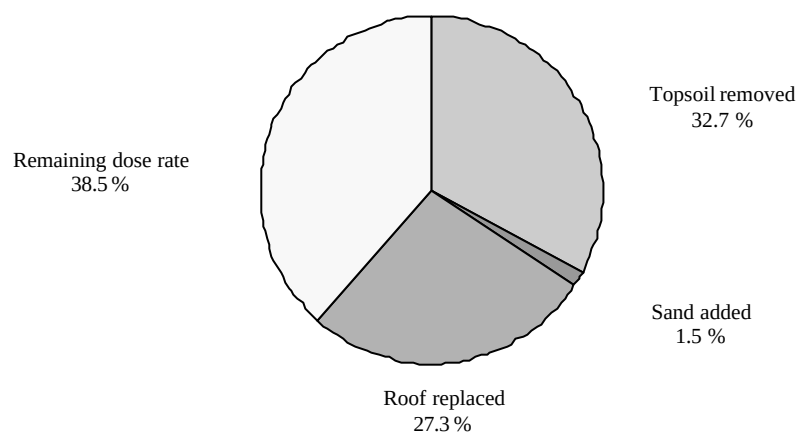


Fig. 4. Reduction in the contribution to dose rate from the contamination, measured on the first floor of house 1.

The overall result of the work was a reduction of the dose rate outdoors in the treated area by almost a factor of 6, and indoors by generally a factor of about 3. This is a remarkably great effect, particularly considering that more than 11 years had at the time of the investigation passed since the area was contaminated, and the contaminant distribution would to some extent have become heterogeneous.

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Poster

A New Technique for Composting Organic Waste by the Hyperthermal and Aerobic Fermentation Method - Changes in the Forms of Heavy Metals in Sewage Sludge Compost

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INTRODUCTION

In Japan, the percentage of severed population has acceleratedly increased, and 56% of household wastewater produced by the total population is treated through sewerage systems. As a result, the production of sewage sludge has kept on increasing, and the quantity reaches 85 million tons. Seventy percent of it is disposed by land filling and 20%, by incineration. The remaining only about 10% is used as composts in greenery areas and farmlands. Though sewage sludge is a highly valuable organic material resource, the resource recovery rate remains very low like this. In recent years, the rising construction costs of landfill sites, final disposal sites, incinerations etc. harass the finances of local governments, and the generation and dioxin and other contaminated poses a large social problem.

To solve the above problem, any unique sewage sludge recycling system tailored for Japan unlike those of Western nations must be created. Only the creation of such a system can achieve the zero emission of sewage sludge. To achieve it, first of all, any excellent compost better in performance than chemical fertilizers must be able to be produced at low cost, simply and quickly.

To clarify the changes in the forms of inorganic materials and heavy metals in the composting process, the forms of inorganic materials and heavy metals were analyzed.

COMPOSTING FACILITIES AND COMPOST FERMENTATION PROCESS

The composting facility is a simple 100-ton 2 m high, 10 m long and 5 m wide tank having only a blower. The number of turning times, the number of days and temperature change in the production process of sewage sludge compost is shown in Fig. 1.

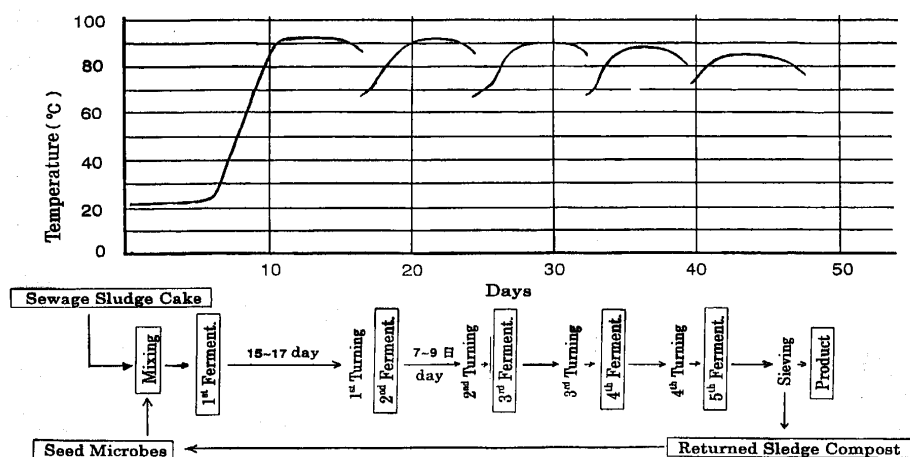


Fig. 1 Schematic of compost production procedure by aerobic hyperthermophiles

The temperature was measured by digital thermometers (sextuple). The compost temperature was measured at 5 places, and the results were averaged. The raw mixture was loaded into the composting facilities (about 100 ton) on May 15, 1997. The period of production was 45 days, and the compost was turned 5 times during the period.

SUMMARY

1) The equipment used for the fermentation is very simple without requiring any large-scale composting facility (with a value of hundreds to thousands of million yen) and also without requiring any deodorizer, raw material introducing hopper and mixed though the sludge is deposited in the equipment. So, the fermentation plant is very inexpensive compared to conventional plants.

2) During the fermentation periods, the temperature reaches 92.6 ° at the highest, and the average temperature during the fermentation period is as surprisingly high as 80°. There is no other composting technique, which can maintain such high temperatures during such a long period of time.

3) The number of bacteria increased sharply after start of fermentation and reached the largest level of 7,700 million after completion of second fermentation, and that also the product after completion of fermentation contained 2,200 million cells. These numbers of bacterial are very large compared to the conventional the number of bacteria.

4) It was found that the quantitatively large exchangeable Ca content clearly decreased in the initial stage of fermentation. On the other hand, the water-soluble Ca content showed little change throughout the fermentation process unlike the exchangeable Ca. These results revealed that the drop of pH value was caused by the decrease of exchangeable Ca.

5) The water-soluble Mg content remarkably increased in the initial stage of fermentation (primary fermentation), and the subsequent increase was very small. On the other hand, the water-soluble and exchangeable K contents increased progressively with fermentation.

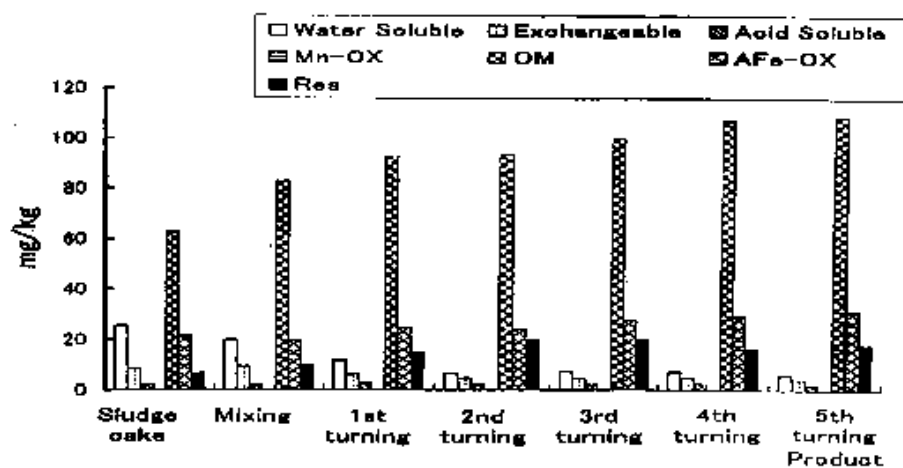


Fig. 2 Cu form dynamics during the compost fermentation process

6) As for the changes in the forms of heavy metal Cu in the fermentation process (Fig. 2), the water-soluble and exchangeable Cu contents decreased with the progression of fermentation, but the slightly soluble Cu content in organic form, iron-containing form and other forms clearly increased progressively with fermentation on the contrary. Therefore, it was suggested that in the fermentation process by hyperthermophilic bacteria, heavy metal Cu was changed into insoluble form.

Trace element issues in developing countries

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Oral

Soil Ingestion Affects the Potential bioavailability of Cu, Mn and Zn

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INTRODUCTION

Geophagia, the intentional and repeated ingestion of soils has been practised for centuries throughout the world. In modern societies it represents a complex eating behaviour and it has been associated with a number of health problems, including Fe deficiency anaemia (Danford, 1982). Geophagia is generally assumed as a source of mineral supplementation, largely based on either bulk mineral nutrient contents of geophagic soils or their partial extractions (Aufreiter et al., 1997). A recently developed test, which included pH, already bioavailable nutrients and solid to liquid ratio broadly similar to that of the gastrointestinal tract, showed that soil ingestion has the potential to remove already bioavailable nutrients (Hooda et al., 2002). Here we report further experiments to assess the effect of soil ingestion on the potential bioavailability of Cu, Mn and Zn.

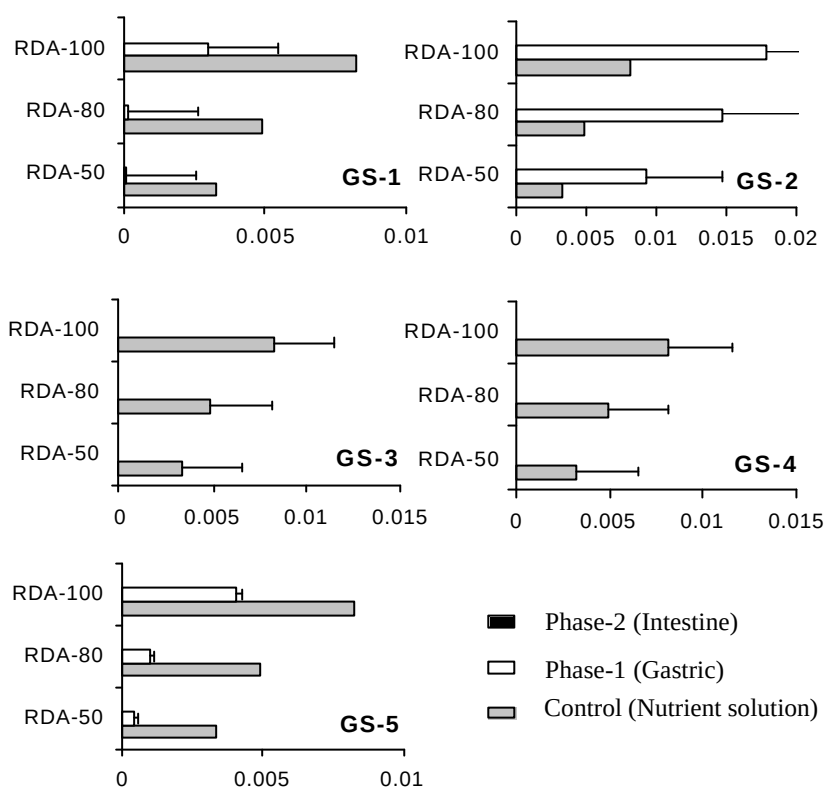
MATERIALS AND METHODS

The work is based on five geophagic soils collected from Uganda, Tanzania, Turkey and India. Although the study is primarily on Cu, Mn, and Zn, other nutrients were included to provide in-vivo like conditions for nutrient adsorption-desorption reactions. Three nutrient solutions: RDA-50, RDA-80 and RDA-100, representing 50, 80 and 100% respectively of the recommended daily allowances (RDA) of the nutrients were prepared to represent a cross section of their dietary intake. The geophagic simulations involving 1-g soil and 20 ml nutrient solution were carried out at two pH values (2 and 10), representing the stomach (phase-1) and the intestine (phase-2) respectively. The soil-nutrient solution mixtures were equilibrated on an end-over-end shaker for 2 h in an incubator at 37°C (body temperature). The potential impact of geophagia in terms of nutrient gain or loss was determined by the difference in nutrient concentrations/contents before and after phase-1 and phase-2 in-vitro simulations.

RESULTS AND DISCUSSION

The solubility of nutrients in the GI tract together with their availability for absorption will largely determine if ingestion of these relatively nutrient-rich soils could become a source of nutrient supplementation. Figure 1 shows example results of geophagic simulations for the five geophagic soils (GS1-GS5) for Cu. The results clearly show significant losses of Cu from the solutions after the phase-1 equilibrium, including the complete removal of already bioavailable Cu by two soils, GS-3 and GS-4. The phase-2 experiment showed that no detectable Cu was left in any of the solutions across all five soils. Similar results were obtained for Mn and Zn.

The findings show that instead of releasing mineral nutrients (supplementation) the soils effectively removed nutrients that were already available in the solutions. The phase-2 soil-nutrient solution pH 10 might be extreme, as pH in the intestine ranges between 7 and 10. However, the fact that large amounts of Cu, Fe, Mn and Zn were retained by the soils even under the highly acidic conditions (phase-1) shows that the findings would not have been any different if the phase-2 pH was < 10. Soil ingestion therefore can potentially reduce the absorption of micronutrients such as Cu, Mn, Fe and Zn, which may exacerbate their deficiency in geophagic individuals regardless of their dietary intake. This is consistent with numerous clinical nutrition studies on geophagic subjects (Danford 1982).



Copper (mg) in 20-ml geophagia test solutions

Figure 1: The effect of geophagia simulation on potentially bioavailable copper. The three nutrient solutions RDA-50, RDA-80 and RDA-100 contained 50, 80 and 100% respectively of the daily-recommended allowances for all essential mineral nutrients represented by respective control solutions. Error bars on phase-1 and phase-2 represent least significant difference (LSD) when a nutrient in the control solution was compared with that following phase-1 and phase-2 geophagia simulation, respectively.

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Urban soil contamination in the Palermo area (Italy): evidence from lead concentrations and isotope ratios

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INTRODUCTION

Recently heavy metals have received increasing attention as toxic pollutant in urban areas. Their main anthropogenic sources include industrial activities, building construction, vehicle emissions, coal burning, and refuse incineration. In particular, because of the recognition of potential health risks associated with Pb pollution, many researchers have focused great interest on levels and behaviour of this element both in natural and contaminated environments. Despite the potential hazard of Pb, until now relatively little attention has been paid to its distribution in Italian soils. Lead pollution is generally marked by elevated contents of this element and other metals in soil surface horizons. However, Pb concentration maps alone may be insufficient for proving contamination, given that strong metal accumulation might also result from natural processes. Lead isotope studies provide an ideal tool for characterizing sources and pathways of Pb pollution. For this study, lead concentrations and isotope compositions were determined on topsoils from the city of Palermo (Western Sicily) in order to assess the metal distribution in urban environment and identify its sources.

MATERIALS AND METHODS

A total of 70 topsoil samples were collected from green areas and public parks within the city of Palermo. Lead concentrations were obtained by absorption spectrophotometry (AAS) and graphite furnace atomic absorption spectrophotometry (GF-AAS) after sample digestion with *aqua regia* in microwave oven. Twenty selected topsoils (17 urban soils, and 3 unpolluted rural soils from Sicily) were analysed for Pb isotopic composition. Lead isotope ratios were measured by inductively coupled plasma mass spectrometry (ICP-MS).

RESULTS AND DISCUSSION

Lead concentrations in the analysed topsoils range between 57 and 682 mg/kg¹ with an average value of 218 mg/kg¹. These values, up to 10 times higher than those recorded in natural soils, suggest anthropic pollutant sources for this metal. The urban soils yield ²⁰⁷Pb/²⁰⁴Pb ratios in the range 15.55-15.79, ²⁰⁸Pb/²⁰⁴Pb ratios between 37.64 and 39.22 and ²⁰⁶Pb/²⁰⁴Pb ratios from 17.89 to 19.00. They show less radiogenic values than those of unpolluted Sicilian soils (averages of ²⁰⁷Pb/²⁰⁴Pb = 15.72, ²⁰⁸Pb/²⁰⁴Pb = 39.38, and ²⁰⁶Pb/²⁰⁴Pb = 19.08). All the urban topsoil samples are distributed along a mixing line, whose end members are represented by an average value of the isotopic composition for European gasoline suppliers and the mean isotopic ratio measured for unpolluted Sicilian soils. Generally, samples with higher lead concentrations are characterized by less radiogenic isotopic compositions. These soils, which also show strong enrichments of other heavy metals, were collected from green areas close to streets with heavy vehicle traffic, thus suggesting automotive emission as main pollution source for the Palermo urban soils.

Platinum Levels in Urban Soils of some Italian Cities

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INTRODUCTION

Platinum content in urban soil is mainly related to anthropic sources and, in particular, to vehicle exhaust emissions as a consequence the introduction (end of 80^s) of the unleaded fuel and cars with catalytic converters.

Pt distribution in soil is not generally influenced by pedogenesis and shows a relatively high mobility with respect to "pedogenetic" metals. This fact created some concern with respect to the dispersion of Pt in the environment.

With the aim to determine Pt background levels and the Pt time dependent accumulation factor in Italian urban and 'natural' soils, starting from 1992 a sampling campaign was carried out in some Italian cities: Rome, Naples, Palermo and Padova. In some cases the relation between Pt concentration and soil grain size fraction was also investigated. These urban areas were selected because they can be representative of the Italian situation. In fact they have different extension, number of inhabitants, and traffic intensity. Besides these four areas show geological and morphological differences very useful to better understand the fate of Pt in Italian urban soils.

MATERIALS AND METHODS

181 soil samples were collected from selected sites in the urban areas of Rome, Naples, Palermo and Padova, taking into account traffic intensity, pollution source distance, green areas extension and morphology. At each selected site 1 kg of soil was sampled at the depth of 0-10 cm (top) and 40-60 cm (bottom) from the relatively undisturbed soil. In dumped materials only "top" samples were collected selecting those closest to the main roads.

Soils samples were dried at 40°C, sieved through a 2 mm sieve and ground. Metals extraction was carried out by means of 'aqua regia' (10 ml) in a microwave oven system. Pt determinations were performed by ICP-MS (Perkin Elmer, Elan 6100). Samples and reagent blanks were prepared following the same procedure. The analytical procedures were previously tested using certified reference materials.

RESULTS AND DISCUSSION

Averages of Pt concentrations in some Italian urban soils are reported in Table 1. Pt in urban soils results, in some cases, to be slightly higher with respect the range of Italian 'natural soils' developed mainly in sedimentary and volcanic rocks. As a general consideration, soils on limestone evidence a wide concentration range for Pt (<1-6 ng/g), reflecting the variable contents of insoluble residue of these rocks.

A comparison between soil samples collected in the same sites in 1992 and in 2001, reveals an evident, even slight, increase of Pt. This finding is noticeable overall in samples from the urban area of Rome, paralleling the decrease of Pb, as a consequence of the large introduction of unleaded fuel.

As a general consideration, in the studied samples Pt concentration are higher in dumped soils (mean 10, max 13.8 ng/g), collected close to main traffic roads, with respect relatively undisturbed soils. Top soils are enriched in Pt with respect to bottom soils, confirming the possible recent deposition of atmospheric particulate containing Pt particles emitted from vehicles exhaust.

While grain-size distribution affects trace elements (generally Pb, Zn, Cd and Hg all increase with decreasing soil grain size), no significant relation was observed for Pt. This fact could be related to the

low Pt concentration in soils that, at the present time, does not permit a clear discrimination between the background levels and the contribution of Pt particles emitted by vehicles catalysts that usually are in the range 5-10 μm . Besides has been observed that platinum associated to particulate matter, is generally not readily mobilized and redistributed in the various grain size fractions of soils.

Table1. Pt concentrations in urban soils of some Italian cities.

	N° of Samples	Pt (ng/g)
Naples	15	8.5 ± 2.5
Rome	64	11.5 ± 4.7
Padova	65	1.41 ± 1.26
Palermo	37	3.60 ± 0.90
Italian natural soils	47	$\leq 1.0 \div 5.0$

CONCLUSIONS

The analysis of data concerning Pt concentration levels in urban soils of some Italian cities show that the concentration range (1-13.8 ng/g) is relatively higher with respect to the values that can be reasonably considered as the geochemical background (1-5 ng/kg). Otherwise, the increase of Pt concentration in soils of Rome (11.5 ± 4.7 ng/g in samples of 2001), result to be three times higher with respect those found in samples collected in 1992 (3.8 ± 1.0 ng/g). This fact evidences the possible existence of early contamination phenomena related to the introduction of catalytic converters. However, notwithstanding this noticeable increase of Pt levels recent literature data show that the increase in Pt content obviously results more evident in airborne particulate matter and road dust than in urban soils.

Trace element issues in developing countries

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Poster

Mercury levels in fish downstream the hydroelectric reservoir at Manso in Mato Grosso, Brazil

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When river water is dammed to construct a hydroelectric reservoir, this causes changes in the aquatic environment and the mercury concentration in ichthyofauna may rise due to transformation of mercury to methyl mercury, its most toxic form. The process of the mercury changes in the aquatic medium is not completely known, especially not in tropical areas with high biological production. Contributing factors are changes in physical and chemical characteristic of water and sediment, where to an increase in dissolved organic matter plays a key role. Thus, more mercury is bioaccumulated and biomagnified in the food chain, most evident in predator fishes. The aim of the present study was to analyze the fish mercury levels and to determine eventual changes in water parameters caused by APM - MANSO hydroelectric power plant in Mato Grosso State – Brazil. Inundation was initiated in November 1999 and the hydroelectric power plant started to work in April 2001.

Fish, sediment and water samples were taken from 4 sites between 14°51'07''S to 14°42'53''S and 55°53'31''W to 56°12'40'', covering Rio Manso downstream the dam to the confluence with the river Cuiabazinho. Rio Manso is a tributary to Cuiabá River, one of the main tributaries to the wetland Pantanal of Mato Grosso. Samples were collected between December 2000 and September 2001. The fishes caught were piscivorous (*Pseudoplatystoma corruscans*, *Pseudoplatystoma fasciatum*, *Salminus maxillosus*, and *Hemisorubim platyrhynchus*) and omnivorous (*Prochilodus lineatus*). The total mercury in fish and sediments was analysed by cold vapor atomic absorption spectrophotometer (CV-AAS). Physical and chemical water parameters determined were pH, dissolved oxygen, water and air temperatures, turbidity and transparency determined by a Secchi disc. Those parameters were determined in both the dry and rainy seasons of 2001. Mercury levels of piscivorous fishes were: in average 527.6 ng g⁻¹ w.w. with a confidence interval of ± 107.7 ng g⁻¹ (n = 45) and in omnivorous fishes 99.1 $\pm$ 17.37 ng g⁻¹ (n=26). The values ranged from 88.1 to 2161.4 ng g⁻¹ in piscivorous species and from 3.07 to 184.9 ng g⁻¹ in omnivorous species (Figure 1).

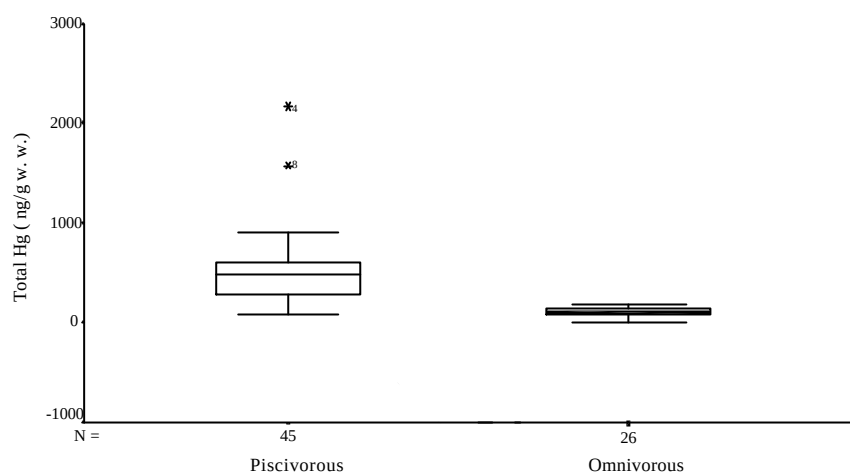


Figure1. Mercury concentrations in piscivorous and omnivorous fish species captured in December 2000 – September 2001 from Rio Manso. The central line in the box plot indicates the median, the upper and lower lines of the box indicate the 75th and 25th percentiles; the upper and lower bar lines indicate the 95.6th and 0.0th percentiles for piscivorous fishes and the 100th and 0.0th for omnivorous fishes, while the points are above these levels.

Descriptive statistical analysis of the mercury concentrations is presented in Table 1. *Salminus maxillosus* (Dourado) showed the biggest values for range, mean and standard deviation of mercury concentrations among the studied species.

Table1. Mercury concentrations in fish captured in Rio Manso, Mato Grosso, Brazil, downstream the hydroelectric reservoir APM- Manso, from December 2000 to September 2001.

specie	Nº	Feeding habits*	Mercury (ng/g)			Values	
			Median	Mean	Standard deviation	Min.	Max.
<i>Pseudoplatystoma corruscans</i> Pintado	23	P	412	440	199	88	907
<i>Salminus maxillosus</i> Dourado	8	P	568	817	703	237	2162
<i>Pseudoplatystoma fasciatum</i> Cachara	9	P	432	499	218	265	864
<i>Hemisorubim platyrhynchus</i> jurupoca	5	P	572	518	155	273	680
<i>Prochilodus lineatus</i> Curimbatá	26	O	100	99	43	3	185

*P = Piscivorous; O = Omnivorous

Dissolved oxygen ranged from 6.0 to 6.9 mg L⁻¹, pH from 6.8 to 8.0, and transparency from 125 to 60 cm. Air and water temperatures were 26° to 30° and 25° to 32° C, respectively (figure 2). The average mercury concentrations in sediment in dry and rainy season was 20,50 and 36,1 ng g⁻¹ dry weight, SD 8,72 and 11,26 ng g respectively. Statistical analyses were used to compare Hg concentrations of piscivorous and omnivorous fishes with the limit value of 500 ng Hg g⁻¹ w.w. recommended by the World Health Organization for human consumption of fish. The results showed significant difference (95% confidence level) only for omnivorous fishes, which were lower than the WHO limit. The piscivorous fishes had a mean level close to the WHO limit value. The results suggest that some physical or chemical parameters have changed after inundation and that the mercury load in piscivorous fishes could represent a health risk for the consumers, especially when considering the large fish consumption in the area. This indicates that the Manso reservoir needs a monitoring program.

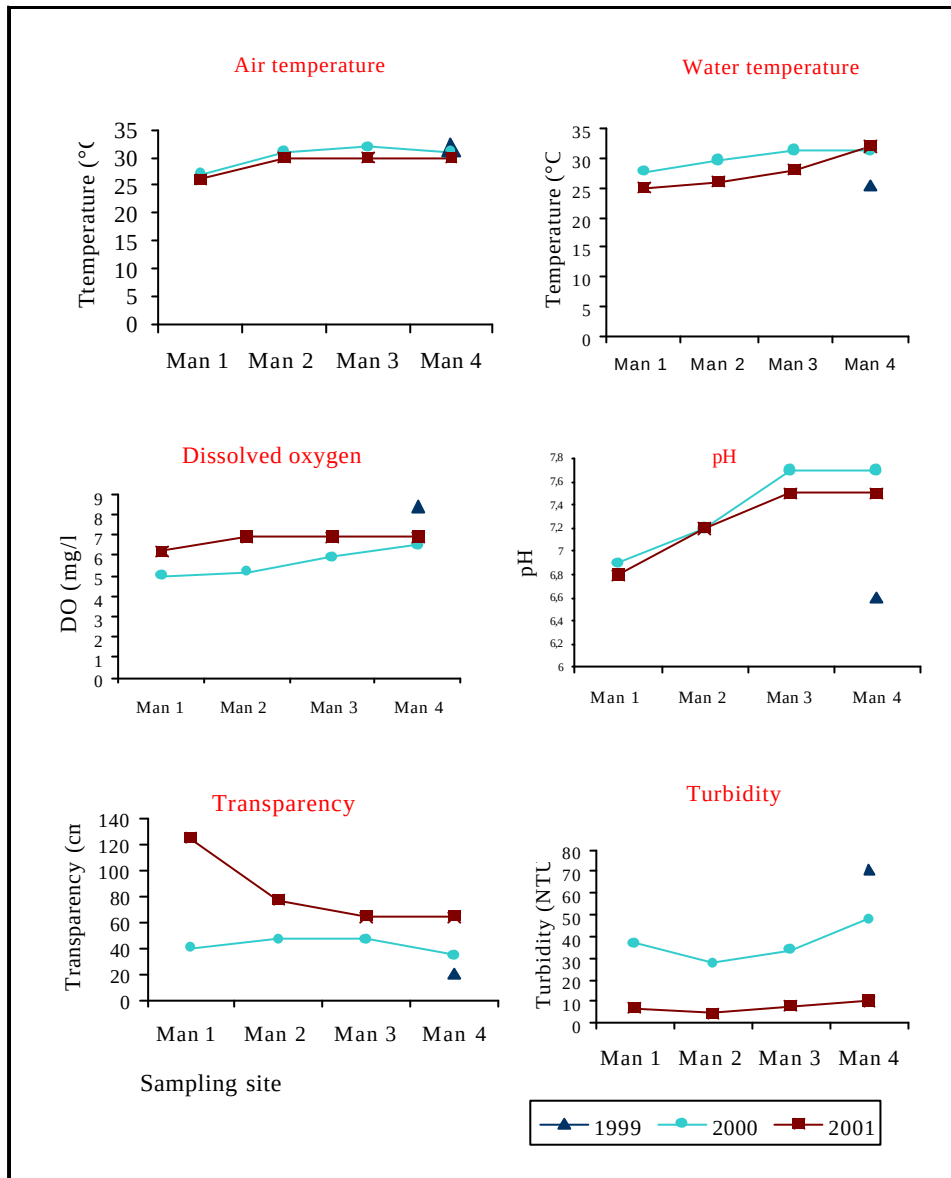


Figure 2. Physical and chemical water parameters downstream Rio Manso before and after inundation of the hydroelectric power plant – APM-Manso.

Mercury fish contamination in Alto Pantanal – Mato Grosso - Brazil

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ABSTRACT

In order to evaluate fish mercury contamination due to release of mercury in upstream gold mining areas in Alto Pantanal wetland (Brazil), samples from 74 piscivorous fishes were analyzed in relation to their total mercury concentrations. The fishes were caught in August to October 2000 at 6 sites located at Rio Cuiabá, Rio Paraguai and Rio Bento Gomes. The samples were digested by nitric and hydrochloric acids and analyzed for total mercury by cold vapour atomic absorption spectrophotometer (CVAAS). Average concentrations $\pm$ SD were 0.18 $\pm$ 0.19 mg/kg w.w. (n=7) for *Pseudoplatystoma corruscans* (pintado), 0.16 $\pm$ 0.12 mg/kg w.w. (n=44) for *Serrasalmus* sp. (piranha), 0.22 $\pm$ 0.20 mg/kg w.w. (n=18) for *Pseudoplatystoma fasciatum* (caxara), 0.05 mg/kg w.w. (n=1) for *Hemisorubini platyrhynchos* (jurupoca), 0.72 $\pm$ 0.00 mg/kg w.w. (n=2) for *Piniranpus pirinanpu* (barbado), 0.72 mg/kg w.w. (n=1) for *Salminus maxillosus* (dourado) and 0.30 mg/kg w.w. (n=1) for *Surubim lima* (jurupensém). The average value of all fish samples was 0.19 $\pm$ 0.18 mg/kg and values ranged from 0.02 mg/kg to 0.80 mg/kg. The highest mercury levels were found in fish sampled in Baía Siá Mariana, Baía Chacororé and Baía Porto de Fora floodplain lakes. There was no statistically significant difference (95% confidence level) between average concentrations of caxara and pintado from Baía Siá Mariana and average values from the others sites. Piranha showed statistically significant difference (95% confident level) between average concentration in Baía Siá Mariana and average values of samples from other sites. The maximum mercury limit for human consumption of predator fishes set in Brazil is 1.0 mg/kg (Ministério da Saúde, Portaria nº 685, August 27, 1998, published in September 24, 1998) and the concentrations were all below this limit. The American FDA (Food and Drug Administration) recommends that consumption should be limited to 14 ounces (about 2 servings) per week of fish species with methylmercury levels averaging 0.5 mg/kg w.w. (Agency for Toxic Substances and Disease Registry – ATSDR. Toxicological Profile for Mercury. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. March 1999). In the present study, 90% of the samples had mercury levels below 0.5 mg/kg. Generally, the maximum Hg content for freshwater fish under natural conditions is 0.2 ppm w.w. (Galvão & Corey, 1987 apud Hylander et al. Mercury Levels in Alto Pantanal: A Screening Study. *Ambio*, 23(8), p. 478 – 484, 1994), and 33% of the samples had Hg concentrations above this value. Although the results were below the Brazilian standard for consumption, a mercury contamination process is suggested. Thus, further research concerning environmental factors determining mercury contamination in the Alto Pantanal region is needed. In addition, it is important to further monitor the region since human surveys indicate that the ingestion of fish with a mercury content below 1.0 mg/kg can cause important health effects depending on fish amount consumed.

Sampling strategy and analytical protocols

Scientific Program 10

Poster

Potential biosensor techniques for monitoring arsenic contamination and remediation

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The classical methods for the analysis of metals such as atomic absorption spectrometry, ion chromatography and mass spectrometric techniques need to be carried out in laboratory after sampling. These techniques are expensive and also not suitable for continuous monitoring in the environment. Optical biosensors for heavy metal detection provide an inexpensive system for environmental control (Durrieu and Tran-Minh, 2002). In Bangladesh and west Bengal region in India, about one million people are exposed to arsenic resulting from consumption of arsenic contaminated groundwater. There is an urgent need for field portable device for on site detection of arsenic contamination. Field portable biosensors might be a good alternative over less reliable colorimetric field kits [Chakraborti et al. 2002].

In addition to a thermal biosensor [Bhand et al. 2003], we have optimized a field portable biosensor for heavy metals detection. The method is based on the inhibition of enzyme butyrylcholinesterase and the chemiluminescent signal generated inside the capillary is detected through a photomultiplier tube. The photomultiplier module provides good sensitivity and can be used for field analysis. Further tests are in progress to monitor arsenic remediation in laboratory column experiments filled with zero valent iron Fe (0) and other materials feasible for in-situ treatment curtains.

MATERIALS AND METHOD

Butyrylcholinesterase (BuChE) (EC 3.1.1.8, from Horse Serum) with a specific activity of 13 U/mg solid, choline oxidase (EC 1.1.3.17 from *Alcaligenes* species) 16U/mg solid and horseradish peroxidase (HRP; EC 1.11.1.7) [1310U/mg solid], butyryl choline chloride (98% purity) were obtained from Sigma Chemical company (St Louis MO) USA. Luminol substrate obtained from PIERCE, USA. Glass capillaries, World Precision Instruments Inc. (Sarasota, FL), ConA Sepharose beads were obtained from Pharmacia Sweden. The photomultiplier tube (PMT) Sensor Module HC 135-01 detection was purchased from Hamamatsu (Hamamatsu Corp., Bridgewater, NJ). All other reagents were of analytical grade.

The basic reaction of the enzyme system used is as follows:

- Step I:
1. *Butyrylcholin chloride (BuChE) → butyric acid + choline*
 2. *Choline (choline oxidase) → betaine + H₂O₂*
 3. *H₂O₂ + luminol (HRP) → 3-aminophthalate + light*
- Step II: *BuChE (on ConA column) + arsenic (incubation) → reduced choline production*

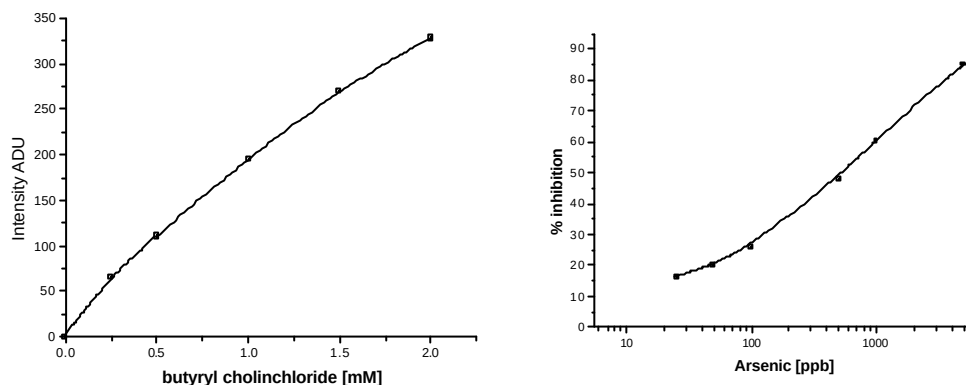
The reduced choline from step-II is transferred to capillary along with the enzymes and measured through PMT module as depicted in step-I. The increase in arsenic (inhibitor) concentration is proportional to reduced signal.

RESULTS AND DISCUSSION

The sensor system was calibrated for substrate concentrations. One unit of the enzyme BuChE was bound to ConA sepharose column and the product collected over 25 min. measured through capillary containing 0.5U choline oxidase and 0.05U peroxidase and luminol in 10 μ l phosphate buffer, pH 7.4. The linear response obtained for substrate butyryl choline chloride is presented in Fig.-1. The calibration for total arsenic inhibition is shown in Fig.-2.

Fig.1: Linear response of the sensor system to substrate, 25min. incubation

Fig.2: calibration for total arsenic



A linear working range for total arsenic was found to be 80ppb to 5ppm with a detection limit of 50ppb and 95% reproducibility. The optimized sensor system might be useful to determine arsenic, uranium and other heavy metals. Influent and effluent samples from the treatment column for arsenic were analyzed employing this sensor system. It is observed that the above system could be explored further for on-site monitoring of contaminants. The assay requires no sample preparation.

ACKNOWLEDGEMENTS

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Geostatistical approach for targeted remediation of metal contaminated soils

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Semivariogram modelling and kriging have been used to characterise the spatial distribution of heavy metal contamination across a site in South London. The aims of this analysis are to illustrate the potential value of geostatistics in the design and implementation of targeted remediation strategies. Contamination of the study area is a direct result of its industrial past and the use of the ensuing waste to reclaim an area of marshland. The unknown and varied origins of this waste have produced a chaotic distribution of contamination.

MATERIALS AND METHODS

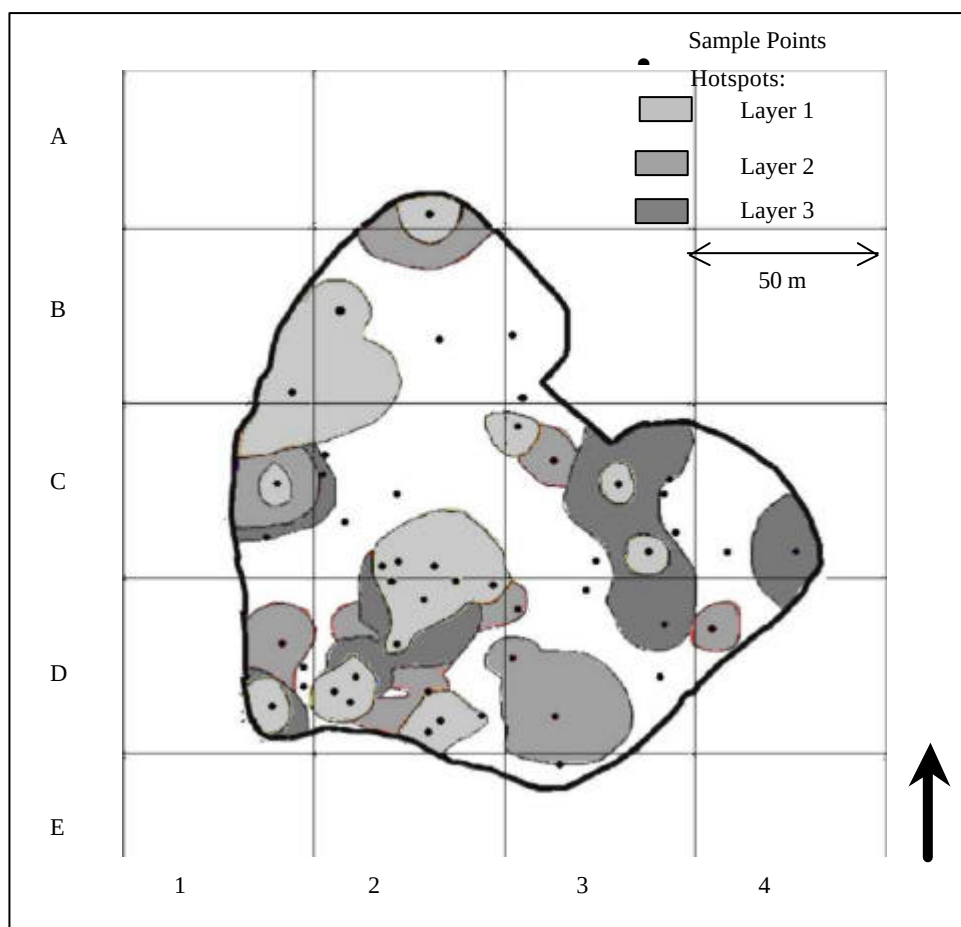
Samples were taken from three depths (0 – 20, 20 – 40 and 40 – 60 cm) at 50 sample locations. The sample strategy used was based on a predefined set of random distances and bearings. However, the inaccessibility of parts of the study area made this difficult to implement in the field. Random sampling is most commonly used at sites, such as this, where the spatial distribution of contamination is anticipated to be highly variable (Byrnes, 1994). The disadvantage of random sampling is that it produces clustering and that parts of the study area remain under-sampled. Clustering of data in this manner, however, has important implications for variogram modelling, as the sample variogram does not provide information for distances shorter than the minimum spacing (lag) between the sample data. Therefore in the case of a regular grid there are no lags at an offset less than the grid interval and very little is known about the shorter lags (Isaaks and Srivastava, 1989).

Samples were analysed for total trace element concentrations as well as for pH and organic matter content. The elements analysed were As, Sb, Hg, Pb, Cd, Mg and Cu.

RESULTS AND DISCUSSION

The most noticeable aspect of the maps produced through this analysis is the lack of cohesion between both elements and layers. There are, however, a number of hotspots common to several elements as well as a combination of layers (Fig. 1). Plotting the hotspots for all of the elements, for all three layers on a single map (Fig. 1) shows that a large portion of the site is highly contaminated; targeted remediation may therefore not be practical. Furthermore, this map emphasises the weakness of the sample strategy employed by this study. Whilst an important element of geostatistical analysis, clustering of data points, primarily the result of the inaccessibility of large portions of the study area, has meant that the areas that appear least contaminated occur where the sample density is also least. When combined with the complex evolution of the site and frugal information available on the probable distribution of contamination, the inadequate distribution of sample points means that there remains the possibility of hotspots being undetected. This problem could be solved through the use of a stratified random sample pattern. In terms of the geostatistical analysis of contaminated land, a stratified random sampling strategy probably represents the most viable practice and could prove a valuable tool in the planning of a suitable remediation scheme. However, the inaccessibility of parts of the study area would have made a stratified random sample strategy difficult to implement in the field. An additional solution involves a secondary sampling, targeted in areas considered under sampled. The data from this phase would then be used to validate the existing maps. In spite of some limitations, it is concluded that, when combined with a suitable sample strategy, kriging offers the potential to analyse the spatial distributions of contamination, contamination trends, identify areas that may potentially require further investigation and can be used as a valuable tool in the design of remediation strategies.

Figure 1 The contamination hotspots located through geostatistical analysis. The information for all three layers has been combined on this map.



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Evaluation of Migration of Heavy Metals with Various Extraction Techniques in a Soil with Texturally Different Horizons

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INTRODUCTION

Soil profiles in Finland commonly have textural differences between horizons. The horizons have been sedimented at different stages of the retreat of the continental ice. These discontinuities in soil profiles make it difficult to determine the transport of heavy metals from a horizon to another only focusing to total metal contents, because the original parent materials contain natively variable heavy metal concentrations. Therefore also other extraction methods than the total should be utilised. Plant available metal fraction in cultivated soils of Finland is extracted with the Lakanen & Erviö (1971) method, the extractant being acidic ammonium acetate EDTA, pH 4.65 (AAAc-EDTA). The four-step sequential extraction procedure proposed by The Standards, Measurements and Testing Programme (formerly BCR) of the European Commission method was designed for the speciation of heavy metals in sediment samples (Rauret et al., 2000). In this study the *aqua regia* total, the BCR and Lakanen & Erviö methods were compared in order to identify the possible metal downward transport in one agricultural soil profile with texturally different horizons near a Cu-Ni-smelter in SW Finland.

MATERIALS AND METHODS

The soil samples (Table 1) were collected by the horizon from the pit excavated to 180 cm. Single extractions were carried out with AAAc-EDTA according to Lakanen and Erviö (1971) and with *aqua regia* (ISO 11466, 1995). The extractants used sequentially in the BCR procedure were dilute 0.11 M acetic acid (**A**; exchangeable and carbonate-bound fraction), 0.5 M hydroxylammonium chloride (**B**; Fe-Mn-oxides-bound fraction) and 8.8 M hydrogen peroxide (**D**; organic-bound fraction) followed by *aqua regia* (residual fraction; **Res**). The solutions were analysed by Perkin Elmer Sciex ELAN 6000 ICP-MS and by Varian SpectrAA-400 with GTA-96 graphite tube atomizer with Zeeman background correction in triplicate.

Table 1. The selected physicochemical properties of the studied soil.

Horizon	Depth/c m	Texture	pH (H ₂ O)	CEC/ cmol(+)kg ⁻¹	Base saturation/ %	Clay/%	Silt/%	Sand/%
I	0-30	Silt loam	6.5	11.3	63	17	65	18
II	30-47	Clay	6.1	25.6	78	69	25	6
III	47-70	Silty clay	6.7	14	70	40	59	1

RESULTS AND DISCUSSION

The texture and CEC varied markedly with depth (Table 1). The total concentrations of Ni, Cr, Pb and Zn were highest in the horizon II falsely suggesting metal leaching in the profile (Fig. 1). Because anthropogenic metals are usually more mobile than lithogenic ones (Chlopecka et al., 1996), the proportion of the sum of the easily soluble fractions A and B should have been high in the horizons II and III, if the metals had been leached from the plough layer polluted with Cd, Cu and Ni. However, the proportion of A+B fractions of the total for Cr, Pb and Zn did not change much with depth but for Cd, Cu and Ni it decreased. Therefore the high total content of the metals in the horizon II is an

implication of the mineralogical composition of this clearly clayey horizon and not of metal leaching. The amounts of Cd, Ni and Zn extracted with AAAC-EDTA were roughly similar to the fraction A of the BCR procedure. Exceptionally, AAAC-EDTA extracted Cd, N and Zn more efficiently from the horizon II (123-140% of the A fraction) than from the other, more coarse textured horizons. The amounts of Cu and Pd extracted with AAAC-EDTA much exceeded the size of the A fraction. Only negligible amounts of Cr were dissolved with both the AAAC-EDTA and the solution A of the BCR procedure.

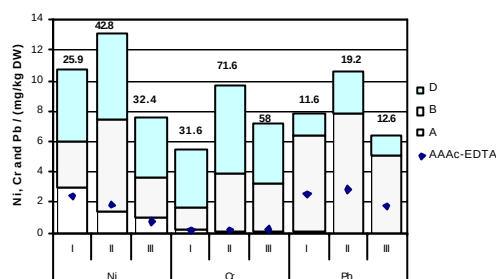


Figure 1. Distribution of metals (Ni, Cr, Pb, Cu and Pb) among different fractions (A, B, and D) of the BCR procedure in the studied soil (horizons I-III). The columns indicate the sum of different fractions and the solid points the single extraction (AAAC-EDTA) results. Total (*ar* extractable) concentrations are presented above the columns in bold. All the concentrations are in mg kg⁻¹ dry weight.

CONCLUSIONS

This study shows the superiority of the BCR sequential extraction and the AAAC-EDTA procedures to identify soil materials polluted with heavy metals, compared to *aqua regia* procedure. From clayey soil AAAC-EDTA extracts metals more efficiently than 0.11 M acetic acid.

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Bioavailability and bioaccumulation

Scientific Program 11

Oral

Zinc uptake by oat from soils amended by sewage sludge

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INTRODUCTION

Czech legislation also contains limits regulating the possible use of sewage sludge in agriculture. There are investigated different amendments to improve quality of sewage sludge and to eliminate its negative effects. The most frequently used is the addition of lime (Balík et al., 1999). Other possibility is the addition of different sorption materials.

MATERIAL AND METHODS

Pot experiments were set up at three different soils [Chernozems ($Zn_{total}=74.5 \text{ mg.kg}^{-1}$), Luvisols ($Zn_{total}=41.5 \text{ mg.kg}^{-1}$), and Fluvisols ($Zn_{total}=26.0 \text{ mg.kg}^{-1}$)], and the addition of eight months incubated sewage sludge (20°C, access and lack of air) treated by different ameliorative compounds were tested. Three sewage sludges were treated by bentonite (added in the amount of 30% of dry matter of sludge), by calcium carbonate (in the amount of 18 %) and by organic materials (two treatments). Treatment signed as Comp. I was made of 50 % sludge + 35 % peat and 15 % straw and treatment Comp. II was made of 50 % sludge + 5 % peat and 45 % straw (expressed in dry matter). All treatments were preincubated for eight months under aerobic (A) and anaerobic (AN) environment at temperature of 20 °C. Fresh sludge in the rate of 33.33 g of dry matter was thoroughly mixed with five kg of soil and with NPK (1 g N, 0.16 g P, 0.4 g K per pot applied as NH_4NO_3 and K_2HPO_4). Mixture was inserted into pots and sown by oat seeds. At each pot there was 20 plants. Green biomass of oat was harvested and analysed. Each treatment was set with three replications.

RESULTS AND DISCUSSION

Added materials contained low amount of Zn (3.2 mg.kg^{-1} in $CaCO_3$, 143.2 mg.kg^{-1} in bentonite, 17.2 mg.kg^{-1} in peat and 94.3 mg.kg^{-1} in straw). The substantial portion of Zn came into soil from sludge. Portion of sludge Zn was almost 100 % at treatments with lime, 91.6 - 97.3 % with bentonite. Treatments signed as Comp.I and Comp.II contained 84.9-98.1 % of Zn from sludge. The rate of sludge was tested before and corresponded in our experiment with 20 tones per ha applied in the field. Treatment A1 (the highest Zn content) supplied $78.8 \text{ mg Zn per pot}$ and treatment A3+Comp.I (the lowest Zn) only 16.4 mg per pot . Comparison of Zn amount in soil and in sludge mixture showed the ratio Zn_{sludge}/Zn_{soil} at Chernozems 4.3-21.2 %, at Luvisols 7.7-38.1 %, and at Fluvisols 12.3-60.8 %. It assumed higher effect of sludge application at Fluvisols.

The effect of individual sludge treatments is presented in figure 1. The lime addition into sludge led to lower mean Zn uptake under addition of both aerobically and anaerobically incubated sludge. Smaller differences were also determined in Zn oat content at treatment A (100 %), A+Comp.I (98 %) and A+Comp.II (99 %). The differences in total applied Zn were substantial. Zn amendment by A+Comp.I was only 56 % and by A+Comp. II 64 % compare to treatment A. Addition of peat and straw led to higher utilization of Zn by plants due to pH fall and increase of mobile Zn fractions.

Comparison of treatments A and AN showed higher Zn accumulation by 17 % at treatments with addition of anaerobically incubated sludge, with only 78 % Zn mean addition compare to A treatments. Tlustoš et al. (2001) showed higher Zn accumulation in exchangeable and oxide fractions and the Zn fall in organic and residual fractions in sludge under aerobic incubation compare to anaerobic one. Mineralization of anaerobically incubated sludge can occur in soil and released Zn was taken up by plants without any binding possibility. Determined differences were also indirectly confirmed by differences in amount of added organic matter. Mean $C_{ox.}$ content was 12.3 % at A and 18.3 % at AN treatments. C_{sludge} contained 5.3 % from $C_{Chernozem}$, 7.9 % from $C_{Luvisol}$, and 12.8 % from $C_{Fluvisol}$ at

anaerobic treatments. All presented calculations showed real assumption of organic matter importance in sludge and the effect of Zn sorbed on organic sludge portion. Highest effect was found at Fluvisols due to their adverse pH and sorption capacity.

Results showed much higher Zn mobility at treatments Comp. I than Comp. II. Higher Zn mobility caused by lower pH (acidification effect caused by peat) was not compensated by higher peat sorption capacity. Differences in Zn utilisation from Comp. I and Comp. II treatments were also caused by different portion of Zn_{straw} in Comp. I (3.2 %) and Comp. II (8.8 %) from total Zn_{Compost} . Figure 1 also showed very low differences between Comp. II treatments incubated under presence or lack of air. The reason is probably high aeration of both treatments by addition of 45 % of straw dry weight.

Bentonite addition showed very mild effect on Zn accumulation. Presented values showed slightly better response of aerobically treated sludge. Mean Zn oat content was 35.8 mg.kg^{-1} at all soils with aerobically treated and ameliorative sludge and 39.3 mg.kg^{-1} at anaerobically treated ones. Applied Zn amount at anaerobic treatments was by 12 % lower compare to aerobic ones with higher pH after preincubation. Important role also played applied carbon and its compounds in Zn soil mobility.

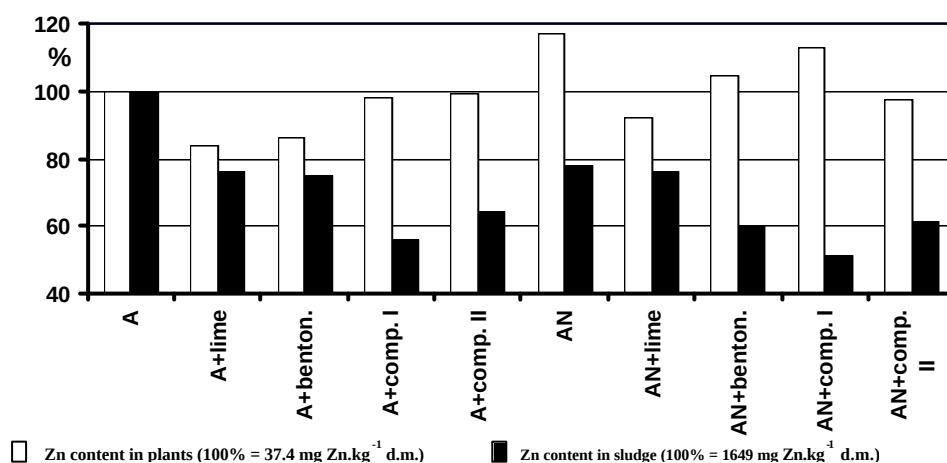


Fig. 1 Relative Zn content in sludge and relative Zn content in plants

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The long term effect of lime application on the bioavailability of Cd for spinach

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INTRODUCTION

Bioaccumulation of elements in plant tissues can be significantly affected by plant species and genotypes and the differences in metal content can reach more than one order for the same plants (Tlustoš et al. 1998). Among vegetable species spinach is able to accumulate large amount of metals (Adriano, 2001). Amount of element in plant tissues is also depended on its bioavailability in soil, that can be regulated by modification of soil properties. Lime application showed best results in lowering of metal accumulation at acid soils (Krebs et al. 1998).

Objective of our research was to investigate direct and subsequent Cd accumulation in spinach biomass at acid soil treated by lime and changes of Cd bioavailability in soil.

MATERIAL AND METHODS

Accumulation of Cd in spinach biomass was investigated in seven subsequent growing cycles in pot experiment at Luvisols (pH 5.2) containing $0.91 \text{ mg Cd}_{\text{TOT}} \cdot \text{kg}^{-1}$. The soil Cd content was elevated by addition of inorganic salt up to $1.5 \text{ mg Cd}_{\text{TOT}} \cdot \text{kg}^{-1}$ to model a moderate soil contamination. The lime powder (2.9 g CaO per kg) was added, thoroughly mixed with the soil and N,P,K, and inserted into pots. Spinach (*Spinachea oleracea* L.), variety Monores was planted and above ground biomass was harvested in full leaf development, washed, dried and analyzed. Soil after planting was air dried, mixed with N,P,K only and used for another planting. After each planting soil for analyses was taken and amount of available Cd was determined after $0.01 \text{ mol.l}^{-1} \text{ CaCl}_2$ extraction. The distribution of Cd among main soil fractions was determined by sequential extraction procedure at the end of experiment. Homogeneous material was analyzed by flame and flameless AAS in Trace element laboratory of Chemistry Department.

RESULTS AND DISCUSSIONS

The addition of lime played a positive role in yield formation of leaf biomass and mean yield of dry matter was about 15 % higher compare to control treatment over all seven cycles. The lime application led to decrease of Cd bioavailability at both unspiked and spiked with Cd treatments and Cd accumulation in spinach biomass was significantly lower at limed treatments in all growing cycles (Fig. 1). Differences between direct lime effect and subsequent ones were similar and caused 70 -80 % fall of Cd spinach content compare to control treatments. Significant positive direct effect of lime addition also found Xiong and Lu (1993) and other authors. The application of lime led to pH increase from 5.2 to 7.5. During continuing planting of spinach soil pH decreased at both control and lime treatments. At control treatment pH dropped to 4.3 and at lime treatment to 5.3, which corresponded to soil pH before beginning of experiment. The fall of pH at lime treatment did not substantially affect changes of Cd plant content. Also soil availability of Cd tested by CaCl_2 solution was significantly affected by lime application (Fig. 2). Limed soil contained about four fold lower Cd available content at all growing cycles. Decrease of soil pH during spinach planting led to slight but consistent increase of Cd availability. Only minor changes were determined in Cd distribution among main fractions determined by sequential extraction.

Fig. 1 Cd content in spinach leaves (mg.kg^{-1}) during seven growing cycles

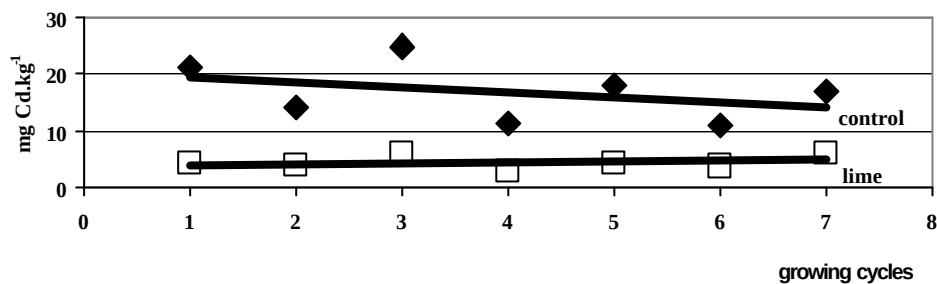
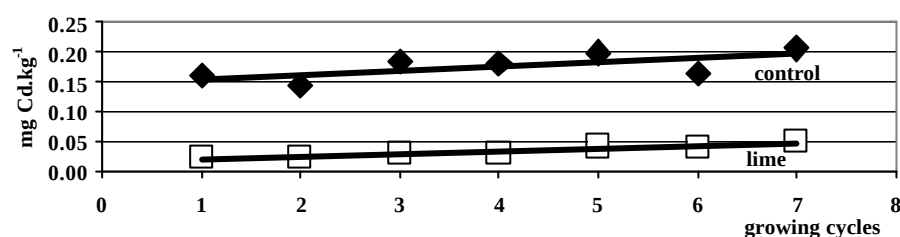


Fig. 2 The amount of available Cd in soil (mg.kg^{-1}) during seven growing spinach cycles



CONCLUSION

The experiment showed consistent long term effect of liming on decreasing of Cd bioavailability at acid Luvisol, confirmed by lower Cd spinach uptake and soil Cd extractability compare to control treatments.

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Bioavailability and bioaccumulation

Scientific Program 11

Poster

Analytical challenges and development for the speciation of organotin compounds in plant

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INTRODUCTION

Because the organic compounds of tin are widely used in many fields of activities, their ubiquitous environmental presence is today undeniably established. Recently, they have been found in rivers, cultivated lands and plants [Bancon-Montigny, 2001]. Unfortunately, some of these species such as the trialkyl- or aryltins are responsible of a significant number of ecological disasters and even suspected to be endocrine disrupters. Accordingly, they have given rise to a great deal of interest in analytical development, challenges being to have sensitive and reliable methods usable in routine conditions in order to monitor the different species in all the parts of the environment. In this way, some international projects of analytical standards for water and soil control have been proposed by ISO.

The food safety is also a crucial point. In order to answer to the socio-economic need, the monitoring of pesticides in fruits and vegetables has to be performed. Nevertheless, very few works exist on organotin speciation in plant. Only one method have been recently proposed: an extraction process has been optimised for the analysis of aquatic plant [Simon, 2002]. However, it seems to be not very well adapted for the organotin determination in vegetable samples such as potatoes, salad or even French beans.

The objective of the present study is to develop and optimise specific procedures suitable for control of organotin content in fruits and vegetables. In order to reach it, different processes of plant extraction of organotins were tested, according to the respect of the original speciation of organic tin in the sample.

MATERIALS AND METHODS

Analytical process. The analysis is based on aqueous ethylation using sodium tetraethylborate (NaBEt₄), separation by gas chromatography (GC) and specific detection, using flame photometric detection (PFPD). This process has been previously validated for environmental analysis [Bancon-Montigny, 2002].

The extraction from environmental solid samples is based on acidic solution, leading to minimum degradation [Bancon-Montigny, 1999]. It was studied considering the use of pressurised solvent extraction (PSE) and compared with the classical solid liquid extraction (SLE) using a mechanical shaking. After derivatization, the analytes are generally collected by liquid/ liquid extraction (LLE) before injection into the GC. Recently, headspace solid-phase micro-extraction (SPME) was proposed as a versatile alternative to LLE. LLE- and SPME- based analyses were compared in order to evaluate the performances of the different processes.

Plants. We chose to study leaves of French beans and tubers of potatoes because they are the most eaten vegetables in France. These plants were cultivated in the INRA Centre in Bordeaux. Their different parts were prepared in our lab, according to the description previously given. Before, they

were spiked with the different organotins of interest, according to the procedure recommended during the intercomparison exercise.

RESULTS AND DISCUSSION

Concerning the acidic extraction, it could seem that the liquid solid extraction made by using a conventional mechanical shaking (SLE) was slightly more quantitative when ethylacetate was used. However, in this case, poor repeatability was sometimes obtained. Methanol/ ethylacetate or methanol was also tested. The comparison of these different extracting solutions shows that the solvent mixture and ethylacetate give the most quantitative extraction, either using PSE or LSE. PSE appears to give the best repeatability and preserve organotin a little more than using SLE.

Some strong matrix effect could occur when LLE is used, since co-extracted products such as chlorophyll or starch lead to disturb at once extraction and analysis. The SPME is confirmed to be a powerful tool since the reliability and efficiency of the SPME- based process are very high. Thus, owing to the headspace process, the matrix effects decrease widely. The high preconcentration power of SPME leads to decrease the limits of detection, especially for the most volatile organotins, reaching sub $\mu\text{g (Sn) kg}^{-1}$.

Different applications made on vegetable materials were performed in order to validate the analytical process developed.

CONCLUSION

The different analytical developments proposed in this study lead to significantly improve the original analytical method based on acidic extraction/ NaBEt_4 ethylation/ LLE/ GC-PFPD. The results obtained from the different analyses made show the exactness and suitability of the proposed process for the monitoring and control of organotin in fruits and vegetables.

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Adsorption/Desorption of Lead, Copper, and Cadmium on Oxidic Soils as Affected by Phosphate Pretreatment

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INTRODUCTION

The mobility of trace elements in soils is essentially related to the factors that contribute to their adsorption/desorption in the solid phase. Metal ion adsorption may be enhanced by the presence of certain anions (McBride, 1994; Guilherme & Anderson, 1998). In highly oxidic soils, if an anion is added to a soil before the metal is added, the anion will affect metal sorption principally by converting S-OH groups into Sanion surface functional groups, possibly altering the net surface charge, and promoting the formation of Sanion-metal surface ternary complexes ($S = Fe^{3+}$ or Al^{3+}). This work evaluated the effect of phosphate pretreatment upon the adsorption and desorption of Pb, Cu, and Cd in A- and B-horizon samples of a Yellow-Red Latosol (LVAd) and a Dark-Red Latosol (LVd) (Table 1).

Table 1. Selected properties of A and B horizons of two Oxisols from Brazil.

Soil sample	Clay	OC [†]	Fe _d [‡]	Fe _o [‡]	Kt [§]	Gb [§]	Gt:H [¶]	SSA [#]
		g kg ⁻¹ soil			g kg ⁻¹ clay			m ² g ⁻¹
LVA	711	23.6±0.3	101	1.20	480	375	10.2	53±4
d _A								
LVA	721	8.0±0.1	114	0.59	480	400	8.3	58±3
d _B								
LVd _A	691	25.3±0.0	99	1.88	350	510	5.0	59±3
LVd _B	753	9.9±0.1	114	0.65	350	510	4.1	61±3

[†] OC is organic carbon measured by the Walkley-Black method.

[‡] Fe_d and Fe_o, respectively, are Fe extracted by dithionite-citrate-bicarbonate and ammonium oxalate (pH 3.1) in the dark.

[§] Kt and Gb, respectively, are kaolinite and gibbsite measured by differential thermal analysis in dithionite-treated clay samples (but expressed on a total-clay basis).

[¶] Gt:Hm is the goethite:hematite ratio in the clay fraction, measured by X-ray diffraction of NaOH-treated samples.

[#] Specific surface area determined by N₂ adsorption isotherms (BET).

METHODS

To assess the effect of the P pretreatment on the adsorption and desorption of these metals, samples of each soil material were reacted for 72 hours with 10.75 mmol L⁻¹ Ca(H₂PO₄)₂·H₂O (21.5 mmol P L⁻¹) at a soil:solution ratio of 2:3 to give a P addition rate of 1000 mg P kg⁻¹ soil. For metal adsorption, 0.3 g of the soil samples with and without P were initially pre equilibrated with 5 mmol L⁻¹ Ca(NO₃)₂ (soil:solution ratio of 1:67; pH 5.5), and then reacted separately with 0.45 mmol L⁻¹ Cu(NO₃)₂, Cd(NO₃)₂, and Pb(NO₃)₂, (final soil:solution ratio 1:100; initial metal concentration 0.15

mmol L⁻¹). Metal desorption was measured after a 72-h reaction of 30 mL of 5 mmol L⁻¹ Ca(NO₃)₂ (pH 5.5) with the remaining soil paste derived from the adsorption experiments.

RESULTS

Sorbed phosphate increased the adsorption of all metals, mostly on B-horizon samples. Cadmium was very weakly adsorbed on untreated B-horizon samples. Metal adsorption followed the sequence Pb ≥ Cu >> Cd (Figure 1). Phosphate pretreatment did not affect Cu fractional desorption (desorbed metal/adsorbed metal), which was always very small (< 0.03). However, the fractional desorption of Pb was about 3.5 times smaller for P-treated samples (0.02 to 0.08) when compared with untreated ones (0.10 to 0.29).

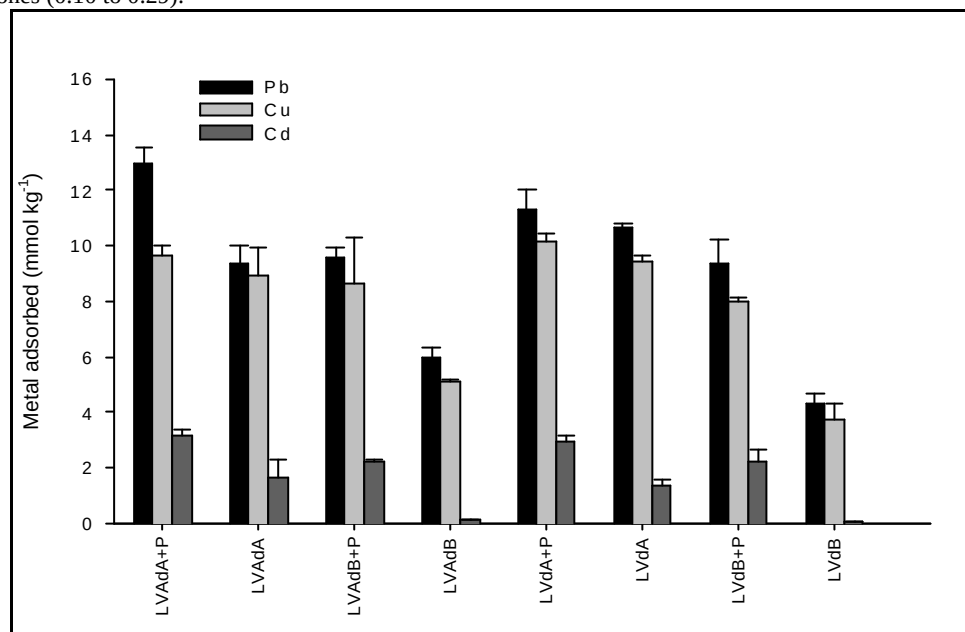


Figure 1. Metal adsorption in P-treated and control A- and B-horizon samples of two Oxisols reacted with Pb, Cu and Cd (initial metal concentration = 0.15 mmol L⁻¹; soil:solution ratio = 1:100; pH = 5.5; LVAd = Yellow-Red Latosol; LVd = Dark-Red Latosol).

The observed general increase in metal sorption and the remarkable decrease in Pb desorption due to the phosphate pretreatment indicates that the presence of P in these soils might contribute for reducing availability of the metals Pb, Cu, and Cd, which can be related to the formation of a ternary surface complex “surface-P-metal”.

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The availability of arsenic, copper and lead fractions after amendment with lucerne substrate

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INTRODUCTION

The organic matter and particularly the microbial pool and its activity can be significantly affected by pollution of soils by heavy metals. The metal bioavailability can only be assessed and is rarely quantified, particularly in microbial investigation (Giller et al. 1998). Many micro-organisms possess a considerable metal accumulating ability. Microbial activities can also play an important role in mobility of toxic elements in soils, but the properties of micro-organisms and their ability to participate in mobilization-immobilization equilibrium of heavy metals in soil-plant system are not often considered. Soil micro-organisms, which are associated with organic fractions of the soil, are expected to influence mobilization-immobilization equilibrium of metals by changing the chemical composition of their immediate micro-environment (Leita et al., 1999).

MATERIALS AND METHODS

The sampling site was situated in the vicinity of lead smelter 60 km SW of Prague in the Czech Republic. The two moist arable soils A and B from contaminated site containing 1138 and 505 mg Pb.kg⁻¹, 35.2 and 25.9 mg.Cu.kg⁻¹, 92.3 and 106 mg As.kg⁻¹ were amended by two rates of lucerne (rate I: 2.500 µgC.g⁻¹soil, rate II: 7.500 µgC.g⁻¹soil). Approximately 1 kg of each soil in plastic jars were placed in three replicates in greater plastic containers with tightly fitting lids at 28°C and the distilled water at the bottom of the containers to avoid drying of soils. A jar with 25 ml of 1M NaOH was placed to take up the CO₂-evolved. After one week of preincubation the soils were treated with lucerne powder. The soils were analysed 5 times (days: 0, 2, 7, 14, and 28) during incubation for the content of microbial biomass, pH and available heavy metal fractions. The measurements of the soil microbial biomass (B_c) were performed using the fumigation-extraction method according to Vance et al. (1987). Available heavy metal fractions were determined by sequential extraction according to SM&T EUR 14763 EN method (Ure et al., 1993).

RESULTS AND DISCUSSION

The metal mobility and microbial biomass dynamics during laboratory incubation with lucerne amendment are described on soil A and rate II in this paper. The microbial biomass content increased in all treatments in the days 1 and 2, thereafter it declined till the end of the experiment (Tab. 1). The pH values increased in the soil A till the day 7 of incubation and the significant correlations were found between microbial biomass and pH during incubation period ($r = 0.8944$, $P < 0.001$). Despite to increased pH values, the portion of elements in exchangeable fractions increased in the first two days (samplings 2 and 3) for all three studied elements (As, Cu and Pb) (Fig. 1). Exchangeable fractions represented only a small portion of the total metal content determined in soils, but significant correlations between element and microbial biomass contents were found, indicating that this fraction might be affected by microbial activities. The correlation coefficients of exchangeable metal fraction with pH were in most cases lower in comparison to microbial biomass. The oxide fraction of As increased in first two days of incubation. The percentage of copper oxide fractions varied between 0.67 – 2.81% confirming a fact that Cu is not an element commonly bound into iron or manganese oxides in soils. The extractability of lead from soil oxides decreased the most in the second day of the incubation (sampling n. 3) indicating that a part of Pb originally bound to soil oxides might be redistributed

preferentially into exchangeable fractions. The highest portion of organically complexed Cu and Pb and partly As was found the second day of incubation when the decrease of less available fractions was observed. This might suggest that during soil incubation the redistribution of element among fractions could occur in soils amended with lucerne in favour of more readily available, eventually of more easily soluble organic complexes. The results obtained suggest that increased microbial activities may alter soil conditions in different ways to enhance heavy metal availability in soils.

Table 1.: Dynamics of microbial biomass and pH during soil incubation with lucerne amendment and the correlation coefficients between heavy metal fractions and microbial biomass and pH (NS – non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Day of incubation		0	1	2	7	14	28
pH	Soil A	6,51	6,85	7,13	7,22	6,62	6,49
Microbial biomass	Soil A	213,81	444,89	540,27	465,83	415,31	354,53
($\mu\text{g C.g}^{-1}\text{ soil}$)							
Correlation coefficients	pH				Microbial biomass		
Soil fraction	exchangeable	oxide	organic	exchangeable	oxide	Organic	
Soil A - As	0,5756*	0,6115**	NS	0,7889***	0,7454***	NS	
Soil A - Cu	NS	NS	NS	0,6894**	NS	NS	
Soil A - Pb	0,6948**	-0,4870*	NS	0,7497***	-0,5946**	NS	

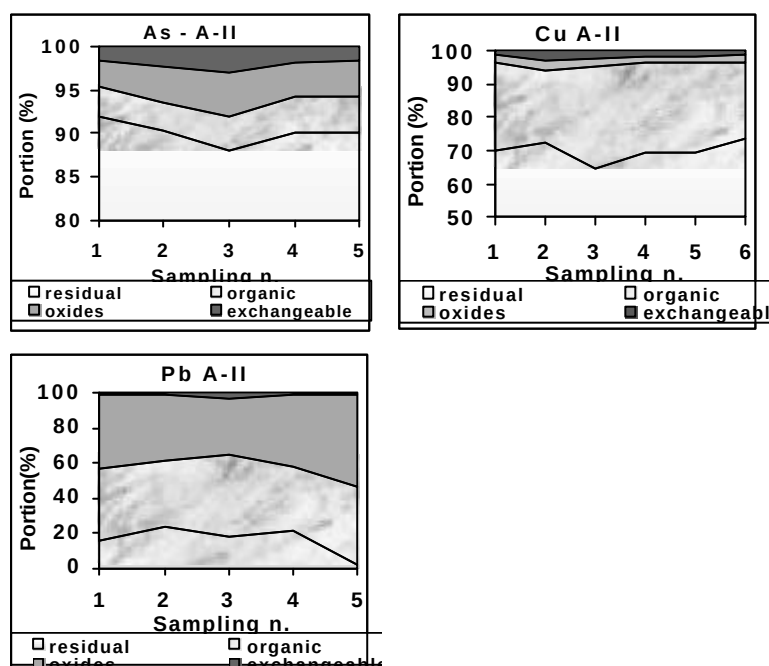


Fig. 1.: Distribution of heavy metals among fractions during incubation of lucerne amended soil

ACKNOWLEDGEMENTS.

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EDTA supplementation enhances legumes remediation ability to remove Pb²⁺ pollution.

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INTRODUCTION

It has been observed for many years that as a result of antropogenic activity a steady increase in environmental contamination with heavy metals, such as Cd, Pb, Cu takes place. In the process of adaptation to the new conditions plants evolved a number of mechanisms which enable them to function normally. Intense research is being conducted on using natural plant resistance in the process of phytoremediation to remove heavy metal ions from soil and water. Apart from the search for useful plant species, some research is also conducted on the application of synthetic chelators, which would make it possible to increase metal uptake and transport to the above-ground parts of plants in the process of the so-called induced phytoextraction. It was shown that among the examined chelating compounds such as DTPA, CDTA, EDDHA, EDTA, the last one, i.e. EDTA, is the most effective Pb²⁺ ion chelator (Huang et al., 1997, Geebelen et al., 2002). It was determined that EDTA not only increases the amount of Pb²⁺ uptake by the plant but also Pb²⁺ ion transport through the xylem and metal translocation from roots to stems and leaves. Our research was aimed to show how EDTA presence affects the resistance of *Pisum sativum* and *Phaseolus vulgaris* plants to Pb and Pb+EDTA presence, and the productivity of accumulation and translocation of Pb²⁺ ions.

METHODS

The research material were *Pisum sativum* and *Phaseolus vulgaris* plants cultivated hydroponically on Hoagland medium supplemented with 1 mM Pb(NO₃)₂ and also with 1 mM Pb + 0.5mM and 1 mM EDTA or only 0.5 mM and 1 mM EDTA. The roots and the aboveground parts of the exposed plants were cut after 2, 8, 24, 48, 72 and 96 h of cultivation.

The amount of Pb in different plant parts was determined with the use of atomic spectroscopy.

The level of thiol compounds in roots of the treated plants were determined by means of the HPLC method combined with the postcolumn reaction with DTNB using Elmann reagent.

RESULTS AND DISCUSSION

Substantial effect of the synthetic chelator was observed on increasing the degree of metal accumulation in roots of plants treated with Pb with EDTA (0.5 and 1mM). *Pisum sativum* roots treated with only 1 mM Pb(NO₃)₂ accumulated over 50 mg Pb/g DW during 96 h of exposition. While in roots of pea plants exposed to Pb²⁺ ions with the addition of 0.5 mM EDTA over 35% more Pb was observed (67 mg/g DW). The presence of EDTA especially at the equimolar concentration also noticeably increased metal uptake and transport to the aboveground parts of plants. In pea plants treated with 1mM Pb(NO₃)₂, only c. 3mgPb/g DW was transported to the aboveground organs, while in plants cultivated with 0,5 mM EDTA supplementation. 3.5mg Pb/g DW was observed in the aboveground parts of *Pisum sativum* treated with 1mM Pb(NO₃)₂ + 0.5mM EDTA. When the amount of EDTA was increased, almost twice (1mM) as much Pb - 6.5mg Pb/g DW - was observed in stems and leaves of plants treated with metal for 96h (in stems and leaves - 4.2 and 2.3 mg Pb/g DW, respectively). Even more ample growth of Pb amount after EDTA supplementation was seen in *Phaseolus vulgaris* plants cultivated in the presence of the chelator. It was demonstrated that in roots of

bean plants treated with $\text{Pb}(\text{NO}_3)_2 + 0.5 \text{ mM EDTA}$ for 96h, 36% of metal uptake was accumulated and over 50% was transported to the leaves.

The effect of EDTA was also observed in the profile of thiol compounds, such as glutathione, homogluthathione and homo- and phytochelatins. In pea roots treated with Pb-EDTA, even three times higher amount of synthesized thiols - glutathione and homogluthathione was demonstrated in relation to plants exposed only to $1 \text{ mM Pb}(\text{NO}_3)_2$. Similarly, in leaves of bean plants (in which homogluthathione is the basic thiol) cultivated with the supplementation of $1 \text{ mM Pb}(\text{NO}_3)_2 + 0.5 \text{ mM EDTA}$, a 2.5 times higher increase of homogluthathione level was observed in relation to plants cultivated only with Pb ions. In roots of pea plants exposed to Pb + EDTA, the first phytochelatins appeared only after 24h of incubation, similarly as the first homophytochelatins, whereas in roots of plants treated with Pb without the chelator, the induction of the synthesis of phytochelatins occurred just after 2h of incubation. Thus, EDTA supplementation delayed noticeably the induction of PCs synthesis. In roots of *Phaseolus vulgaris* plants, it was demonstrated that the induction of homophytochelatins synthesis occurred after 24h of exposure both in roots of plants treated with Pb alone and in roots with Pb and the chelator. Increasing Pb transport to the aboveground plant parts, EDTA supplementation increases markedly remediation abilities of the two examined legumes, simultaneously limiting their phytotoxicity in relation to Pb.

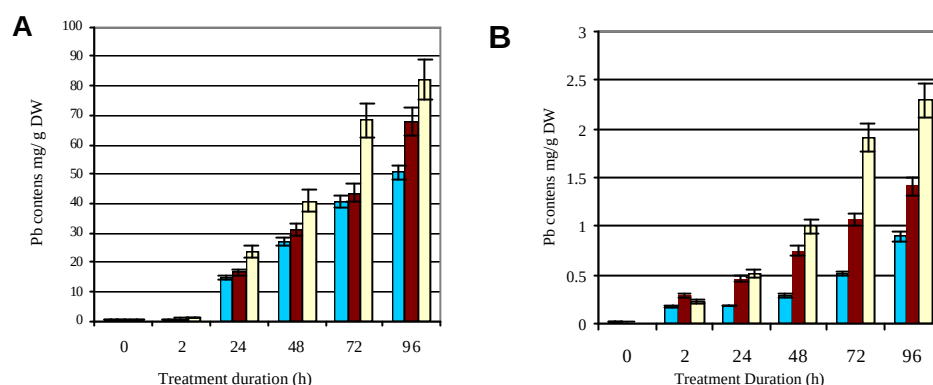


Fig.1 The changes in lead accumulation (mg/g DW) in roots A and leaves B of pea plants treated with $1 \text{ mM Pb}(\text{NO}_3)_2$, $1 \text{ mM Pb}(\text{NO}_3)_2 + 0.5 \text{ mM EDTA}$ and $1 \text{ mM Pb}(\text{NO}_3)_2 + 1 \text{ mM EDTA}$

CONCLUSIONS

The presented research results lead to the conclusion that the application of the chelator with Pb^{2+} ions caused a decrease in metal phytotoxicity. It was also shown that EDTA significantly affects not only Pb^{2+} accumulation and metal transport to the aboveground plant parts but also the profile and the amount of thiol compounds such as glutathione, homogluthathione or homo- and phytochelatins synthesized by plants.

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Biotic and abiotic processes in the rhizosphere

Symposium 2

Poster

The Influence of Cell-surface Electrical Potential on Plant-ion Interactions

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Plant plasma membranes (PMs) incorporate negative charges in their constituent molecules. These charges may be screened by free ions in the adjacent solutions or they may be altered by the binding of ions to the PM surface. The charges establish an electrical potential (ψ_0) at the PM surfaces that is different from the transmembrane potential (E_m). Figure 1 illustrates these potentials, but the figure does not show the negatively charged cell wall, which is also responsive to ions in the medium. The value of ψ_0 may be computed with a Gouy-Chapman-Stern model that combines electrostatic interactions and ion binding. The requisite parameters for the model, based upon experimentation and simplifying assumptions, are now available for the computation of ψ_0 in response to the ionic composition of the external medium.¹

Some ions are more effective than others in altering ψ_0 . Cations are effective in the reduction of surface negativity in this order: $Al^{3+} > La^{3+} > H^+ > Ca^{2+} \geq Mg^{2+} > Na^+ \approx K^+$, and Al^{3+} , La^{3+} , and H^+ can convert ψ_0 to positive values. Figure 2 illustrates the close agreement between computed ψ_0 and surface potential measured by electrophoresis (ζ potential). Once ψ_0 has been determined, the PM-surface activities of ions may be computed with the familiar Nernst equation, $\{I^Z\}_0 = \{I^Z\} \exp[-ZF\psi_0/(RT)]$, which relates the activity of ion I with charge Z at the PM surface (subscript 0) to the activity of the ion in the external medium (no subscript). $F/(RT) = 1/25.7$ when ψ_0 is expressed in mV and the temperature is 25°C.

Recent experimental work demonstrates that $\{I^Z\}_0$ is a much better predictor of plant-ion interactions than $\{I^Z\}$. For example, Ca^{2+} uptake is inhibited by Al^{3+} (and by other cations). This inhibition is commonly interpreted as a blockade of Ca^{2+} channels by Al^{3+} , but Figure 3 illustrates that the inhibition is more likely the result of Al^{3+} -induced reduction of surface negativity and the consequent reduction of $\{Ca^{2+}\}_0$. Conversely, SeO_4^{2-} uptake is enhanced by Ca^{2+} (and by other cations). This enhancement is likely the result of Ca^{2+} -induced reduction of surface negativity and the consequent increase of $\{SeO_4^{2-}\}_0$. A recent theoretical treatment of ion fluxes proposes that electrostatic effects may account for rate, saturation, *cis*- and *trans*-inhibition, rectification, voltage gating, shifts in voltage optima, and other phenomena also attributable to other mechanisms.²

Electrostatic effects also account for interactions between plants and toxic ions and for the alleviation of toxicity by ameliorative ions such as Ca^{2+} . Figure 4 illustrates the commonly seen enhancement of root elongation by low levels of Al^{3+} followed by inhibition at higher levels. The figure also illustrates the enhancement of elongation by Ca^{2+} or Mg^{2+} . All of these effects can be adequately interpreted in terms of PM-surface activities of the ions, especially H^+ .

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Rhizosphere effect on distribution P, B, Pb and Cd in the root zone of tree seedlings in reclaimed oil-shale mining detritus

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In Northeast Estonia, forest rehabilitation of skeletal calcareous detritus of open cast oil-shale mining has been carried out since 1960 in area over 8000 ha. Coarse calcareous limestone fragments formed mainly the newly exposed surface; proportion of fine earth (< 1 mm) reached up to 25 g kg⁻¹, latter contains mostly oil shale remains. The average content of organic solids formed 20.5 g kg⁻¹. Considering alkaline soil reaction with pH 8.1, the deficiency of P might occur in field conditions. Also low total nitrogen content 0.31 g kg⁻¹ and low water storage capacity of stony soils serve quite harsh environment for tree seedlings after planting.

The aim of the study was to estimate the effect of roots and associated microbial communities ('rhizosphere effect') on distribution of two essential nutrients for plant development (B and P) and two toxic trace elements (Pb and Cd) in the root zone of tree seedlings planted into calcareous detritus.

Sieved soil (3 mm) was used in pot experiment with one-year-old seedlings of Norway spruce (*Picea abies* (L.) Karst.), Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth). Samples for analyses (12-15 pots per tree species) and bare soil as control were taken in the middle of growing season. Soil and roots were separated in three fractions: soil-root interface (SRI), rhizosphere and bulk soil according to Gobran and Clegg (1996). Element concentrations have been measured with ICP-AES; values for bulk soil are given in Table 1.

Table 1 Concentrations of P, B, Pb and Cd mg kg⁻¹ in fine detritus (< 1mm)

Bulk soil	P	B	Pb	Cd
Bare soil	400	18	12	0.05
<i>Picea abies</i>	390	15	9.7	0.10
<i>Pinus sylvestris</i>	400	14	11	0.15
<i>Betula pendula</i>	390	15	18	0.08

Phosphorus concentrations were low (Table 1) comparing to the average values of Estonian soils. The soluble P determined in ammonium lactate formed 59 mg kg⁻¹, which was unexpectedly high. The boron content was twice so high as estimated for Estonian Rendzinas, which indicated that the extensive leaching has not yet taken place. Also Cd and Pb content remained slightly lower than it has been estimated for North Estonian cultivated Rendzinas.

For P no difference was found between rhizosphere and bulk soil, but the mean concentrations of P in SRI were about twice as high and formed for spruce, pine and birch 880, 670 and 630 mg kg⁻¹, respectively. Roots and associated microbial communities acidified the surrounding soil, because the difference in pH between bulk soil and SRI formed from 1.45 to 1.79 pH units. There was significant increase in B concentration from soil to roots, the mean values for bulk soil, rhizosphere and SRI were 17.7, 18.0 and 43 mg kg⁻¹, respectively. Boron concentrations in SRI were high; for mature trees of Scots pine and Norway spruce growing in Estonian forest soils the values for fine roots D < 2 mm varied between 7 and 26 mg kg⁻¹, however the boron demand for seedlings may differ from those of mature trees.

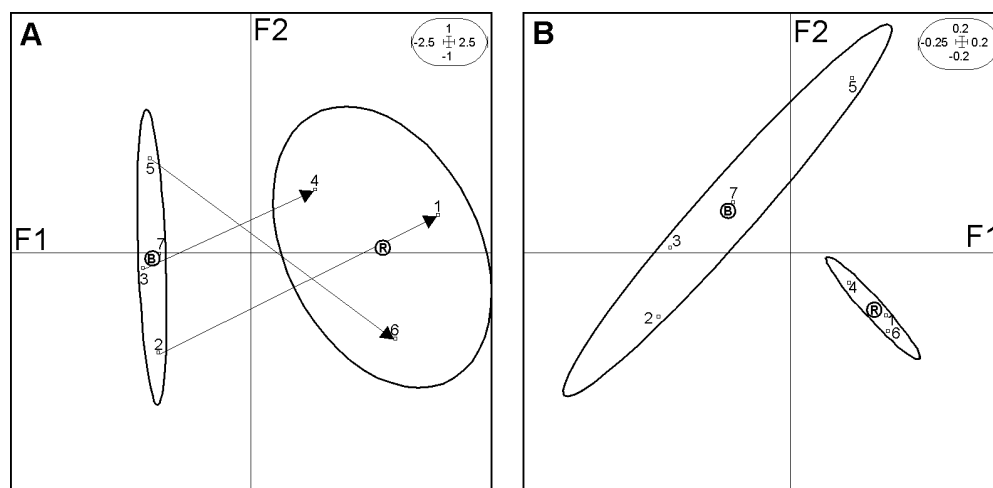


Figure 1. Principal component analysis (PCA) plots of substrate utilization patterns of SRI and bulk soil microbial communities estimated using BIOLOG EcoPlates data. (A) PCA based on covariance matrix of Biolog non-standardized data. The first and second principal components described 87.0% and 4.5% of the overall data variation, respectively. Arrows are connecting SRI and bulk soil samples of the same tree species.

(B) PCA based on covariance matrix of Biolog data adjusted for average well color development (AWCD). The first and second principal components described 44.6% and 27.7% of the overall data variation, respectively. Symbols: 1 – pine SRI, 2- pine bulk soil, 3 – birch bulk soil, 4 – birch SRI, 5 – spruce bulk soil, 6 – spruce SRI, 7 – bare soil (control without plant), B – group centroid of bulk soil samples, R – group centroid of SRI samples.

The analysis of substrate utilization patterns of SRI and bulk soil microbial communities estimated using BIOLOG EcoPlates data show distinctive differences between SRI and bulk soil microbial communities. Figure 1A shows results of ordination of soil samples based on non-standardized BIOLOG data. In such case main gradient (axis F1) in data set is related to differences in microbial community density. While bulk soil samples have practically similar microbial abundances, the SRI microbial communities exhibit bigger differences in density values. Highest densities can be attributed to microbial communities in SRI of Scots pine and Norway spruce. The effect of microbial community density is removed from Biolog data when initial values are adjusted for average well color development (AWCD). This transformation yielded ordination of microbial communities, which indicates that structure of SRI communities is very similar (Fig. 1B). The structure of microbial communities of bulk soil samples shows rather big variation.

In general, SRI microbial communities differ from bulk soil communities both by density and structure. Higher activity of microbial communities in SRI, higher acidity and accumulation of essential nutrients P and B are the result of different rhizospheric processes. Toxic trace elements Cd and Pb were evenly distributed in considered fractions of soil-root continuum, no differences between bulk soil, rhizosphere and SRI were revealed.

ACKNOWLEDGEMENTS

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Trace elements in Sugarcane crop field experiments with irradiated biosolids fertilization.

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INTRODUCTION

Biosolids have been extensively used to increase soil fertility and crop yields. Land application is controlled by specific regulations all over the world to meet hygienic criteria and to prevent harmful environmental effects due to the pathogen microorganisms, heavy metals and organic toxics contents. Irradiation treatment is recognized as a reliable tool to eliminate the risk due to pathogen agents, and it is likely useful for decomposition of macromolecules; but no effect is expected on the elements.

A Sewage Sludge Irradiation Project has been developed in Northwestern Argentina, based on Co-60 gamma radiation sources, for disinfection of anaerobically digested sludges, product of the Tucuman City Wastewater Treatment Plant. The aim is the agriculture reuse of biosolids on surrounding sugarcane fields. Four years of annually experimental sugarcane crop have been evaluated to assess benefits as well as limitations of biosolids application on land.

Heavy metals concentration (Cd, Cu, Cr, Ni, Pb, Zn) in the sludges and sludge-amended soils were measured as it is usual to meet the regulations in every country. Some of them, among other trace elements (Ca, Mg, Mn, Mo, Fe, Al) also play significant roles as micronutrients for sugarcane crop. In this paper, measurements of these elements in soil, plant tissue and sugarcane juice are described to evaluate the incidence of accumulated sludge-borne elements on the plant composition and finally on the consumption product: sugar.

MATERIALS AND METHODS

Physico-chemical characteristics and elements concentrations in the biosolids were well studied before and after experimental irradiation process. No significant effects of the process were found on the chemicals (except available N). Radiation absorbed dose was limited to the disinfection requirements, 3.5 to 6.5 kGy.

Sugarcane crop fields previously planted, were used for experiments at Tucuman City. Fertilization rates were based on the ratio: sugarcane N requirement over N% in the biosolids, applying 100%, 200%, and 300% ratio. For the purpose of this study only the maximum rate (10 ton d.m./ha) was considered, and compared to control plots without fertilization. Soil samples were collected from three depth levels (0-10 cm, 10-20 cm, 20-30 cm), finely ground and chemically digested for measurement. Plant tissue (green leaves) samples were collected at plant maturity (8 months) and digested in acid mixture (modified method of Mc Grath et al.). Sugarcane juice extracted from the plants were also acid digested after soft oven drying to constant weight. The elements concentration in the acid extracts were measured by ICP AES.

RESULTS AND DISCUSSION

Heavy metals Cd, Cu, Cr, Ni, Pb and Zn concentration in soil, increases due to sludge-borne contamination; there are some differences among the elements, about the distribution in depth according to different mobility (less Pb in deepest zone, for example). Ca, Mg and Mn concentrations are also increased in biosolid amended soil respect to control soil. Molybdenum is hardly detected in

soil samples. There are no increased value in the sugarcane leaves samples of biosolids amended plots compared to leaves of control plots plants, at least after four consecutive maxima fertilizations.

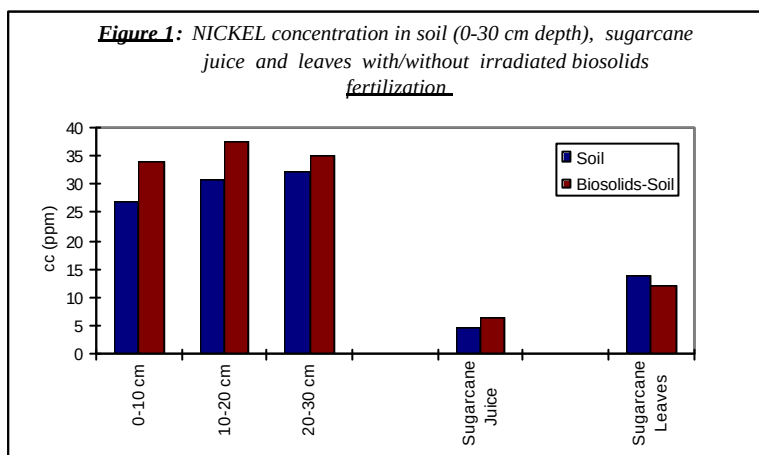
There are three elements Cu, Ni and Mg significantly increased in the sugarcane juice samples, respect to juice samples of plants from control plots. Cu and Ni, as well as Zn are from the group of elements that were identified as “strongly absorbed” by sugarcane in literature data. Factors affecting the metals behaviour (soil texture and acids composition, climate) also include plant species and management.

Anyhow, the increase (Cu: 3.9 ppm to 5.0 ppm; Ni: 4.8 ppm to 6.4 ppm and Mg: 783 ppm to 896 ppm) might be negligible on the final consumption product: sugar, as it is demonstrated by a theoretical simple calculation. On the other hand, routine tests of sugar quality (Brix, Pol, Purity) revealed no deleterious effect by biosolids application on soil.

CONCLUSION

The project is dealing with cautious agronomic rate of biosolids application on land, and heavy metals contents in sludge are far enough the regulation limits. But after four annual applications of maximum rate, in a few cases the concentration in soil is exceeding the limits. Nevertheless, higher concentrations of trace elements detected in sugarcane juice due to biosolid fertilization are still rarely significant. That is an encouraging assessment to promote the reuse of biosolids for this kind of industrial crop fertilization.

Note: Figure 1 is given as an example of one of the twelve elements studied.



METAL COMPLEXING CAPABILITIES OF PHYTOSIDEROPHORES: IMPLICATIONS FOR TRACE-METAL PHYTOTOXICITY AND FOOD CHAIN TRANSFER

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INTRODUCTION

Phytosiderophores (PS) (including the mugineic acids) are amino acids that are universally excreted by the roots of grasses when subjected to iron deficiency in soils of alkaline pH. The mugineic acids are important for Fe solubilization and uptake, including in a number of cereal and grain crops, but may also be important in the acquisition of other essential metals (Zn, Cu) or in the mobilization of metals that pose health concerns (Cd). We are studying the metal-binding chemistry of PS to clarify how effectively other metals can compete with Fe(III); this in turn will disclose whether soil metals (Cd) are likely to be mobilized into the food chain by MAs, and whether excessive Cu or Ni could induce Fe deficiency in grasses.

MATERIALS AND METHODS

To simulate the Fe(III), Cd, Zn, Cu and Ni solubilizing properties of 2'-deoxymugineic acid (DMA) in the rhizosphere, we used empirically based solubility information modeled in GEOCHEM-PC (Parker et al., 1995). The solubility of Fe(III) in soils was described by a simple oxyhydroxide phase with a $\log K = -2.7$ (Lindsay, 1991). For the other metals we used the concept of a "mythical soil ligand" with characteristics based on metal solubility in soil models determined by other researchers to determine stability constants (Gray et al., 1999; McBride et al., 1997; Sauve et al., 2000). These models take the form of:

$$\log (M^{2+}) = a + b \text{ pH} + c \log M_T + d \log \text{OM}$$

where the a term is an empirical constant (intercept), while the other three coefficients reflect the dependence of solubility on pH, total soil metal content (M_T), and organic matter (or carbon) (OM), respectively. For metal-DMA complexation, we used stability constants determined by Murakami et al. (1989). No stability constants have been determined for Cd (determinations are currently underway in our laboratory), hence, the $\log K$ for Cd was assumed to be as per Zn. We used DMA concentrations of 25 μM as likely to be representative of the rhizosphere.

RESULTS AND DISCUSSION

To test the ability of Fe(III) to compete for DMA with other metals, a system of two metals was modeled. The simulations suggest that while Fe(III) was able to effectively compete with Zn, Cd, Cu or Ni at low pH and complex the majority of the DMA present, at higher pHs the other metal dominated DMA complexation (see Figure 1). Regardless of whether field-grown plants excrete PS in response to deficiencies of micronutrients other than Fe, metal-binding by MAs may have implications for the solubilization and enhanced uptake of metals from contaminated soils, and hence the potential to increase food-chain transfer hazards. Cadmium is highly toxic to humans and animals, and is thus of particular concern and binding of metals such as Cu, Ni and Zn by DMA could interfere with the ability of the PS to provide adequate Fe to meet plant nutritional needs.

Further GEOCHEM-PC simulations with 25 μM concentrations of DMA, Fe(III) and Cd/Zn/Cu/Ni, no precipitation and no "mythical soil ligand" were of the same general shape as those in Figure 1 but had different crossover points. For example, Cu and Fe(III) were at equimolar concentrations at a pH of ~ 3.5 (c.f. ~ 4.5 in Figure 1) and Fe(III) and Cd at ~ 8.5 (c.f. ~ 7.5). This indicates that the assumed solubility of the soil solid phase will influence the speciation of DMA in the rhizosphere

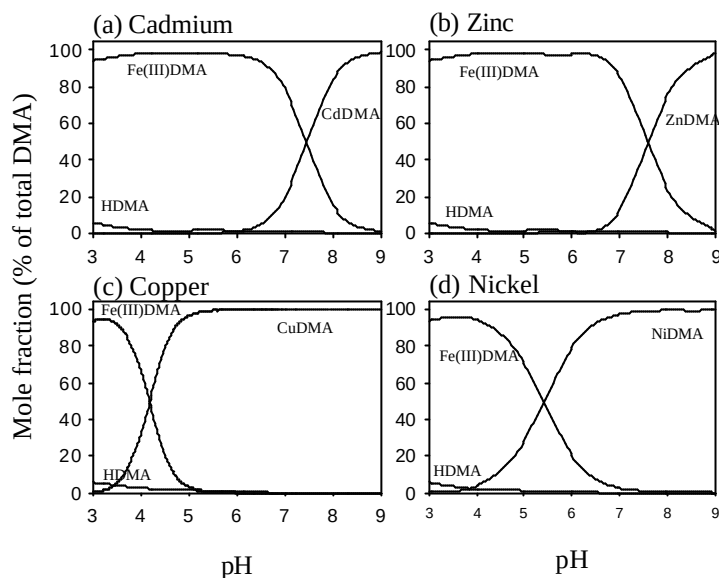


Figure 1. GEOCHEM-PC simulations of the pH dependent competition between Fe(III) and (a) Cd, (b) Zn, (c) Cu and (d) Ni for binding to DMA. Metal solubility characteristics were simulated using empirical solubility models (for explanation see text). Soil moisture contents were 25 %. DMA was added to give a final soil solution concentration of 25 μM and the ionic strength was 0.03 M.

CONCLUSIONS

Binding of other metals besides Fe(III) appear to play an important role in PS dynamics in the rhizosphere with implications for plant nutrition and food-chain transfer. To further investigate this area our laboratory is in the process of reevaluating the stability constants for Fe, Zn, Cu and Ni using high purity chemical synthesized DMA and expanding the range of ionic strengths from previous research. In addition we will provide the first stability constants for CdDMA. We also aim to empirically test our modeling results in soils over a range of metal loadings.

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Natural remediation-bioavailability interactions in contaminated ecosystems: Concepts and applications

Symposium 5

Oral

EFFECTS OF pH ON SOLUBILIZATION AND BIODEGRADATION OF PHENANTHRENE WITH BIOSURFACTANT

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INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are on the U.S. Environmental Protection Agency (EPA) priority pollutant list, because some are known carcinogens and mutagens. PAHs are hydrophobic and most are practically insoluble in water contributing to their persistence in the environment. Their removal efficiency can be limited in low mass transfer phases, such as PAHs-contaminated soils, and recent research has examined the possibility of enhancing the bioavailability of low solubility and highly sorptive compounds by the addition of solubilization agents, such as a surfactant, to the system. Biosurfactants are attractive because they are natural products, which are biodegradable and have potential for use in in-situ remediation and the morphology of biosurfactant can be significantly affected by changes in pH. The purposes of this study were to examine: 1) the effect of pH on phenanthrene solubilization using a biosurfactant solution, and 2) the effect of pH on the rate of biodegradation of phenanthrene in the presence and the absence of the biosurfactant.

MATERIALS AND METHODS

The effect of pH on solubilization of phenanthrene.

The effect of pH on the solubility of phenanthrene in the biosurfactant solution was determined by batch tests. Phenanthrene (100mg) was added to 45ml centrifuge tubes and 20ml of the biosurfactant solution (240-mg/l & 20000-mg/l) in deionized water at the various pHs was added. The rhamnolipid solutions pHs were adjusted and triplicate samples were placed on an orbital shaker for 48 hours at room temperature (25°C). Samples were centrifuged at 4000 rpm for 10 minutes, and the supernatants were analyzed by HPLC.

The effect of pH on cell growth with biosurfactant.

The biodegradation of phenanthrene was quantified in two ways: 1) direct measurement of phenanthrene loss, used to determine substrate utilization, and 2) spread plate cell counts, used to evaluate cell growth. For phenanthrene utilization experiments, 4mg of phenanthrene was dissolved in chloroform and added to the 125ml flasks. Mineral salt medium (MSM) was made, and the pH of the solution was adjusted with HCl or NaOH. Twenty milliliters of MSM was added to each flask, and the samples inoculated with 0.2ml aliquots of the phenanthrene degraders from precultures. The inoculated samples were incubated at 25 °C, in a water bath shaker at 80 rpm. The cell growth was measured by spread plate cell counts on R2A agar. At the end of the experiment, the contents of each flask were extracted with 30-ml of chloroform. The amount of phenanthrene in the extract was quantified by high-performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

Effect of pH on solubilization of phenanthrene

The phenanthrene solubility in the rhamnolipid solutions, at each pH, as determined by the centrifuge vial tests are presented in Figure 1. The highest solubilities were detected in the pH range 4.5 to 5.5 for both concentrations. The apparent solubilities at pH 5.5 with the 2000-ppm and 240-ppm

rhamnolipid were 36 and 25.7 times greater, respectively, than those at pH 7. The effect of pH on phenanthrene solubility seems to correlate to the type of rhamnolipid aggregate present in the solution. Based on our result, vesicles may have a larger solubilizing capacity for phenanthrene.

The effect of pH on cell growth with biosurfactant.

The effect of rhamnolipid and pH, on cell growth and biodegradation of phenanthrene, were monitored as described in the growth experiments above. The specific growth rates at different pHs are shown in Figure 2. Compared to the μ values obtained without the biosurfactant, the specific growth rates were increased, and the maximum μ values shifted from around pH 6 without the biosurfactant, to around pH 5 which showed a maximum enhancement in the solubility for the abiotic experiment. Zhang et al. (1997) reported the addition of rhamnolipids markedly increased the rate of dissolution of phenanthrene, but increased the rate of biodegradation more moderately. Also, the uptake of micellar phenanthrene requires the transfer of substrate from micelles to cells, which should be inversely related to the affinity of the substrate for micelles. In our study, the increase in the specific grow rates observed were not as high as the increase in the solubilization. The 1.44 times increase in μ values at pH 5 was lower compared to the 3.6 times enhancement of solubility. This is supported by the fact that all of the solubilized phenanthrene is not bioavailable to the microorganisms. The trends in the μ values were validated by the total substrate loss (%) at the end of the experiment, and this analysis shows similarly with the shift in the maximum value from pH 6 to 5 (Figure 2).

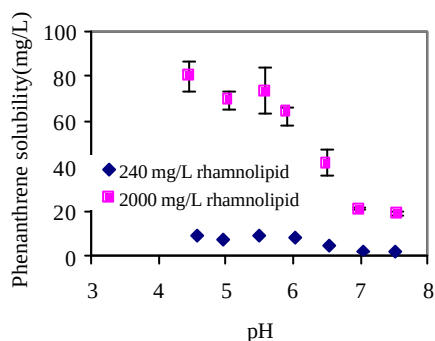


Fig. 1. The effect of pH on phenanthrene solubility

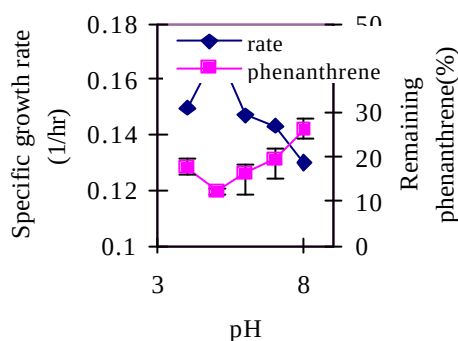


Fig. 2. The effect of pH on specific growth rate, and phenanthrene biodegradation

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Chemistry, bioavailability and recycling within biota
of artificial and natural radionuclides: Towards an
integrated approach in mobility assessment

Symposium 6

Oral

Applying Radioecology in a World of Multiple Contaminants

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INTRODUCTION

The discipline of radioecology grew as a response to society's need for the best possible scientific understanding of the impacts of nuclear materials on the environment. It is now a mature, hybrid science with the advantages of interdisciplinary links between the primary disciplines of physics, chemistry, biology and geology. It has a strong foundation of quantitative and predictive science, based on 50 years' experience of some of the most interesting and challenging environmental problems to face mankind. Radioecologists have developed extensive data bases of both environmental activity concentrations and transfer parameters which have underpinned the development of numerical models of radionuclide transfer and impact in a wide variety of ecosystems. The achievements of radioecology are impressive.

On the other hand, a fundamental weakness of radioecology has been its tendency to consider radioactive contamination of the environment in isolation from other branches of environmental science, particularly those concerned with the impacts of contaminants on the environment. This has resulted in the parallel evolution of radioecology and conventional pollution ecology. The opportunities for convergence of these evolutionary paths have always existed but the interests of radioecologists have lain firmly within the boundaries of specific problems associated with a small number of cases such as global fallout, Sellafield and Chernobyl. Now that the focus on such problems is reducing it is timely to consider common issues which confront radioecology and pollution science and ways in which a convergence of interests and activities might be achieved.

ENVIRONMENTAL EXPOSURE – A COMMON CAUSE

Radioecology and conventional pollution science are ultimately concerned with the impact of contaminants, radioactive or non-radioactive, on the biotic world. This is, at least in part, a selfish interest since humankind is an integral part of this biotic world. From the beginnings of radioecology the impact of radionuclides on non-human biota were of interest, from studies on the effects of radiation from the Hanford reactors on salmon in the Columbia River, USA, to the broader ecological effects of the Kyshtym accident in the former Soviet Union. When considering the biological effects of potentially toxic substances released to the abiotic world, tracing the pathways and, ultimately, the bioavailability of both radioactive and non-radioactive contaminants is a key consideration. If organisms do not come into contact with contaminants, or the degree of contact is slight, then the impacts of contaminating substances are likely to be small or even non-existent. Understanding the degree of exposure of organisms to contaminants of all types is therefore key to a successful understanding of their environmental impact.

Occasionally, the environmental exposure pathways *via* which organisms become exposed to individual contaminants will be unique to a single contaminant or a specific group of contaminants. Usually, however, contaminants move along common environmental exposure pathways, though the rate and degree of movement differ between substances. Thus, whether we are studying the environmental impact of potentially toxic metallic elements or fission and activation products from diverse locations within the Periodic Table, the pathways of transfer, dispersion and accumulation within the environment are broadly the same. This should lead us to the conclusion that the techniques we use for understanding, quantifying and predicting these pathways should also be similar, at least at the conceptual level. A significant case in point is the concept of bioavailability. In many respects

bioavailability of radionuclides and other potentially toxic substances released to the environment is the most important problem for radioecologists and pollution ecologists, alike. Bioavailability controls the ultimate exposure of organisms to contaminants and hence the final potential environmental impact of those substances. However, despite several decades of efforts to improve our understanding, and even the definition, of bioavailability much has still to be learned in this area. Conceptually and methodologically, this is a key problem which should not be solved separately for different classes of environmental contaminants, but collectively as a unifying principle in environmental pollution science.

RADIONUCLIDES – STILL THE BEST TRACERS?

If the exposure pathways *via* which metals and radionuclides move through the environment are similar, a major dissimilarity between these groups of contaminants is the ease with which they can be measured. The vast number of measurements of ^{137}Cs amassed since the Chernobyl accident is testament to the ease with which radionuclides, particularly gamma emitters, can be measured in complex environmental media. Despite the advent of modern techniques such as ICPMS, which provide high sensitivity for multiple elements in single samples, destruction of the sample matrix is still required to obtain analytical results. Measurements, in general, tend to disturb the system being measured, but non-destructive, *in situ* measurements cause less disturbance than destructive, *ex situ* measurements. Such measurements are still easier to obtain using appropriate radiotracer techniques although, in recent years, the advantages of such techniques in informing us about biosphere processes has often been overlooked. One major advantage is the presence in environmental systems of natural cosmogenic tracers such as ^{14}C , ^{36}Cl and primordial tracers such as ^{40}K and ^{210}Pb . In addition, experimental application of techniques such as *in situ* tracers, isotopic dilution, radioimmunoassay and the labelling of organic compounds continues to provide a powerful array of methods by which measurements can be made of contaminant behaviour in environmental systems, often non-invasively.

Conceptually, radioecology and conventional pollution science have much to share. The existence of multiple contaminants at many locations around the world dictates that assessment and remediation exercises should be carried out in an integrated fashion, as a partnership between scientific disciplines. Perhaps the major contribution that radioecology can make to this partnership is its methodology which, in many respects, remains unique.

Chemistry, bioavailability and recycling within biota
of artificial and natural radionuclides: Towards an
integrated approach in mobility assessment

Symposium 6

Poster

The Effect of applying EDTA on the phytoextraction of metals by rainbow pink growing in the soils contaminated by cadmium, zinc and lead

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ABSTRACT

Using green plants to uptake the toxic elements from contaminated soils is an economical and friend remediation strategy and the application of synthesize chelating agents to soils contaminated by metals appear to increase the concentrations of heavy metals either in soil solution or in the shoot of plants in the last decade, especially with EDTA. The garden flower, rainbow pink (*Diathus chinensis*), can uptake high concentration of cadmium after planting in the Cd-contaminated soil of north Taiwan only in 5 weeks from previous studies in Taiwan. The soils for pot experiments were seriously combined contaminated by cadmium (20 mg Cd/kg soil), zinc (500 mg Zn/kg soil) and lead (1,000 mg Pb/kg soil). In this study, three concentration levels of EDTA solution (0, 5 and 10 mmol/kgsoil) were added to the contaminated soils after 7 days of planting to study the influence of applying EDTA on the phytoextraction by rainbow pink growing in multiple metals contaminated soil. The experiments were conducted in three replicates. Plants were harvested after 7 days of addition the EDTA solution. The soil solution was sampled directly by Rhizon soil moisture sampler (RSMS) from the pot.

The results indicated that the concentrations of Cd, Zn, and Pb in soil solution significantly increased after application of EDTA treatments ($p < 0.05$) (Fig 1). The concentrations of Cd and Pb in shoot of plant also significantly increased after application of EDTA treatments ($p < 0.05$), but not significantly increase for Zn (Fig 2). For biological uptake of metals from contaminated soils, the EDTA treatment only significantly increase the total uptake of Pb in the shoot of plant compared with the control treatment ($p < 0.001$), but not significantly increase for Cd and Zn uptake by plant ($p > 0.05$) (Fig 3). This indicated that the EDTA treatments could be evaluated as more efficient amendment method to remove the Pb from the contaminated soil.

The correlation coefficient (R^2) between the concentrations of three elements in soil solution and those in the shoot of plant are 0.23 (Cd, $p > 0.05$), 0.50** (Zn, $p < 0.01$) and 0.91*** (Pb, $p < 0.005$), respectively (Fig 4). The results indicated that there are significant relationships between the concentration of Zn and Pb in soil solution and those in the shoot of plant of rainbow pink. From this study, we conclude that the addition of EDTA solution into the seriously multiple metals contaminated soil can affectively increase the concentration of Cd, Zn, or Pb in the soil solution and their concentration in the shoot of rainbow pink growing in contaminated soils. This amendment also can significantly reduce the soil remediation time by rainbow pink growing in contaminated soils.

Key words: EDTA, Rainbow pink, Phytoextraction, Cadmium, Zinc, Lead, Soil solution

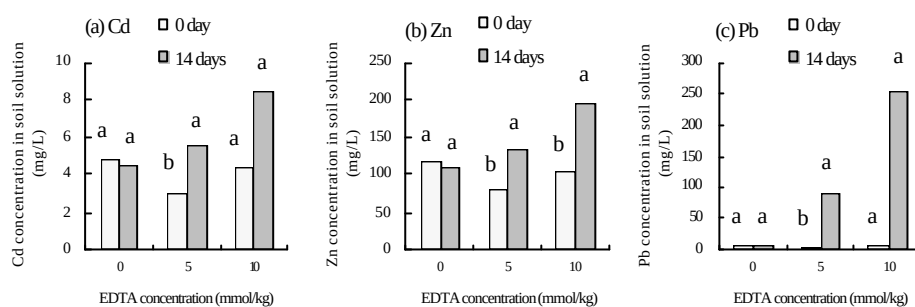


Fig 1. The change of concentration including (a) cadmium, (b) zinc and (c) lead in the shoot of rainbow pink which was planted in the day 0 and EDTA solution was applying 7 days after planting. The significant is at the 0.05 probability level.

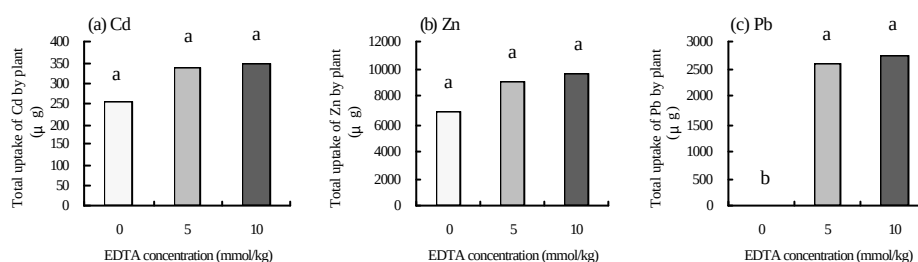


Fig 2. The change of concentration including (a) cadmium, (b) zinc and (c) lead in the shoot of rainbow pink which was planted in the day 0 and EDTA solution was applying 7 days after planting. The significant is at the 0.05 probability level.

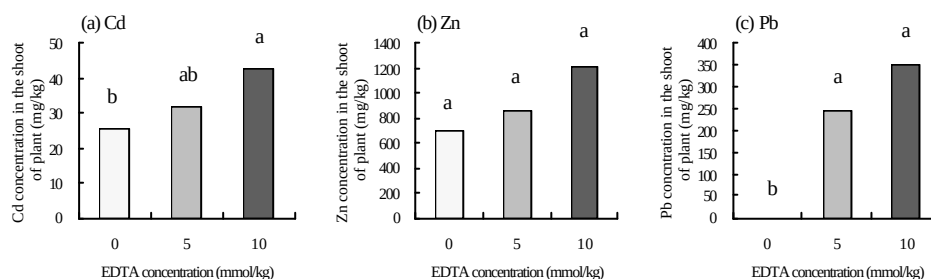


Fig 3. The total uptake of (a) cadmium, (b) zinc and (c) lead in the shoot of rainbow pink which was planted in the day 0 and EDTA solution was applying 7 days after planting. The significant is at the 0.05 probability level.

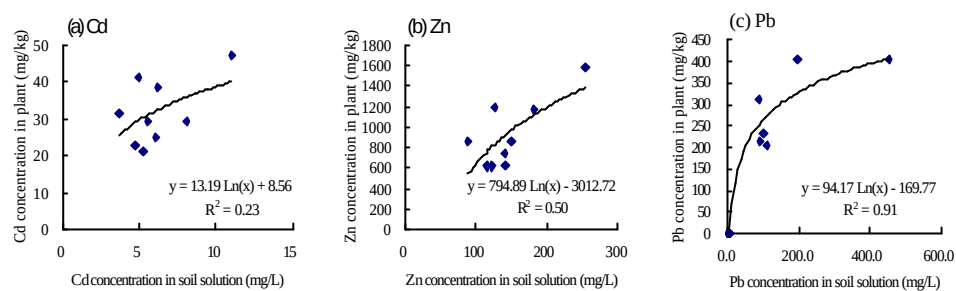


Fig 4. The relations between the concentration of (a) cadmium, (b) zinc and (c) lead in soil solution and those in the shoot of rainbow pink which was planted in the day 0 and EDTA solution was applying 7 days after planting.

Phytostabilization of cadmium, zinc and lead in contaminated soil by vetiver grass

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ABSTRACT

Specific plants, like vetiver grass, with strong and long root tissue were used for the phytostabilization of metal contaminated soil to decrease the mobility of the metal, especially when contaminated soil was eroded by heavy rain or strong wind. Vetiver grass (*Vetiver zizanioides*) was able to accumulate 250~750 mg/kg in the shoot when growing in the Pb contaminated sandy loam soil in previous study, but it resulted in the decrease on growth, biomass, and total chlorophyll content. In this study, pot experiment were conducted to investigate the potential phytostabilization of using vetiver grass to stabilize the soils which were seriously contaminated by cadmium (20 mg Cd/kg soil), zinc (500 mg Zn/kg soil) and lead (1,000 mg Pb/kg soil) in Taiwan. Three concentrations of EDTA solution (0, 5 and 10 mmol/kg soil) were added to the contaminated soils after 7 days of planting to study the influence of applying EDTA on the uptake by the shoot of vetiver grass (*Vetiver zizanioides*) growing in the multiple metals contaminated soil. The experiments were conducted in three replicates. Plants were harvested after 14 days of the planting. The soil solution was sampled directly by Rhizon soil moisture sampler (RSMS).

The results indicated that the concentrations of Cd, Zn, and Pb in soil solution are significantly increased after application of EDTA treatments ($p < 0.05$), especially for applying 10 mmol of EDTA solution (Fig 1). Even the concentrations of the three metals in soil solution changed drastically, but the concentrations of Cd and Zn in the shoot of vetiver grass varied from 20 to 30 mg Cd/kg and from 390 to 520 mg Zn/kg, respectively, and only significantly different for Zn ($p < 0.05$) (Fig 2 & Fig 3). The results also indicated that the growth of vetiver grass is not affected by the toxicity of seriously contaminated metals. It grows very well after planting for one month. The applying of EDTA solution only slightly decreased the biomass of vetiver grass and slightly decreased the total removal of heavy metals from the contaminated soils ($p > 0.05$) (Fig 4.).

We conclude that vetiver grass (*Vetiver zizanioides*) can grow well in the soils contaminated by Cd, Zn, and Pb, and the growth is not significantly affected by the change of metal concentrations in the soil solution after the addition of EDTA solution in 10 mmol/kg soil. The concentration of Cd, Zn, and Pb in the shoot of vetiver grass is still lower than those of the hyperaccumulator species found in the world. This genotype of vetiver grass is suitable for phytostabilization of soil contaminated by multiple metals although the metal concentration of soil solution increased drastically after soil amendments.

Key words: Phytostabilization, Vetiver grass, Cadmium, Zinc, Lead, EDTA, Soil solution

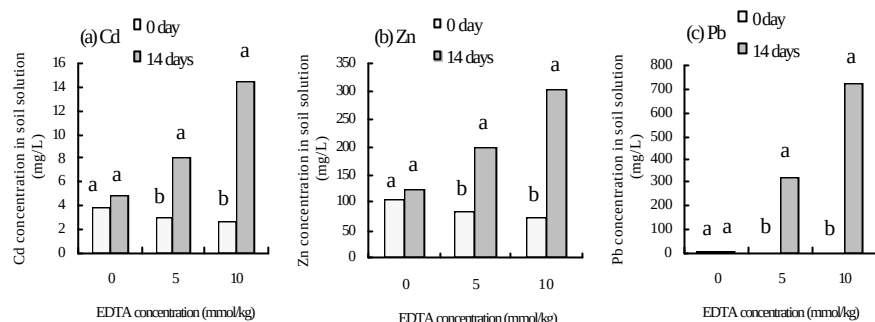


Fig 1. The change of concentration of heavy metals including (a) cadmium, (b) zinc and (c) lead in the shoot of vetiver grass, which was harvested at the day 14th after planting. The significant is at

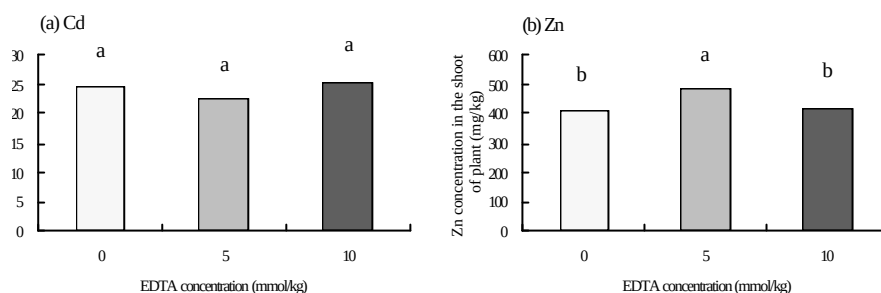


Fig 2. The change of concentration of heavy metals including (a) cadmium and (b) zinc in the shoot of vetiver grass, which was harvested at the day 14th after planting. The concentration of Pb in the shoot is not detectable (ND). The significant at (b) Zn is at the 0.05 probability level.

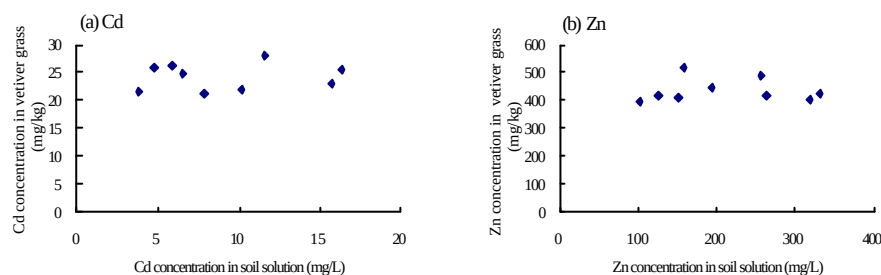


Fig 3. The correlation between the concentration of (a) cadmium and (b) zinc in soil solution and that concentration in the shoot of vetiver grass, which was harvested at the day 14th after planting. The concentration of Pb in the shoot is not detectable (ND).

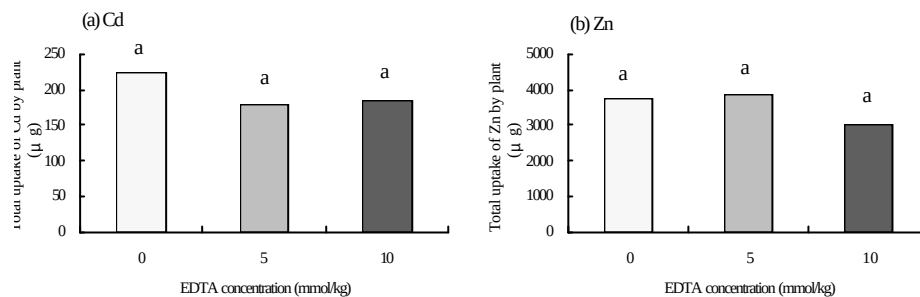


Fig 4. The total uptake of (a) cadmium and (b) zinc in the shoot of vetiver grass, which was harvested at the day 14th after planting. The concentration of Pb in the shoot is not detectable (ND), therefore the total uptake of Pb is uncalculated. There are no significant differences between treatments at the 0.05 probability level.

Uranium distribution and cycling in Scots pine (*Pinus sylvestris* L.) on a revegetated U-mining heap

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INTRODUCTION

The *in situ* remediation of uranium waste rock piles usually include their reprofiling and covering with a layer of mineral soil. The soil cover is then revegetated with grasses or forest trees for stabilisation and aesthetic purposes. At these sites, the long term minimisation of the solution phase transport of radionuclides is primordial and depends obviously on the stability and self-sustainability of the engineered and planted soil cover. Thanks to its high biomass and perennial character, the forest vegetation plays an important role against soil erosion as well as in stabilisation of the water cycling. Because of its high biomass turnover, forest vegetation can also absorb and recycle a considerable amount of nutrient when compared to the soil bioavailable reserve. In afforested contaminated area such as U-mining heaps, the extent of radioactive contaminant availability to perennial plants and ecosystem cycling could thus have important implications for the radioactivity dissemination as well as for the possible economic valorisation of the forest products. This study will focus on uranium in a Scots pine stand growing on U-mining debris. Despite numerous studies on U content in vegetation, there is very little information concerning its uptake and storage by perennial vegetation, especially by forest trees planted on reclaimed mining sites. This investigation aims at quantifying the U transfer to the different compartments of Scots pine and at assessing its cycling dynamics.

METHODOLOGY

The vegetation sampling was performed in a 37-year old Scots pine plantation established on the plateau of a U-mining heap situated near Schlema in Saxony (Wismut Company, Germany). The distribution of trunks circumferences at 1.30 m (breast height, CBH) was determined from a systematic record of all trees located within the stand. Four trees were then selected in the average CBH category and felled. Each tree was then compartmented into major annual and perennial parts: stemwood, inner and outer bark, needles of the year and >1y., twigs and branches. The total content and relative distribution of U in the aboveground biomass were calculated using the adequate biomass functions for Scots pine selected in Goskom (1984), together with the U concentrations measured by ICP-MS for treated sub-samples of the different tree compartments.

The methodology used to correctly assess the element cycling was progressively refined and applied to the study of the functioning of various forest ecosystems (Ulrich, 1973; Cole and Rapp, 1981; Ranger and Bonneau, 1984; Ranger et al., 1997). On the basis of the changes observed in biomass production and element content in the various tree compartments, the biological cycling in the ecosystem can be quantitatively described with three main annual fluxes: the demand, the uptake and the translocation (Ranger et al., 1997).

RESULTS & DISCUSSION

The relative distribution of biomass and U in the pine stand are compared in Fig. 1. The foliage act as a sink for U while its biomass represents a minor part of the aboveground biomass. To the opposit, stemwood strictly accumulates with time and represents a dominant reservoir for tree biomass but only a few percent of the U in tree is retained in this compartment. This picture suggest that U is easily transported to the foliage via transpiration fluxes. The trunk xylem few react with U along its transport most likely because of strong soluble U-complexes in xylem sap (Günther *et al.*, 2002). After

several decades, the total U incorporated in aboveground biomass amounts to about 4 g ha^{-1} that is low compared to the average U level of the rooting zone in soil ($30 \text{ cm} - 165 \cdot 10^3 \text{ g ha}^{-1}$). The stump and roots accumulate, however, 100 times more U than the upper part (data not shown).

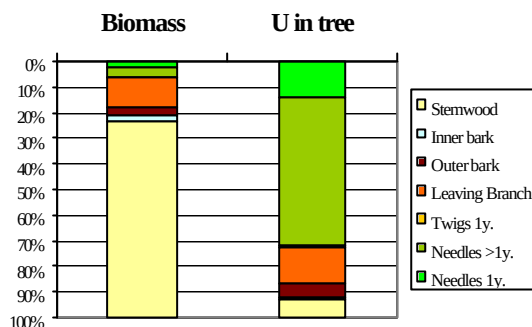


Fig. 1. Biomass and U distribution in pine cycling

FLUXES	U ($\text{g ha}^{-1} \cdot \text{y}^{-1}$)
(1) "Demand"	0,58
(2)=(3)+(4) Uptake	2,97
(3) Return	2,90
(4) Immobilisation	0,07
(5) Internal transfer	-2,20

Table 1. Annual fluxes of the U biological cycling

There is no role for U in plant nutrition. The amount of U associated with the mobilisation of element for new biomass production (demand) is extremely low (Table 1). The internal transfer of U i.e. the U transferred from senescing organs to support new production, presents a negative value and amounts to 74% of the uptake. That means that most of the U annually absorbed through root uptake accumulates in senescing components, the needles in particular. Accordingly litterfall returns to the soil 98% of the U uptake. This lead to a high turnover of U and a very limited annual immobilisation in perennial ligneous organs (2 % of the uptake).

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Trace elements of natural origin in soils

Symposium 7

Poster

CHANGES IN THE FRACTIONATION OF HEAVY METALS IN A CONTAMINATED SOIL FOLLOWING THE ADDITION OF ORGANIC AMENDMENTS WITH DIFFERENT MINERALISATION RATES

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The use of organic amendments is a useful strategy for re-establishment of vegetation on contaminated sites. This relies upon the ability of the organic matter to re-distribute heavy metals of the soil between the different forms. Mature composts have a high proportion of humified organic matter, which can decrease the availability of heavy metals in soil by adsorption, and by forming stable complexes with humic substances. However, fresh organic wastes, such as animal manures, are rich in soluble organic compounds which can increase the short-term solubility of metals, though formation of soluble organo-metallic complexes. This can be an alternative to synthetic chelators (such as EDTA) used for enhanced phytoextraction. The effects of compost and manure on heavy metal availability has been studied in pot experiments with plants (Walker et al., 2002). We concluded that other factors such as phosphate content and pH can have more effect on metal availability than the nature of the organic matter.

The aim of this work is to study the short-term effect of organic amendments, having different organic matter mineralisation rates on the availability of soil heavy metals, in an incubation experiment.

A soil from an ancient Pb-Zn mine area at La Unión (Murcia, SE Spain) was selected. The soil has high Pb and Zn concentrations (1572, 2602 mg kg⁻¹). Two organic amendments were used: a fresh cow manure, and a mature compost prepared from a mixture of olive leaves and olive-mill wastewater during 9 months.

An incubation experiment was carried out using 50 g soil as a control and two organic treatments: manure (50 g soil + 1.45 g manure) and compost (50 g soil + 1.07 g compost). The proportion of manure and compost added to the soil was equivalent to an addition of 1 g organic-C per 100 g soil. Deionised water was added to the soils in order to bring their moisture content to 60% of the water holding capacity. The incubations were carried out in darkness in a temperature-controlled incubator, at 26°C, for 56 days. After 14, 28 and 56 days, the soils were sampled and different fractions of heavy metals were determined by AAS after sequential extraction (McGrath and Cegarra, 1992). The dynamic of C-mineralisation was determined in a separate incubation, by measuring the CO₂ evolved during the incubation time, trapping it in a NaOH solution, in a small tube placed on the top of the soil, inside the incubation vessels.

The amount of CO₂ evolved during the incubation was low in the control soil (212 ± 27.5 µgC g⁻¹) and in the soil treated with compost (585 ± 23.5 µgC g⁻¹). Manure treatment showed the highest microbial activity with a CO₂ evolved of 2619 ± 3.3 µgC g⁻¹. However, these results were lower than those obtained in other soils with organic amendments. This is due to the heavy metal contamination, as microbial activity is one of the most sensitive parameters to soil metal toxicity.

Regarding metal distribution, in comparison with control soil, Cu linked to the organic matter fraction slightly increased in the samples with organic treatments during the incubation. Manure treatment showed that EDTA-extractable Cu (associated with the carbonates) decreased while the NaOH-extractable fraction increased, and in the compost treatment this fraction increased at both

residual and EDTA-extractable expense. This shows how Cu associates easily with soil organic matter and how both organic amendments favour Cu solubilization.

The organic treatments had Pb immobilisation effects during the incubation. The percentage of metal in the residual fraction rose in all the samples with organic treatment with respect to the control, mainly in those receiving manure. In this treatment EDTA-extractable Pb decreased, probably due to insoluble salts precipitation, such as phosphates, because P was present as a high concentration. In manure treated samples an initial (14 days) decrease of the concentration of Pb linked with the organic matter (NaOH-extractable) was observed. This fraction increased with incubation time, maybe because of the formation of intermediate compounds coming from the degradation of the organic matter, with higher affinity for Pb complexation. Compost had a limited effect, showing metal fixation in the residual fraction.

Compost increased the concentration of Zn in soluble and exchangeable forms (CaCl_2 -extractable), as a consequence of the increment in the cation exchange capacity due to compost addition (Walker et al., 2002). The main effect of manure was the decrease of NaOH and EDTA-extractable Zn, as this metal could have precipitated as inorganic compounds during manure mineralisation (Walker et al., 2002), although Narwal and Singh (1998) stated that the effects of organic amendments on Zn availability were related to soil pH.

CONCLUSIONS

Organic amendments favoured metal fixation to soil, indicating their suitability for bioremediation techniques by phytostabilisation. Only Cu increased its bioavailability through organic matter chelation. In this soil Cu concentration was not high, then this mobilisation could not imply an environmental risk. Organic amendments could be useful on the fixation of Zn and especially Pb in this soil, mainly when cow manure was used.

The main differences between manure and compost behaviour result from the different mineralisation of their organic matter, affecting redox conditions in soil, inorganic salt formation and soil microbial activity.

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Groundwater contamination and remediation

Symposium 8

Poster

Compound-specific Stable Isotope Analysis of Organic Groundwater Contaminants: Analytical Challenges and Environmental Applications

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Compound-specific isotope analysis (CSIA) is a very promising approach to help determining both in-situ transformation processes of pollutants in contaminated aquifers as well as sources of groundwater contaminants. Various laboratory studies have demonstrated the potential of this tool in contaminant hydrology (1–3). To date, the use of CSIA in field studies is, however, confined to near source zones of groundwater contamination with high pollutant concentrations. The method detection limits in CSIA are in the order of 150 µg/L for chlorinated solvents and solid phase microextraction as preconcentration method (4). Hence the method sensitivity is currently insufficient to investigate tail and fringe zones of contaminant plumes, which would be most helpful to assess natural attenuation processes in the field.

We evaluated different injection and concentration techniques in combination with isotope ratio mass spectrometry (IRMS). The determined method detection limits for the different analytical techniques are shown in Table 1. To improve the method detection limits of CSIA, we applied online purge&trap (P&T) as well as solid-phase microextraction (SPME) as preconcentration techniques and evaluated their influence on the isotopic signature of the analytes. Isotopic fractionation effects of the various processes involved in P&T and/or SPME (i.e., evaporation, sorption, desorption, and condensation of the analytes) have been studied for a series of analytes.

Table 1: Method Detection Limits for GC/IRMS Various Injection Techniques in CSIA

Compound	On Column [mg/L]	Splitless [mg/L]	Split 1:50 [mg/L]	SPME [mg/L]	P&T [mg/L]
1,1-dichloroethene	n.d.	n.d.	n.d.	78.8×10^{-3}	3.57×10^{-3}
trans-1,2-dichloroethene	74.9	94.5	864	127×10^{-3}	1.49×10^{-3}
methyl, tert-butyl ether	24.4	29.2	326	15.7×10^{-3}	0.63×10^{-3}
cis-1,2-dichloroethene	71.0	82.6	836	91.7×10^{-3}	1.13×10^{-3}
chloroform	170	155	1950	173×10^{-3}	2.25×10^{-3}
tetrachloromethane	215	223	2800	276×10^{-3}	4.96×10^{-3}
benzene	19.3	18.4	214	21.7×10^{-3}	0.28×10^{-3}
trichloroethene	84.3	80.4	1060	94.4×10^{-3}	1.41×10^{-3}
toluene	9.47	14.3	218	9.02×10^{-3}	0.25×10^{-3}
tetrachloroethene	74.4	74.3	1190	66.3×10^{-3}	2.24×10^{-3}

The fractionation effects, if present, were found to be compound-specific but showed a high reproducibility. The detection limits for $\delta^{13}\text{C}$ -determinations of volatile organic compounds could drastically be lowered to <5 µg/L with the developed P&T-GC/IRMS method. The effect of increasing purge times on the $^{13}\text{C}/^{12}\text{C}$ ratio of benzene is shown in Figure 1. For extraction efficiencies of less

than 40%, the P&T procedure causes a significant fractionation because the heavier isotope is enriched in the gas phase (inverse isotope effect).

The SPME-technique allowed the determination of $\delta^{13}\text{C}$ -values of MTBE and its degradation product tert-butyl alcohol in a highly contaminated aquifer in Brasil. The results suggest that the contaminated aquifer has two different zones, one with isotope fractionation linked to MTBE-degradation and a second where the observed concentration decrease is mainly due to dilution or other non-fractionating processes. Furthermore the stable isotope data allow the identification of different spill zones at the site.

P&T-GC/IRMS was successfully applied to study the fate of halogenated solvents in a contaminant plume down-gradient of a municipal landfill. Contrary to previous evaluations based on concentration data alone, our P&T-GC/IRMS data strongly suggest the absence of in-situ degradation of trichloroethene (TCE) despite the presence of cis-dichloroethene, a known metabolite of TCE.

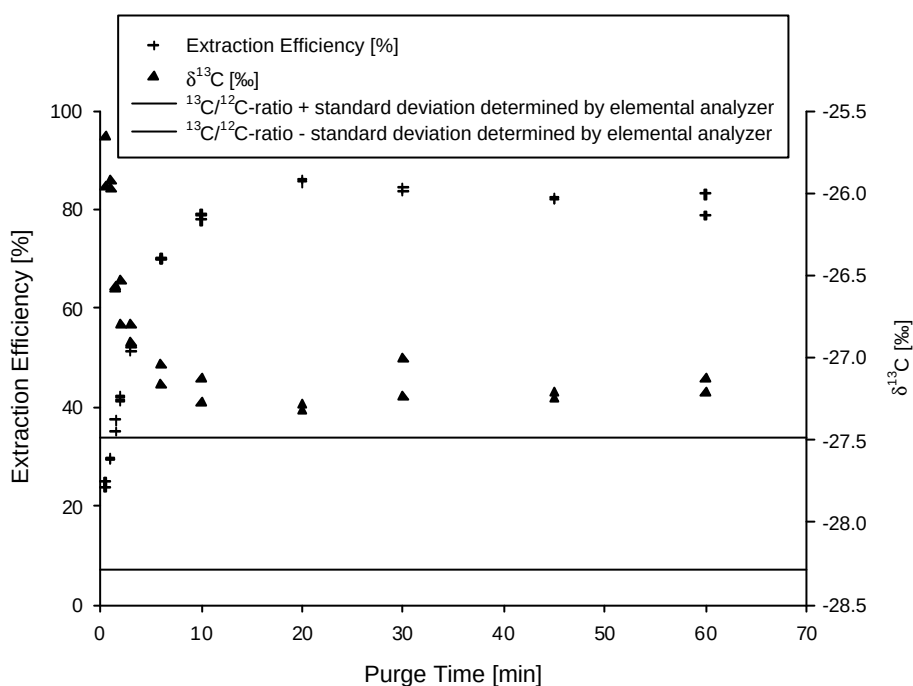


Figure 1. Purge&Trap extraction of benzene. The extraction efficiency increases with increasing purge times. The $^{13}\text{C}/^{12}\text{C}$ -ratio determined by P&T-GC/IRMS approaches the value determined by EA-IRMS for extraction efficiencies of more than 40%. The slight but reproducible fractionation can be corrected for using compound-specific external standards.

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Chemical composition of the soil and ground water along a primary succession gradient located on an acid sulphate soil in western Finland

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INTRODUCTION

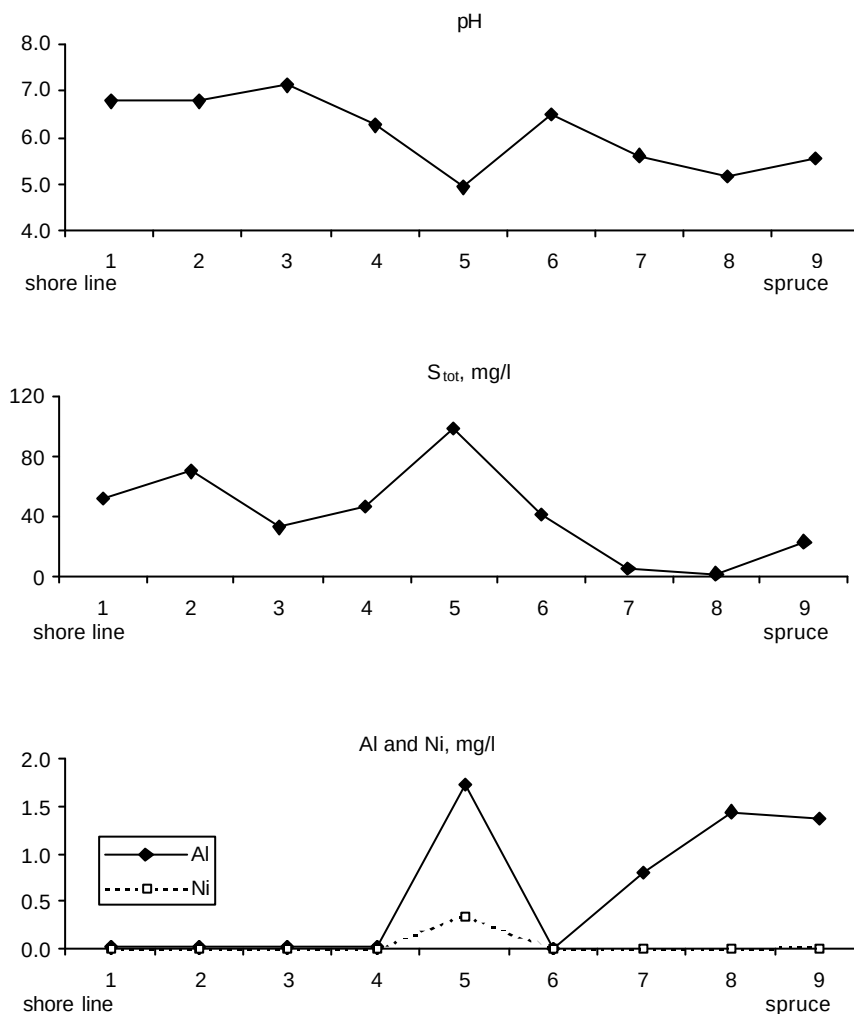
Many soils along the western coast of Finland, which is a region subjected to considerable land uplift (ca. 90 cm/decade), are so called acid sulphate soils. These soils are typically strongly acidified as a result of the oxidation of sulphide-rich sediments into sulphuric acid. Sulphide oxidation occurs as a result of the sinking of the ground water table, caused by the rapid land uplift in the area. The aim of this study was to determine how the processes related to acid sulphate soil formation are reflected in the ground water quality, and to identify changes in soil acidity and the C/N ratio along a primary succession gradient on the western coast of Finland.

MATERIAL AND METHODS

Ten sampling points were established along a primary succession transect (ca. 200 m long) running inland from the shoreline of the Gulf of Bothnia (Baltic Sea). The sampling points were located in sulphate soil, and the elevation of the nine points varied from ca. 0.5 - 5 m above sea level. The soil texture on the points was till with a relatively high proportion of fine material. The vegetation cover along the transect consisted of species typical of primary succession close to the shoreline: the tree cover along the transect consisted of separate succession zones comprising alder (*Alnus incana*), birch (*Betula spp.*) and Norway spruce (*Picea abies*). Ground water samples were collected during 2001 and 2002 using tension lysimeters (porcelain cups) located below the ground water table. The pH and Al, Ni and S_{tot} concentrations of several metals were determined. Samples were taken from the organic layer in 2002, and the pH and C/N ratio determined on the dried, milled material.

RESULTS AND DISCUSSION

The chemical composition of the ground water in the primary succession zones is affected by three factors: the inflow of ground water from inland sources, the inflow of NaCl-rich ground water/seawater from the sea, and the inflow of water derived directly from precipitation in the study area. The input from precipitation also includes cations and anions leached from the overlying soil layers where sulphide oxidation has already partially or completely taken place. Acid sulphate soil formation was clearly reflected in the chemical composition of the ground water furthest away from the shoreline. There was a clearly decreasing trend in the pH of the ground water on moving towards the spruce stand (Figure), and a strong acidification peak (low pH) in the middle of the transect. This acidification peak was associated with strongly elevated S_{tot} , aluminium and nickel concentrations. The low pH has resulted in the weathering of Al and Ni from the mineral material.



The C/N ratio in the organic layer was low along most of the transect, indicating that there is a relatively abundant supply of nitrogen for plant growth. As the pH values were relatively high, nitrification is likely to be an important process, at least along part of the transect. The C/N ratio in the spruce stand was higher owing to the gradual decrease in nitrogen availability, which is typical of the organic layer in coniferous stands. There was a clear acidification gradient (decreasing pH values) along the transect, reflecting the processes involved in the formation of an acid sulphate soil and the acidifying effect of nutrient uptake by the trees.

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Study of radionuclide transport with ground water (on some of the Semipalatinsk Test Site areas)

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INTRODUCTION

The analysis of radioactive contamination of the geological environment caused by underground nuclear explosion (UNE) is of particular interest for study of the radiation-ecological situation of the region. It is supposed carrying-out of complex investigations to explore the possibilities of radionuclide transfer with ground water for elaboration of recommendations on efficient nature management in the Semipalatinsk test site (STS) vicinity. In connection with the urgency of the mentioned problem authors of article suggested a project "Study of radionuclide transport with ground water to predict possible effects of drinking water and geological environment radioactive contamination at the STS" supporting the International Science and Technology Center (ISTC).

MATERIAL AND METHODS

This project will be implemented through fulfilling the following tasks:

- generalize and systematize information on some of the STS areas should be well studied from the hydrogeodynamic standpoint;
- develop a mathematical model describing radionuclide transfer with ground water;
- develop a procedure for sampling and laboratory analysis of representative water samples and radionuclides;
- carry out expeditions to some STS areas to choose of responsible engineering-geological factors for radionuclide transfer;
- analyze data obtained to predict possible consequences of STS groundwater contamination.

Early special researches on studying influence UNE on the mountain massif spent incidentally on separate objects. The complex of area and hole engineering - geological, hydrogeological and geophysical researches was carried out in two stages - before and after UNE.

A number of objects with unfavofable geoecological situation indicating the hydraulic connection between an UNE cavity and ground water will be identified based on the results of generalization and systematization of unique archival information about the geological enviroment response to the explosion impact at the STS.

Review results of observing hydrogeodynamic and radiation situation in areas of underground testing at the STS will allow to select areas most promising for studying radionuclide transport by ground water. Based on preliminary analysis of archival materials the arears with undeveloped

aquifuge will be outlined and areas of hidden groundwater discharge will be identified as they are possible radionuclide migration pathways.

Schematize the main stages of hydrogeological, hydrogeochemical situation dynamics during an UNE and classify conditions of radionuclide transfer with ground water will be promote to create a mathematical model describing the radionuclide transport with ground water. The contamination has a complicated spatio-temporal nature. The basic pathways of radionuclide transfer depend upon particular features of the mountain massif structure and tectonics and upon the filtration and sorption properties of the water-bearing medium.

Select the main types of hydrogeological and geophysical work will be direct to study filtration structure of the underground current determining radionuclide transfer. Develop recommendations on systematic assessment of reliability of data obtained using experimental checks of the observation hole condition is supposed. Recommendations on laboratory analysis of representative groundwater samples for radionuclide content will be formulated.

When establishing a stationary network to monitor and timely detect radionuclide releases, equipment of additional observation stations capable to strip key water-bearing beds and informative will be installed and used along with existing prospecting and observation holes.

RESULTS AND DISCUSSION

Carrying-out of expedition will allow to select allocation of the most probable ways of radionuclides transport on the chosen sites. The existing areas of groundwater contamination will be outlined using specified "marking" components (tritium, cesium, strontium, plutonium and americium isotopes) in the course of work conducted under the project.

Carrying-out of geophysical investigations and plan pilot filtration work in holes will be directed to select time periods mostly appropriate for studying radionuclide transfer with ground water. Perform pilot migration studies to determine direction and rate of groundwater flow. Arrange stationary observations of the groundwater table and collect representative groundwater samples for radionuclide analysis is supposed.

The searching nature of the field expeditions assumes the successive refinement of forecasting estimates of the geological environment contamination. To obtain correct results a comparative assessment of parameters determined with background values recorded in undisturbed (similar to natural) conditions will be carried out.

Analyze data obtained will make it possible to determine the radionuclide formation mechanism and distribution patterns taking into account engineering geological factors. The comparison of results obtained by borehole studies and experimental work will form a basis for making diagrams of groundwater movement more precise.

As a result of the integrated study, regularities and peculiarities of radionuclide transport will be determined and possible ways of radionuclide migration will be identified. Process and systematize experimental data on the present hydrogeological and hydrogeochemical situation in areas of underground nuclear testing is supposed. Analyze the regularities of changes in the groundwater regime will be determine the possibility of groundwater contamination.

CONCLUSION

The analysis of information obtained on some STS objects will form a basis for developing preventive measures to mitigate unfavorable effects of UNE.

The arrangement and implementation of integrated investigations of radionuclide transport by ground water within some STS objects will assist in solving such a national and international technical problems as control of radiation-ecological situation in areas of enhanced risk (underground nuclear test sites, radioactive waste disposal sites, NPP sites).

Levels and mobility of thallium in mining waste deposits - a case study.

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INTRODUCTION

As an element scarcely examined according to its natural occurrence and behaviour, thallium is still a mystery. In the close future, several problems connected to the high toxicity of the element can appear.

The use of thallium in the industrialised world is increasing. In the past thallium was used in medicine as a therapeutical drug from the end of 19th century and to the early 20th, today it is used as a contrast medium. During the 20th century, thallium was used as an active component in pesticides, which was banned by WHO in the 70's. In 1919, one of the first technological uses of thallium was patented, since then thallium salts have been a well used component in high quality glasses, thermometers, alloys, semiconductors etc.

NATURAL LEVELS

The average natural abundance of thallium in the lithosphere is low and reported levels are between 0.1-3 mg/kg. Sulphidic rocks, acidic granites and cambrosiluric limes are known to contain high levels of thallium and thereby affect the surrounding environments through weathering processes. An average concentration of thallium in groundwater of 7 ng/l has been reported in Norway, but concentrations as high as 120 ng/l has been found. In polluted fresh water levels up to 1000 ng/l have been reported in a stream close to sulphidic mine waste in Sweden, close to Ämmeberg.

SPECIATION

Thallium is a redox active metal with an E° of 1.28V, it has two redox states, Tl(I) and Tl(III), in surface waters. Which one that dominates is difficult to predict, since there appears to be some controversy between theoretical predictions and empirical measurements. Difficulties lie in the unspecific empirical methods used and the unknown natural conditions controlling the redox equilibrium. Another factor influencing the presence of thallium in the environment is the formation of metallic thallium, which can occur in environments (e.g. sediments) with E° of $-0.33V$ or lower.

In this study we report some concentrations as well as distribution coefficients for thallium in ground- and surface waters exposed to trace metal pollution from sulphidic mine waste.

EXPERIMENTAL

Surface water samples were collected in polyethylene bottles by immersing them by hand. Groundwater was sampled with an Amazon pump 1–1.5 meters below the groundwater surface. All metal analyses were performed using ICP-MS (Agilent, 4500) placed in a clean room (class 1000). Distribution coefficients were determined after equilibrating an aqueous solution of the test material at pH 3.5 and 9.3.

RESULTS; SURFACE WATERS - MINING DEPOSITS*Ämmeberg*

A old deposit with sulphidic mine waste that was in use from 1862 to 1976, located on the shore of Lake Vättern has been the subject for surface- and groundwater measurements as well as a partitioning experiment. The deposit mainly consists of a sand fraction from extraction of zinc and lead from the ore.

Surface water from small water ponds on the deposit contained 0.3 µg/l thallium, the same levels were found in the groundwater. Average total thallium concentration in the sand was 0.6 g/kg. About 1% of the thallium in the sand was found in solution after equilibration for 24 hrs at pH 3.5. This results in a distribution coefficient of 1.6×10^3 l/kg. The corresponding distribution coefficient was 2.4×10^6 l/kg and 2.2×10^6 l/kg for the natural surface- and groundwater environments (pH 7.8). Consequently, at a pH that corresponds to oxidative weathering of pyrite the mobility of thallium increases by approximately three orders of magnitude in relation to a calcareous soil environment.

Bersbo

The copper mine in Bersbo was in operation from medieval times to 1902. In 1987 approximately 1 Mtonne of sulphidic tailings was covered by a highly impermeable layer of clay or cephill in order to prevent any future weathering and accompanied metal release.

Sampling of surface water in 2002 shows that the former deposit still has a major impact on trace metal concentrations in the watershed. Upstream of the deposit the concentration of thallium in a small brook was 220 ng/l. It increased; however, to 6800 ng/l and a pH of 3.5 as the brook passed the deposit, a significant signal that contaminated water still reaches the environment. Further downstream the concentrations of thallium were consecutively lowered with increasing discharge area. Approximately 10 km from the deposit the concentrations were 16 ng/l, which is close to background levels in Sweden (Karlsson and Bäckström, 2002). Since only a few observations are available the behaviour of thallium does not resemble the easily hydrolysable elements aluminium, iron, and lead as the acidic drainage water mixes with soil/groundwater with pH of 7.5. On the contrary, according to these observations thallium behaved in a more conservative fashion, similar to zinc, which would indicate that the thallos state was dominating. Thus, it appears as if the element is not prone to participate in any redistribution processes once it is released from the tailings deposit.

CONCLUSION

Both sites show higher levels in the surface water than natural uncontaminated surface water. In Ämmeberg there was no difference between surface- and groundwater. In Bersbo the concentrations of thallium were very high and the element behaved in a conservative manner.

Colloids and biogeochemistry of trace elements

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Poster

Medical Geology: Comparative Anthropogenic Metals in Inner City and Suburban Communities of Detroit and New Orleans

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INTRODUCTION

The anthropogenic activity of redistributing metals within residential communities of modern society is the medical geology topic of this study. We evaluate mixtures of 9 metals (Pb, Zn, Cd, Mn, Ni, Cu, Cr, Co, and V) in inner city and suburban census tracts of Detroit, Michigan and New Orleans, Louisiana. Detroit is located near the Great Lakes where glacial geomorphic processes deposited tills during the last Ice Age. New Orleans is located in the lower Mississippi River Delta on alluvial soils that have been deposited by flood events connected with fluvial geomorphic processes of the river. The processes forming tills and alluvium in these two cities are expected to yield different geochemical characteristics of the soil. We compare the quantities of soil metals within inner city and suburban residential communities of Detroit and New Orleans to test the hypothesis that geomorphology is the basis for geochemical differences of soils of the two cities.

MATERIALS AND METHODS

a. Soil Sampling. Census tracts from inner city and suburban Detroit, which has not been surveyed for soil metals, were picked from a map of the city. Census tracts in New Orleans, which have been fully mapped, are from known extremes in the city (Mielke et al 2000). Surface (2.5 cm) samples were collected according to a protocol used to map New Orleans (Mielke et al., 2000). Four types of soil samples were from: busy streetsides (4 samples) residential streetsides (9 samples), open spaces (3 samples) and foundations (3 samples) (n=19). Documentation for each sample includes, census tract number, GPS coordinates, sample type, and site characteristics. All samples were air-dried and sieved (2 mm screen).

b. Metal Analysis. Soil metal extraction was done using a 5:1 ratio of 1 mol L⁻¹ nitric acid (room temperature) to soil, shaken for 2 hours, centrifuged (1000 x g - 15 min.) and filtered. Metals were determined using inductively coupled plasma-atomic emission spectrometry.

c. Statistical Analysis. General characteristics for the two databases are described by presenting percentiles of the central tendency results (Mielke and Berry, 2001). The results provide information about the soil metal trends for inner city and suburban residential communities of both Detroit and New Orleans.

RESULTS AND DISCUSSION

The two cities cannot be compared on an absolute basis because Detroit census tracts were selected without reference to a known soil metal map while New Orleans census tracts were chosen from the most contaminated inner city and least polluted suburban census tracts of the city. Tables 1 and 2 show the trends for inner city and suburban census tracts of the two cities. Note that for every metal measured in both cities, the inner city soils always have higher quantities than the soils in suburban census tracts. This pattern was previously described for Pb (Mielke, 1993) and is here shown for a suite of metals. From Table 1 of the inner city results, note that Pb and Zn are exceptionally elevated in New Orleans compared with Detroit. Also note that Cd, Ni, Cr, Co and especially Mn are elevated in Detroit compared with New Orleans. Cu and V are about the same for both cities. From Table 2 suburban census tracts results, Cd, Mn, Cr and Co are elevated for Detroit while Zn is elevated for New Orleans. There are no differences in the Pb, Cu and V of the suburban census tracts of these two cities. The differences between soil Mn and Zn may reflect geochemistry of till compared with alluvial parent materials of the two cities.

Table 1. Central tendency of inner city Detroit and New Orleans soil metals (in mg/kg).

DETROIT	Pb	Zn	Cd	Mn	Ni	Cu	Cr	Co	V
25	97	76	4	252	10	17	4	7	5
50	185	147	5	372	12	33	6	8	6
75	338	355	8	532	14	43	12	9	8
NEW ORLEANS									
25	420	234	3	110	8	20	2	4	5
50	656	373	4	134	10	32	3	6	6
75	1323	784	5	223	15	60	6	7	8

Table 2. The central tendency of Detroit and New Orleans suburban soil metals (mg/kg).

DETROIT	Pb	Zn	Cd	Mn	Ni	Cu	Cr	Co	V
25	5	12	3	172	8	5	1	6	3
50	11	16	4	189	9	5	2	6	3
75	24	46	4	232	10	8	3	7	4
NEW ORLEANS									
25	8	22	1	50	5	4	1	3	2
50	12	33	2	72	6	5	1	4	3
75	26	52	2	128	7	7	1	4	3

CONCLUSIONS

Tables 1 and 2 summarize soil metal results for inner city and suburban communities of Detroit and New Orleans. In general, the results indicate significant differences in accumulated metals between inner city and suburban residential communities in both cities. On the basis of the quantity of soil metals of inner city compared with suburban census tracts, anthropogenic processes appear to overwhelm geomorphic processes in distributing metals in the city. Note that this trend applies especially to Pb, Zn, Mn and Cu, and to a lesser extent Cd, Ni, Cr, Co, and V. These results reject the hypothesis that geochemistry of the parent materials are a major factor influencing the metal differences between communities of urban environments. Soil geomorphic processes may only account for the trend of elevated Mn in Detroit and elevated Zn in New Orleans. A critical issue of medical geology concerns the sensitivity of children to environmental contamination (Mielke et al., 1999). Anthropogenic processes appear to have contributed stressful accumulations of Pb and other metals (or other types of toxic substances) that in turn may negatively influence human health responses, especially in inner city environments (Di Giulio and Benson 2002).

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Cinti D.	SP09or	Cruz M.C.P.	SP09or
Cinti D.	SP09p	Csathó P.	SP01p
Cintra Alcides A.D.	SP05or	Csillag J.	SP11p
Citeau L.	SYM09or	Cuello G.	SYM09or
Clarens M.	SP07or	Cullaj A.	SYM01p
Clark J.	SYM03or	Curi N.	SP11p
Clemente R.	SYM07p	Curtis G.P.	SYM08or
Clijsters H.	SYM05or		
Close M.	SYM03p	D	
Clozel B.	SYM07p	D'annibale L.	SP09or
Clucas L.M.	SP11or	da Costa F.G.	SP11p
Cofield N.	SP02p	da Silva E.	SP07p
Coleman M.L.	SP02p	Daigneault E.	SYM08or
Collins C.	SP02p	Daniels W.L.	SP07p
Collins C.D.	SYM06or	Danielsson B.	SP10p
Collins R.N.	SYM04or	Danjuan M.	SP02p
Colpaert J.	SYM05p	Danker R.M.	SYM10or
Compton H.	SYM03or	D'Annibale L.	SP09p
Compton H.	SYM03p	Das D.K.	SP09or
Conder J.	SYM03or	Datta S.	SYM08or
Condron L.M.	SP02or	Davée Guimarães J.-R.	SYM04or
Conil P.	SYM01or	Davis J.	SYM08or
Corns W.	SP02p	Davis J.A.	SYM08or
Corns W.	SP03p	Davis M.R.H.	SYM03p
Cornu S.	SYM07or	Davison W.	SP01p
Cornu S.	SYM07p	Davydchuk V.	SP14p
Cornu S.	SP01p	Dayton E.A.	SYM03or
Corradini F.	SP01or	De Brouwere K.	SP02p
Cortes-Maramba N.P.	SP03p	de Castro e Silva E.	SP09p
Cortina J.	SP02p	de Castro e Silva E.	SP09or
Cosio C.	SP06p	de Groot A.	SYM03or
Costa A.	SP07p	de Jager A.	SP05or
Costanza-Robinson	SYM01or	de Jager A.	SP05p
M.S.		de la Fuente L.M.	SP11or
Cotelle S.	SYM06or	de Melo G.M.P.	SP02p
Cotter-Howells J.	SP09p	de Melo W.J.	SP02p
Courchesne F.	SP05or	de Melo W.J.	SP03p
Courchesne F.	SYM02or	de Melo V.P.	SP02p
Cousin I.	SYM07p	de Oliveira A.H.	SP02p
Coves J.	SP11p	de Oliveira A.H.	SP03p
Creamer R.E.	SP02or	de Oliveira L.J.	SP09p
Cremers A.	SYM06or	De Schamphelaere	SP12p
Cremisini C.	SP09or	K.A.C.	
Cremisini C.	SP09p		

de Vries W.	SP01or	Diyab C.	SYM02p
de Vries Wim	SP05or	Dolin V.V.	SP14p
Declerck S.	SYM02or	Domeizel M.	SP11p
Deda T.	SYM01p	Dong N.	SYM02p
Degryse F.	SYM04or	Donisa C.	SYM07p
Degryse F.	SYM06or	Douay F.	SP02p
Deiana S.	SYM02or	Douay F.	SYM07or
Deiana S.	SYM02p	Downum K.R.	SYM01or
del Carmen M.	SYM02p	Doyle P.	SP12p
Del Gaudio S.	SYM01or	Doyle P.	SYM03or
del Pico Y.	SP01p	Driouich A.	SP05or
Del Re A.A.M.	SP03p	Du Laing G.	SP11p
Del Re A.A.M.	SP11p	Duarte A.C.	SP02p
Del Río B.	SP02p	Duarte A.C.	SP03p
Delbeke K.	SYM03or	Duarte A.C.	SP06or
Delgado J.	SP01p	Duarte A.	SP05p
Dellantonio A.	SYM01or	Duarte A.	SP10p
Delolme C.	SP02p	Dubbin W.E.	SYM02p
Delolme C.	SYM09p	Dubourgier H.-C.	SP02p
Delolme C.	SP02or	Duffa C.	SP12p
Delwar Hossain M.	SYM01p	Dumat C.	SP11p
Delvaux B.	SYM07p	Dumat C.	SYM02p
Denaix L.	SP05or	Dunham S.J.	SP06p
Denaix L.	SP02p	Dunn J.	SYM05p
Denaix L.	SP11or	Duro L.	SYM08or
Denaix L.	SP11p	Duxbury J.M.	SYM01or
Denaix L.	SYM03or	Duxbury J.M.	SYM01p
Deneux-Mustin S.	SYM02or	Dwyer R.	SYM03or
Denis M.	SYM02p	Düwel O.	SYM07or
Denison Francis H.	SP05or	Düwel O.	SYM07p
Dère C.	SP01p	Düwel O.	SP10p
Derome J.	SYM08p		
Derome K.	SYM08p	E	
Deschamps E.	SYM01p	Ebinghaus R.	SP01or
Deville-Caveline G.	SP14p	Echevarria G.	SP05p
DeVolder P.	SYM03or	Echevarria G.	SP06p
Dhillon S.K.	SP09or	Edmonds R.	SYM01p
Di Lella L.A.	SP14p	Eidhammer Sjøbakk T.	SYM07p
Dias C.L.	SP12p	Eimbeck M.	SYM07p
Díaz-Barrientos E.	SP02or	Ekström L.	SYM08p
Díaz-Barrientos E.	SP07p	Elahi S.F.	SP09p
Dickinson N.	SP01p	Elektorowicz M.	SP05or
Dickinson N.	SP06p	Elices B.	SP05p
Dickinson N.E618	SYM05or	Elices Beatriz	SP05p
Dictor M.	SP07or	Elkhatib E.A.	SP06p
Dictor M.C.	SYM01p	Elkhatib E.A.	SP07p
Diez J.	SP06p	Elkhatib H.A.	SP07p
Diez S.	SP10p	Elless M.P.	SP06p
Dimitriou J.	SP06p	Ells S.J.	SYM03or
Dioquino C.C.	SP03p	El-Motaium R.A.	SP03p
Disante K.	SP02p		

Emnova E.	SYM02p	Fifield L.K.	SP14p
Enedi Boaretto A.	SP11p	Filimonova Z.V.	SP02p
Enel M.	SYM09p	Fiori A.	SYM02p
Eriksson J.	SP02p	Fischerova Z.	SP11p
Eriksson J.	SP03p	Fitz W.J.	SP11p
Eriksson J.	SYM07or	Fitz W.J.	SYM02or
Ermis I.	SP03p	Fleischbein K.	SP01or
Eruslankina L.V.	SP01p	Flores-Tavizón E.	SP06p
Eruslankina L.V.	SP02p	Flühler H.	SP14p
Escaig F.	SP11p	Follet-Gueye M.	SP05or
Escolar A.	SYM07p	Ford R.G.	SYM01or
Escudero P.	SP02p	Fornasier F.	SP11p
Evans K.	SYM08or	Fortin Claude	SP05or
Eyckmans H.	SYM06p	Fortunati P.	SYM06p
F		Foster A.	SYM01or
Fabre B.	SP07p	Foster S.D.	SYM03or
Fairbrother A.	SYM03or	Fotovat A.	SP11p
Fajon V.	SP01p	Fourrier H.	SYM07or
Fajon V.	SP01p	Foust Jr.R.D.	SYM01or
Fangueiro D.	SP05p	Francis A.	SP05or
Farid A.T.M.	SYM01p	Francisco-Rivera A.T.	SP03p
Farid T.M.	SYM01or	Francois M.	SP02p
Farooqui A.	SP11p	Françoise P.	SYM02p
Fauziah C.I.	SP03p	Franzen C.	SP01p
Fedotov P.S.	SP02p	Frazier S.W.	SYM06or
Fedotov P.S.	SP11or	Frech W.	SP05p
Feldmann J.	SP03p	French C.	SP06p
Feng M.	SYM01p	Frey B.	SYM02p
Feng X.	SP01p	Friesl W.	SP07or
Ferard J.-F.	SYM06or	Fritioff Å.	SP06p
Ferdyn M.	SP02p	Frossard E.	SP14p
Fernandez J.M.	SYM06p	Frossard E.	SYM04or
Fernández Covelo E.	SP05p	Fryxell G.E.	SYM10or
Fernández M.T.	SP02p	Fuentes D.	SP02p
Fernández Marcos M.L.	SP02p	Fujii R.	SYM01p
Fernández-Sánchez R.	SP11p	Fukuyama T.	SP14p
Fernández-Sanjurjo	SP02p	Furrer G.	SP07p
M.J.		Furrer G.	SYM09or
Feroze Ahmed M.	SYM01p	G	
Ferrand E.	SP11p	Ga ka B.	SYM01p
Ferrari Aimar F.	SP02p	Gabrio T.	SYM01p
Ferrari Aimar F.	SP03p	Gaeta Lemos M.M.	SP12p
Ferreira A.G.	SP01p	Gagnon C.	SYM02or
Ferreira E.	SP02p	Gago C.	SP02p
Ferro A.M.	SP06p	Galceran J.	SP05p
Feurtet-Mazel A.	SP05or	Galicz É.	SYM05p
Février L.	SYM02p	Galle P.	SP11p
Fiasconaro M.	SYM01p	Gallerand M.O.	SP06p
Fick W.	SYM03or	Gamarra R.	SP02p

Gao S.	SYM01p	Gomez E.	SYM06p
Gardea-Torresdey J.L.	SYM06p	Gonçalves G.M.	SP01p
Garg S.K.	SP02p	Gonçalves P.	SP02p
Garnier J.M.	SYM09p	Gong Z.	SP01p
Garnier-Laplace J.	SP05or	Gong Z.	SP02p
Garrett R.G.	SYM07or	Gongalsky K.B.	SP02p
Garrido F.	SP07or	Gonzales C.R.	SYM09p
Garrido F.	SYM01p	González P.J.	SP02p
Garrido T.	SP02p	Gonzalez S.	SP05or
Gaspari M.	SP07p	González-Chávez A.	SYM02p
Gatsios F.A.	SP11p	González-Elizondo S.	SP06p
Gaudet J.-P.	SYM09p	Gorbunova E.M.	SYM08p
Gautier M.P.	SP11p	Gorfer M.	SP06or
Gavshin V.M.	SP14p	Gorokhovatska M.	SP03p
Gbadebo A.M.	SYM01p	Goulet R.	SYM03or
Geebelen W.	SYM05or	Goulet R.R.	SP12p
Gelabert A.	SP05or	Govorova A.F.	SP02p
Gemignani S.	SP01or	Grant C.A.	SP02p
Genevini P.L.	SP02p	Grawé K.P.	SP03p
George G.N.	SYM01or	Grawé K.P.	SYM03p
Geraedts K.	SP14or	Gravett A.E.	SYM06or
Gerzabek M.H.	SP07or	Gray C.W.	SP11or
Gerzabek M. H.	SP05or	Grcman H.	SP06or
Gessa C.	SP01p	Grcman H.	SP11p
Gessa C.	SYM02or	Green A.	SYM03or
Gessa C.	SYM02p	Green I.D.	SP11p
Ghanbaja J.	SP05p	Greger M.	SP01p
Ghani M.A.	SYM01p	Greger M.	SP06p
Gharbi F.	SP06p	Greger M.	SP11or
Ghidini M.	SP02p	Greger M.	SP11p
Ghidini M.	SP02or	Greger M.	SYM02or
Ghuman G.S.	SP02p	Gregorauskiene V.	SYM07or
Giandon P.	SYM07p	Grennberg K.	SP02p
Giger W.	SYM01or	Grigatti M.	SP01p
Gill T.E.	SP14p	Grivé M.	SYM08or
Gilles V.	SP05or	Groenenberg B.	SP05or
Gilson B.	SP07p	Groenenberg J.E.	SP01or
Gilucis A.	SYM07or	Grytsyuk N.	SP14p
Ginocchio R.	SP11or	Gräfe M.	SYM01or
Giorgioni M.E.	SP01p	Gröhn J.	SP09or
Giovanni Bidoglio	Keynote	Grønflaten L.K.	SP11p
Giro Frédéric	SP05or	Guha J.	SP02p
Girondelot B.	SP06or	Guilherme L.R.G.	SP11p
Glavin R.	SP10p	Guimarães J.R.	SP07p
Gobbo L.	SP11p	Guinberteau J.	SP06p
Gobran G.R.	SP06p	Gulson B.L.	SP03p
Gobran G.R.	SYM02p	Gulz P.A.	SP06p
Godzik B.	SP06or	Gunadi B.	SYM03or
Golia E.E.	SP11p	Gupta S.K.	SP06p
Goller R.	SP01or	Gustafsson J.P.	SYM09p
Golubeva E.I.	SP02p	Gustafsson J.P.	SP05or

Gustafsson K.	SP03p	Heikkinen T.	SYM06p
Gustafsson K.	SYM03p	Hendershot W.	SP01p
Gustafsson Ö.	SP11p	Hendershot W.	SP05or
Günthardt-Goerg M.S.	SP02or	Hendry J.	SYM09or
Günthardt-Goerg M.S.	SYM05or	Henner P.	SP11p
Günther D.	SP11or	Herbert Jr.R.B.	SYM08p
Gäbler H.-E.	SYM07p	Hermle S.	SP02or
Görransson A.	SP11or	Hernández J.A.	SP02p
Göthberg A.	SP11p	Hernández-Córdoba M.	SP10p
		Hesterberg D.	SYM03or
H		Heulin T.	SP11p
Haberhauer G.	SP05or	Hiemstra T.	SP05or
Haderlein S.B.	SYM08p	Hilscher A.	SYM01p
Hafez Darbani M.R.	SP11p	Himmelbauer M.	SYM02or
Hakkila K.	SP02p	Himmelbauer M.L.	SYM02p
Hale B.	SP02p	Hindar Atle	SP10p
Hallfrisch J.	SYM05or	Hinsinger P.	SYM05or
Hallfrisch J.	SP05or	Hintelmann H.	SP01or
Hamilton-Taylor J.	SYM09or	Hisamatsu S.	SP11p
Hammer D.	SP06or	Hisamatsu S.	SYM06p
Hamon R.E.	SP02or	Hodson M.E.	SP02p
Hamon R.E.	SYM03p	Hodson M.E.	SP06p
Hamon R.E.	SYM04p	Holm E.	SYM06p
Han F.	SYM02p	Holm K.	SP11p
Hanc A.	SP11or	Holm P.E.	SP02p
Hanc A.	SP05p	Holm P.E.	SP02p
Hanc A.	SP11p	Holm P.E.	SP05or
Hansen B.	SP05or	Holmer L.E.	SP11p
Hansen H.	SP07p	Holmes S.	SP07or
Hansen H.C.B.	SP05or	Holmes S.	SYM05p
Hanslík E.J.	SYM06p	Hooda P.S.	SP10p
Haque M.A.	SYM01p	Hooda P.S.	SP09p
Hardy M.	SYM07or	Hooda P.S.	SP09or
Hardy M.	SYM07p	Hopgood M.J.	SP06p
Hardy S.E.	SP02p	Horvat M.	SP01p
Hart B.R.	SP11p	Hossain K.M.	SYM01p
Hart B.R.	SYM10p	Hossain M.B.	SYM01or
Hartikainen A.	SP03p	Hossain M.F.	SP09p
Hartikainen H.	SYM01p	Hseu Z.-Y.	SYM09p
Hasegawa H.	SYM06p	Hu Z.	SP05or
Hasneen A.	SYM01p	Huang B.	SP02p
Hassinen V.	SP06or	Huang B.	SP01p
Hassler C.S.	SP11or	Huang B.	SP05p
Hassler S.	SYM01p	Huang J.-H.	SP01p
Hattori H.	SP06p	Huang P.M.	SYM02or
Hawson C.	SYM03or	Huber D.M.	SYM02p
He Y.	SYM02p	Hudnik V.	SP11p
Heidkamp A.	SYM07p	Hulshof A.M.	SYM08or
Heier L.S.	SYM06p	Hultman B.	SP01p
Heijerick D.G.	SP12p	Huq S.M.I.	SYM01or
		Hussam A.	SYM01p

Hussein H.	SP06p	Jann S.	SYM08or
Hutchison J.	SYM10or	Janssen C.R.	SP11p
Hylander L.D.	SP02p	Janssen C.R.	SP12p
Hylander L.D.	SP09or	Janssen C.R.	SYM03or
Högbom L.	SP14p	Janssen C.R.	SP05p
Höhn R.	SYM08or	Janssens K.	SP14or
		Jansson G.	SP02p
I		Jansson G.	SYM09or
Iakimenko O.S.	SP01p	Janta-Koszuta K.	SP02p
Iancu V.	SP02p	Japenga J.	SP06or
Idris R.	SYM02p	Japenga J.	SP06p
Inaba J.	SP11p	Jarvis N.	SP01or
Inaba J.	SYM06p	Jarvis N.	SP05or
Inaba S.	SP11p	Je H.-K.	SP02p
Ingri J.	SP11p	Je H.-K.	SP14p
Ingri J.	SP14or	Jenkins C.R.	SP02p
Isenbeck-Schröter M.	SYM08or	Jensen P.E.	SP07p
Islam M.A.	SYM01p	Jensen T.F.	SYM08or
Islam M.R.	SYM01p	Jézéquel K.	SYM07p
Islam M.S.	SYM01or	Jiménez J.	SP10p
Itoh Y.	SP01p	Johansson K.	SP01or
Ivanov K.	SP11p	Joner E.	SYM02or
Ivanov Y.A.	SYM06p	Jordão C.B.	SP09or
Ivanov Yuri A.	SP05p	José M.	SYM02p
Ivanova T.	SP11p	Jose-Yacaman M.	SYM06p
Ivanova T.N.	SP14p	Jover E.	SP10p
Ivanovová D.	SYM06p	Juang Kai-Wei	SP10p
Ivask A.	SP02p	Juhasz A.L.	SP11or
Ivask A.	SP05or	Juhasz A.L.	SP11p
		Juillot F.	SYM01or
J		Juillot F.	SYM02p
Jack C.	SP02p	Juillot F.	SP05or
Jacks B.	SP09p	Juncos C.	SYM06p
Jacks G.	SP09p	Jung G.-B.	SP03p
Jacks G.	SYM01or	Juntunen R.	SYM01p
Jacks G.	SYM01p	Jurkiewicz A.	SP06or
Jacks G.	SYM08or	Jönsson J.Ö.	SP02p
Jackson B.	SYM09or	Jönsson J.	SP05or
Jackson B.P.	SYM10or	Jørgensen N.	SP02p
Jacob D.L.	SP09p		
Jahiruddin M.	SYM01p	K	
Jahiruddin M.	SYM01or	Kaasalainen M.	SP10p
Jahn R.	SYM02p	Kabala C.	SYM07p
Jaillard B.	SYM05or	Kabata-Pendias A.	SP01or
Jakubowski N.	SP03p	Kaekhtin D.A.	SP02p
Jalil M.A.	SYM01p	Kahelin H.	SP06p
Jambor J.L.	SYM08p	Kahru A.	SP02p
Jana J.	SYM01p	Kahru Anne	SP05or
Jankowski J.	SP05or	Kalbasi M.	SP05or
		Kalbasi M.	SP11p

Kalinowski B.E.	SP11p	Kim V.Z.	SP02p
Kamruzzaman M.	SYM01p	Kimlenka I.	SP11p
Kanal A.	SYM2or	Kimura K.	SP02p
Kanazawa S.	SP07p	King D.	SP12p
Karathanasis A.D.	SYM10or	King D.	SYM07or
Karavanova E.I.	SP05p	Kinraide T.B.	SYM02or
Karczewska A.	SP02or	Kirpichtchikova T.	SYM05p
Karczewska A.	SYM01p	Kj��ller C.	SYM08or
Karczewska A.	SP05or	Klassen R.A.	SYM07or
Karimian N.	SP11p	Klassen R.A.	SYM07p
Karlsson S.	SP02p	Klassen W.	SYM10p
Karlsson S.	SP03p	Kliaugiene E.	SP05p
Karlsson T.	SP05p	Kliaugiene E.	SP05or
Karlsson U.	SYM08p	Klinck B.	SYM01p
Karpova E.	SYM01p	Klinphoklap S.	SP02p
Karthikeyan K.S.	SP02p	Klinphoklap S.	SP09or
Kaschl A.	SYM08p	Klitzke S.	SYM09or
Kasimov N.S.	SYM07p	Klonowska A.	SP11p
Kaupenjohann M.	SYM09or	Knox A.S.	SYM10or
Kautsky L.	SP06p	Knox J.D.	SYM10or
Kazuhiko K.	SP02p	Koistinen K.	SP06or
Kchaou R.	SP06p	Kolomenskyi E.N.	SP12p
Keller C.	SP06or	Konopleva I.V.	SYM06p
Keller C.	SP06p	Konovalov V.E.	SYM08p
Kent D.B.	SYM08or	Konovalov V.E.	SYM09p
Kereszt��ri P.	SP06p	Koo B.-J.	SYM02or
Kereszt��ri P.	SYM05p	Koo B.-J.	SYM10or
Keron S.D.	SP06p	Koochi M.	SP11p
Ketterer M.E.	SP14p	Kookana R.	SYM09p
Khademi H.	SP11p	Kookana R.S.	SYM09p
Khadivi I.	SP11or	Koolhaas J.E.	SP11p
Khajehpour M.R.	SP11p	Koolhaas J.E.	SYM03or
Khaled H.M.	SYM01p	Koptsik G.N.	SP01p
Khan A.G.	SP06p	Koptsik G.N.	SP02p
Khandelwal P.	SP07p	Koptsik S.V.	SP02p
Khorokhorina E.V.	SP02p	Korzeniowska J.M.	SP11or
Khoshgoftar A.H.	SP05or	Kos B.	SP06or
Khoshgoftarmanesh A.H.	SP11p	Kos B.	SP06p
Kidd P.	SP11p	Kosaki T.	SYM02or
Kidd P.S.	SP06p	Kosheleva N.E.	SYM07p
Kikuchi R.	SYM10p	Kostic N.	SP05p
Kilian R.	SP01p	Kotnik J.	SP01p
Kim K.	SYM05or	Kov��cs B.	SP03p
Kim K.	SP07p	Kovacsovics B.	SP02p
Kim K.-H.	SP02p	Kraemer S.M.	SYM02p
Kim K.-H.	SP11p	Kraemer S.M.	SYM06or
Kim K.-M.	SP03p	Krajewski J.	SYM01p
Kim K.-W.	SP02p	Kralj T.	SP11p
Kim K.-W.	SP11p	Kr��m P.	SP01p
Kim W.-I.	SP03p	Kramer T.	SYM01p
		Krenn A.	SP07or

Kreshtapova V.N.	SP02p	Lakatos G.	SP06p
Kretzschmar R.	SP02p	Lakimenko O.S.	SP01p
Kretzschmar R.	SYM02p	Lalor G.C.	SYM07or
Kretzschmar R.	SYM06or	Lamy I.	SP05p
Kristensen I.	SP07p	Lamy I.	SP01p
Krivolutsky D.A.	SP03p	Lamy I.	SP12p
Kroglund F.	SP10p	Lamy I.	SYM05or
Kruyts N.	SYM07p	Lamy I.	SYM09or
Krüger G.C.T.	SP01p	Land M.	SP14or
Kubota H.	SP06p	Landberg T.	SP06p
Kucharski R.	SP06p	Landberg T.	SP11or
Kuimova N.G.	SP11p	Landi L.	SP02p
Kukla H.	SP07or	Lang F.	SYM09or
Kukla H.	SP07p	Langenkamp H.	SYM07p
Kumar G.	SP07p	Langenkamp H.	SP10p
Kumpiene J.	SP06p	Langore L.	SYM01p
Kuno R.	SP09p	Lanno R.P.	SYM03or
Kunttu K.	SP05or	Lanno R.P.	SYM05or
Kuo S.	SP05p	Lansac A.	SP11p
Kuperberg J.M.	SP06p	Lanzirotti A.	SP01or
Kuperman R.G.	SYM03or	Lanzirotti A.	SYM01or
Kuzyakov Y.	SP07p	Laperche V.	SYM01or
Kuzyakov Y.	SYM02p	Laroche L.	SP11p
Kvasnikova E.V.	SP14p	Larraz C.	SP07p
Kvasnikova E.V.	SYM08p	Larsen F.	SYM08or
Kwiatkowska J.	SP07p	Larsson J.	SP11p
Küçük M.	SP09p	Lauren J.G.	SYM01p
Kyzio J.	SP07or	Lauro G.P.	SYM02p
Kyzio I J.	SP02p	Laursen J.	SP02p
Kärenlampi S.	SP06or	Lavado R.S.	SP03p
Köhler S.	SYM05or	Lavado R.S.	SP11p
Köllensperger G.	SP11p	Lazo P.	SYM01p
		Le Faucheur S.	SP11p
L		Le Faucheur Séverine	SP05p
Labejof L.P.	SP02p	Le Guern C.	SYM01or
Labejof L.P.	SP03p	Le Hécho I.	SP05or
Laboudigue A.	SP06or	Le Hécho I.	SP11p
Laboudigue A.	SYM05p	Leal R.M.	SP11p
Lacatusu R.	SP02p	Lebeau T.	SP07p
Ladeyn I.	SYM06or	Leclerc-Cessac E.	SP05p
Ladonin D.V.	SP05p	Leclerc-Cessac E.	SP06p
Ladonin D.V.	SP05or	Leclerc-Cessac E.	SP11p
Lafferty B.J.	SYM01p	Ledgham F.	SP11p
Laffray	D.	Lee A.	SP06p
Lahermo P.	SYM01p	Lee C.	SYM07or
LAI H.	SYM06p	Lee C.R.	SP06p
Lai S.-Y.	SP14or	Lee Dar-Yuan	SP10p
Laiho J.V.-P.	SP06p	Lee J.-S.	SP02p
Lair Georg J.	SP05or	Lee J.-S.	SP03p
Lakatos G.	SYM05p	Lee J.-S.	SP11p
		Lee S.-E.	SP02p

Macouillard D.	SYM06p	Martin-Garin A.	SYM02p
Madejon E.	SP07or	Martinoia E.	SP06p
Madejon E.	SP07p	Martins A.L.C.	SP09p
Madhavan N.	SP09or	Martins S.C.	SP09p
Madhavan N.	SYM01p	Márton L.	SP03p
Madrid F.	SP02or	Marzadori C.	SYM02p
Madrid F.	SP07or	Masami N.	SP02p
Madrid F.	SP07p	Mason S.D.	SYM04or
Madrid L.	SP02or	Massiani C.	SP11p
Madrid L.	SP07p	Masson P.	SYM03or
Maes A.	SP14or	Massoura S.	SP05p
Maes André	SP05p	Massoura S.	SP06p
Magiera T.	SP02p	Mastretta C.	SP06p
Magnavacca C.	SYM2or	Mastretta C.	SYM05p
Magyar M.	SP01p	Masud A.Al	SYM01p
Mahdy A.M.	SP06p	Masue Y.	SYM01p
Mahimairaja S.	SP09p	Mateo R.	SYM01or
Maier M.	SYM08or	Mateus E.P.	SP07or
Majer V.	SP01p	Matinian N.N.	SYM07or
Makino Tomoyuki	SP10p	Matorin D.	SP09p
Malina G.	SYM08p	Matschullat J.	SYM01p
Malm O.	SP02p	Matsunaga H.	SYM01p
Manceau A.	SYM05p	Mattiazzo M.E.	SP11or
Mander Ü.	SYM2or	Mattiazzo M.E.	SP11p
Mangabeira P.	SP11p	Mattigod S.V.	SYM10or
Mangabeira P.A.O.	SP02p	Mattsson S.	SP02p
Mangabeiras P.A.O.	SP02p	Matzner E.	SP01p
Mangabeiras P.A.O.	SP03p	Maubert H.	SYM06p
Mankad V.	SP02p	Maurice C.	SP06p
Mano D.	SP07p	Maxemchuk A.	SYM03p
Manoucheri N.	SP05or	Maxted A.P.	SP06or
Manta D.S.	SP09or	Mayer K.U.	SYM08or
Maqueda C.	SP05p	Mays M.D.	SYM07or
Marc B.	SYM02p	McCarthy E.M.	SYM01or
Marcet P.	SP05or	McCarty G.W.	SP07p
Marcet P.	SP05p	McCleskey R.B.	SP06or
Marcic C.	SP05or	McCleskey R.B.	SYM01or
Marcic C.	SP11p	McCleskey R.B.	SYM01p
Marcus M.	SYM01or	McCleskey R.B.	SYM08or
Marcus M.	SYM05p	McGrath S.	SYM03p
Marcussen H.	SP05or	McGrath S.	SYM03or
Marimón Santos J.	SP01p	Mcgrath S.P	SP06or
Marinussen M.P.J.C.	SYM09p	McGrath S.P.	SP06p
Mariotti A.	SYM02p	McGrath S.P.	SYM01or
Marseille F.	SP06or	McGrath S.P.	SYM02or
Martin R.A.	SYM01or	McGrath S.P.	SYM03p
Martin R.R.	SYM02or	McLaren R.G.	SP02or
Martínez J.L.	SP02p	McLaren R.G.	SP11or
Martínez Sánchez M.J.	SP01p	McLaughlin M.J.	SP02or
Martínez-Cortizas A.	SP01p	McLaughlin M.J.	SP02p
Martínez-Sánchez M.J.	SP01p	McLaughlin M.J.	SP07or

McLaughlin M.J.	SYM03or	Mingozzi L.	SP07p
McLaughlin M.J.	SYM03p	Minkina .T	SP05p
McLaughlin M.J.	SYM04or	Minkina T.	SYM05p
McLaughlin M.J.	SYM04p	Minouflet M.	SYM06or
McLeod K.	SP01or	Mirlean N.	SP09p
Medina J.	SP01p	Mitsios I.K.	SP11p
Megharaj M.	SYM05p	Moharam M.L.	SP07p
Meharg A.A.	SP09p	Molina I.	SYM02or
Meharg A.A.	SYM01or	Moncur M.C.	SYM08p
Meili M.	SP02p	Mondini C.	SP11p
Meili M.M.	SP01or	Monné J.	SP05p
Meisner C.A.	SYM01p	Monni S.	SP06p
Meisner C.M.	SYM01or	Monterroso C.	SP06p
Meisner C.R.	SYM01p	Monterroso C.	SP11p
Melfi A.J.	SP09or	Montes C.R.	SP09or
Mellis E.V.	SP09or	Montuori P.	SP10p
Melo W.J.	SP07p	Moon H.	SP07p
Melo W.J.	SP02p	Moore M.R.	SP02p
Melo W.J.	SP11p	Moreira C.S.	SP09p
Melo W.J.	SP05or	Morel J.L.	SP06p
Mench M.	SP05or	Morel J.L.	SP05p
Mench M.	SP02p	Morel J.L.	SP06p
Mench M.	SP06p	Morel J.L.	SYM04or
Mench M.	SP11or	Moreno D.A.	SP03p
Mench M.	SP11p	Moreno G.A.M.	SP02p
Mench M.	SYM03or	Moretto L.M.	SP01or
Mench M.	SYM03p	Moretto L.M.	SP07p
Mench M.	SYM05or	Morillo E.	SP05p
Mendoza J.	SP02p	Morin G.	SP05or
Merckx R.	SP02p	Morin G.	SYM01or
Merckx R.	SYM04or	Morin G.	SYM01p
Merckx R.	SYM06or	Morin G.	SYM02p
Merilä P.	SYM08p	Motelica-Heino M.	SP01p
Merkel B.J.	SYM08or	Mott C.J.B.	SP06p
Merrington G.	SYM04or	Motuzova G.	SP11p
Mertens J.	SP02or	Motuzova G.	SYM01p
Mesquita M.E.	SP05p	Moulin C.	SYM06or
Mészáros I.	SP06p	Moulin V.	SYM06or
Mészáros I.	SYM05p	Mrvic V.	SP03p
Metzler D.M.	SP11p	Mueller R.	SP07or
Meylan S.	SP11or	Mukherjee A.B.	SYM01or
Miah M.A.M	SYM01or	Mukherjee A.B.	SYM01p
Miah M.A.M.	SYM01p	Mukherjee P.K.	SYM01p
Michel L.	SP05or	Munthe J.	SP01or
Mielke H.W.	SYM09p	Muraoka T.	SP11p
Mielke M.	SP11p	Muris M.	SYM09p
Mielke M.S.	SP02p	Musrafi I.	SP11p
Millán R.	SP02p	Mustafa G.	SYM09p
Miller W.P.	SYM10or	Mühlbachova G.	SP11p
Mimmo T.	SYM02p	Mylonas V.	SP11p
Mingelgrin U.	SYM02or	Mårtensson A.	SP14p

Mörth M. SYM01or

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Nadian H. SP11p
 Naftel S. SYM02or
 Nag S.K. SYM08or
 Nagaraju A. SP06p
 Naidu R. SP11or
 Naidu R. SP11p
 Naidu R. SYM01or
 Naidu R. SYM05p
 Nannipieri P. SP02p
 Nannoni F. SP14p
 Narang U. SP02p
 Narwal R.P. SP11or
 Nassisi A. SP01p
 Nastarin M. SP05or
 Nath B. SYM01p
 Naus C.A. SYM08or
 Navarro Hervás C. SP01p
 Navarro M.C. SP01p
 Neel C. SP05p
 Néel C. SP05p
 Neis E.C. SP09p
 Nelissen H.J.M. SP06p
 Nemecek J. SP05p
 Nemeth T. SP11p
 Németh T. SP01p
 Neretnieks I. SYM08or
 Neri R. SP09p
 Neri R. SP09or
 Neuschütz C. SP06p
 Ng J.C. SP02p
 Nieminen T. SP06p
 Nieminen T.M. SP02p
 Nietfeld H.W.F. SYM02or
 Nikkarinen M. SP03p
 Nilsson I. SP01p
 Nkoane B. SP10p
 Nobuntou W. SP02p
 Nobuntou W. SP09or
 Noecker B.L. SP07p
 Nolan A.L. SYM04or
 Nolan A.L. SYM04p
 Nordberg E. SYM01or
 Nordling S. SP11p
 Nordstrom D.K. SP06or
 Nordstrom D.K. SYM01or
 Nordstrom D.K. SYM01p
 Nordstrom D.K. SYM08or

Nowack B. SP02p
 Nowack B. SP06p
 Nowack B. SP07or
 Nowack B. SYM02p
 Nowack B. SYM09p
 Novak A. SYM03or
 Nowak B. SYM05or
 Novosad M. SP05or
 Nunes C. SP07p
 Nunes da Rocha U. SP07p
 Nurmi J. SP11p
 Nuttall C.Y. SP06p
 Nybroe O. SP11or
 Nyquist J. SP06p

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O'Brien A.M. SYM01or
 O'Shea B. SP05or
 Oeyen L. SYM05p
 Ogrinc N. SP01p
 Ogrinc N. SP01or
 Oliveira A. SP07or
 Oliveira A.H. SP11p
 Oliveira C. SP09p
 Oliveira Katarzyna W. SP05or
 Oliveira Lázaro J. SP09or
 Oliver I. SYM03or
 Oliver I. SYM03p
 Oliver I.W. SP02p
 Oliviera K.W. SP02p
 Oorts K. SP02p
 Oprean L. SP07p
 Ore S. SP02p
 Orlando D.L. SP14p
 Orlova M.I. SP11p
 Orłowska E. SP06or
 Ornes W.H. SP01p
 Ortego L. SYM03or
 Osborn D. SYM01or
 Oskarsson A. SP11p
 Ostonen I. SYM02or
 Osvaldo B.F. SP14p
 Osztoics A. SP01p
 Osztoics E. SP01p
 Othman.O.C. SP09or
 Otte M.L. SP09p
 Ottosen L. SP07p
 Ottosen L.M. SP07or
 Ottosen L.M. SP07p
 Oughton D.H. SP14p

Outola I.	SYM06p	Paz González A.	SP09p
Owens G.	SP07or	Paz-González A.	SP11p
Ovsiannikova S.	SP11p	Pearson D.A.B.	SYM03or
Ozdemir F.	SP03p	Pechová Pavlina	SP05or
P		Pedersen A.	SP07p
Packer A.P.	SP11p	Pedersen K.	SP11p
Page A.L.	SYM02or	Pedrinho J.	SP02p
Pakhomov O.Y.	SP01p	Pedrinho J.	SP03p
Paksi V.	SP06p	Peel J.W.	SP06p
Paksi V.	SYM05p	Peijnenburg W.	SYM03or
Palazzo A.J.	SP02p	Pelletier A.	SP01p
Palermo E.F.	SP02p	Pelletier M.	SP05or
Palestra I.	SP02p	Penfield R.	SP14p
Palumbo B.	SYM01p	Peplow D.	SYM01p
Pampura T.V.	SP01or	Peralba M.	SP09p
Panaullah G.M.	SYM01or	Peralta-Videa J.R.	SYM06p
Panaullah G.M.	SYM01p	Percival H.	SYM03p
Pandya K.	SYM03or	Percival H.J.	SYM03p
Panfili F.	SYM05p	Pereira E.	SP02p
Pang L.	SYM03p	Pereira E.	SP03p
Panganiban L.R.	SP03p	Perepelyatnikova L.V.	SP14p
Papadopoulos S.	SP11p	Perez de Mora A.	SP07or
Papadopoulos S.	SP05or	Perez de Mora A.	SP07p
Paramasivam S.	SP01p	Pérez Sirvent C.	SP01p
Paramasivam S.	SP02p	Pérez Zamora F.	SYM02or
Parat C.	SP05or	Pérez-Sirvent C.	SP01p
Paratay G.	SP11p	Perrin J.	SP07p
ParatC.	SP05or	Petit J.	SYM03p
Pardo R.	SP01p	Petruzzielli G.	SP02p
Pardo R.	SP05p	Petruzzielli L.	SP11p
Parker D.R.	SYM02or	Pezzarossa B.	SP02p
Parker D.R.	SYM04or	Pham H.V.	SYM01or
Parsons J.G.	SYM06p	Pham K.T.	SYM01or
Parthasarathy N.	SP05or	Philippe H.	SYM02p
Pasanen T.	SYM01p	Phillips C.T.	SYM03or
Pasieczna A.	SYM07or	Pickering I.J.	SYM01or
Pasini M.A.	SP02p	Pico F.	SP07or
Patel K.S.	SP02p	Piechalak A.	SP11p
Patel K.S.	SP03p	Pierangeli M.A.P.	SP11p
Patel S.R.	SP02p	Pierangeli M.A.P.	SP11p
Pato P.	SP02p	Pierzynski G.M.	SYM03or
Pauly S.	SP05p	Pietruch C.	SP01or
Pavan Kumar G.	SP11p	Pigna M.	SYM01or
Pavlíková D.	SP11or	Pigna M.	SYM02or
Pavlíková D.	SP05p	Pinati Casarini D.C.	SP12p
Pavlíková D.	SP06or	Pind N.	SP02p
Pavlíková D.	SP11p	Pinto Mergulhão A.P.	SP07p
Payton M.	SYM03or	Piquet A.	SP05p
Paz Ferreira J.C.	SP09p	Piquet-Pissaloux A.	SP01or
		Piquet-Pissaloux A.	SP01p
		Pires A.J.	SP11p

Pires A.M.M	SP11or	Pullinen A.	SYM01p
Pirrone N.	SP01p	Punshon T.	SP01or
Plancque G.	SYM06or	Punshon T.	SYM10or
Planer-Friedrich B.	SYM01p	Puschenreiter M.	SYM02or
Plassard C.	SYM06or	Puschenreiter M.	SYM02p
Plestenjak S.	SYM02p	Putwain P.	SP06p
Plumlee G.S.	SYM08or	Puy Jaume	SP05p
Plyaskina O.V.	SP05or		
Podlesáková E.	SP05p	Q	
Pokarzhevskii A.D.	SP02p	Qasaimeh A.	SP05or
Pokarzhevskii A.D.	SP03p	Qi L.	SP02p
Pokrovsky O.S.	SP05or	Qiao X.L.	SP09or
Pongsakul P.	SP02p	Qiu G.	SP01p
Pongsakul P.	SP09or	Quaghebeur M.	SYM02or
Ponizovsky A.A.	SYM03p	Queiruga J.	SP05p
Ponizovsky A.A.	SYM05or	Quilici H.	SP02p
Ponthieu M.	SP05or		
Poschenrieder C.	SP11p	R	
Poschenrieder C.	SYM02p	Raab A.	SP03p
Postma D.	SYM08or	Rábago J.A.I.	SP02p
Potin-Gautier M.	SP05or	Raber B.	SYM07or
Potin-Gautier M.	SP11p	Rachou J.	SP01p
Powell E.T.	SYM09p	Racine C.	SP02p
Powell M.A.	SP11p	Ragazzi F.	SYM07p
Powell M.A.	SYM10p	Raha P.	SP11p
Praharaj T.	SYM10p	Rahman G.K.M.M.	SYM01or
Prasad M.N.	SP07p	Rahman G.K.M.M.	SYM01p
Prasad M.N.V.	SP06p	Rahman L.	SP02p
Prasad M.N.V.	SYM01p	Rahman L.	SP03p
Prasad M.N.V.	SP06or	Rahman M.A.	SYM01p
Prasad M.N.V.	SP11p	Rahman M.S.	SP09p
Premoli A.	SYM02or	Rahman M.S.	SYM01p
Premoli A.	SYM02p	Rais D.	SP06p
Preus E.M.	SP11p	Rais D.	SYM07or
Prevost F.	SYM03or	Rajesh K.G.	SP06p
Prince R.C.	SYM01or	Ramocha A.	SP10p
Prister B.	SP14p	Ranville J.	SYM09or
Prister B.S.	SP14p	Recalde N.	SP06p
Proix N.	SYM07or	Recalde N.	SYM05or
Prone A.	SP11p	Reddy K.J.	SP06p
Prosiannikov E.V.	SP02p	Reddy K.J.	SP07p
Protano G.	SP14p	Reddy K.J.	SYM05p
Protasowicki M.	SP02p	Redman A.	SYM09p
Protic Nebojsa	SP05p	Reeves R.D.	SP06p
Prudent P.	SP11p	Reichard P.	SYM06or
Ptacek C.J.	SYM08or	Reichard P.U.	SYM02p
Ptacek C.J.	SYM08p	Reichman S.R.	SYM02or
Ptitskaya L.D.	SYM08p	Reiller P.	SYM06or
Puente P.	SP07p	Reimann C.	SYM07or
Puglisi E.	SYM03p		
Puhakainen M.	SYM06p		

Reimann C.	SYM07p	Routh J.	SYM08or
Reinoso R.	SP02or	Roux M.	SP11p
Rejeb M.N.	SP06p	Rovdan E.N.	SP14or
Rejeb S.	SP06p	Royle A.	SP01p
Renella G.	SP02p	Rozita O.	SP03p
Rengel Z.	SYM02or	Rubin A.	SP09p
Rentsch D.	SYM09or	Rubinchik A.	SP11p
Renzi A.	SP02p	Rufyikiri G.	SYM02or
Reva O.A.	SP11p	Ruley T.	SP06p
Revoredo Marcos D.	SP05or	Rusk J.	SP02or
Rey-Castro C.	SYM09or	Rusk J.	SYM03p
Ribas N.	SP10p	Russom C.L.	SYM03or
Ribeiro A.	SP07p	Ryan J.	SYM05or
Ribeiro A.B.	SP07or	Ryan J.A.	SYM05or
Riccobono F.	SP14p	Ryan Jim	SP05or
Richardson J.P.	SP02p	Rytwo G.	SYM02or
Riff M.	SYM03or	Ryzhikova I.	SP02p
Rigo C.	SP11p	Rääf .C	SP02p
Rimmer D.L.	SP02or	Römkens P.F.A.M.	SP06or
Ritschel J.	SP07or	Römkens P.F.A.M.	SP05or
Ritter K.	SYM09p	Rönnegård J.	SP11p
Rizou Z.S.	SP11p		
Roberts A.H.C.	SP02p	S	
Robinson J.E.	SYM10or	Sadovnikova L.K.	SP05p
Roche A.L.	SP02or	Sadurski W.	SP07or
Rodgers J.D.	SP06p	Sadzawka A.	SP02p
Rodrigues S.M.	SP06or	Sæther O.M.	SYM01p
Rodríguez P.H.	SP11or	Sager M.	SP01p
Rodríguez-Rubio P.	SP05p	Sahi S.V.	SP06p
Roed J.	SP07or	Sahut C.	SYM06p
Roed J.	SP07p	Saiyasitpanich D.	SP02p
Roelofs J.G.M.	SP02p	Saiyasitpanich D.	SP09or
Roig A.	SYM07p	Sajwan K.	SP01p
Roman-Ross G.	SYM09or	Sajwan K.S.	SP01p
Romero L.	SP03p	Sajwan K.S.	SP02p
Romic D.	SP02p	Sakurai Y.	SP10p
Romic M.	SP02p	Salbu B.	SP14or
Rommens C.	SP12p	Salbu B.	SP14p
Rooney C.	SYM03p	Salbu B.	SYM06p
Rooney C.	SYM03or	Salemaa M.	SP06p
Rooney C.P.	SP02or	Saleque M.A.	SP09p
Rose J.	SYM07p	Sall M.	SP09p
Rosen K.	SP14p	Salvador-Blanes S.	SYM07or
Rosina A.	SP09or	Salvador-Blanes S.	SYM07p
Rosina A.	SP09p	Salvagio Manta D.	SP09p
Roth K.	SP01p	Samokhin A.	SP05p
Rothan C.	SYM03p	Samokhin A.	SYM05p
Roudaut A.	SP02p	Samonova O.A.	SYM07p
Rouleau C.	SP11p	Samson D.	SP07p
Roussel-Debet S.	SYM02p	Sánchez M.	SYM02or
Routh J.	SYM01p		

Sánchez P.	SP11or	Schulin R.	SYM05or
Sand C.	SP07p	Schulin R.	SYM09p
Sandberg R.	SP03p	Schulze D.G.	SYM02p
Sandberg R.	SYM03p	Schwab P.	SP02p
Sandlu S.S.	SP02p	Schwartz C.	SYM07or
Santore R.C.	SYM03or	Schwartz C.	SP06p
Santos E.	SP05p	Schwarzenbach R.P.	SYM08p
Santos G.C.G.	SP11or	Schwenk M.	SYM01p
Sanzharova N.I.	SYM06p	Schwesig D.	SP01p
Sappin Didider V.	SP11or	Scopece P.	SP07p
Sappin-Didner V.	SP06p	Scroggings R.	SP12p
Saraswathy A.	SYM01p	Scroggins R.	SYM03or
Sarret G.	SYM05p	Seagren E.A.	SYM05or
Sartori G.	SP01or	Seaman J.	SYM09or
Sas-Nowosielska A.	SP06p	Seaman J.C.	SYM10or
Satake K.	SP02p	Sedlá r ová B.	SYM06p
Satoh H.	SP06p	Séguin V.	SYM02or
Sauvé S.	SP01p	Selim H.M.	SP05p
Sauvé S.	SP05or	Sellami R.	SP06p
Sauvé S.	SYM03or	Semeria A.	SP07p
Savithri P.	SP09p	Sen R.	SYM01p
Sawula G.	SP10p	Senchugova N.	SP03p
Schamphelaere	SP05p	Senesi N.	SP02or
D.K.A.C.		Senette C.	SYM02or
Schat H.	SP06p	Sengupta S.	SYM01p
Scheid S.	SP02p	Sens-Zanetto C.	SP11p
Scheinost A.C.	SYM05or	Sepúlveda N.	SP11or
Schertenleib R.	SYM01or	Sergeant A.	SYM03p
Schettini C.A.F	SP01p	Serpillo M.	SYM02or
Schlossberg M.J.	SYM10or	Serrão M.G.	SP05p
Schmid t.	SP02p	Sessitsch A.	SYM02p
Schmidt H.	SYM01p	Setia R.K.	SP09p
Schmidt P.	SYM06p	Severo M.I.G.	SP02p
Schmidt T.C.	SYM08p	Severo M.I.G.	SP03p
Schmitt-Kopplin P.	SP07or	Severo M.I.G.	SP11p
Schmitt-Sirguy C.	SP06p	Shagalova E.	SYM06p
Schnepf A.	SYM02or	Shah A.	SYM09p
Schnepf A.	SYM02p	Shah S.A.	SP11p
Schoeters I.	SYM03or	Shalabi J.	SP02p
Scholz C.	SYM08or	Shamshuddin J.	SP03p
Schott J.	SP05or	Shapiro Anna D.	SP05p
Schrefl T.	SYM02or	Shariatmadari H.	SP11or
Schroder J.	SYM03or	Shariatmadari H.	SP11p
Schröder T.J.	SYM09or	Shariatmadari H.	SP05or
Schulin R.	SP02p	Sharma K.N.	SP09p
Schulin R.	SP06p	Sharma N.C.	SP06p
Schulin R.	SP07or	Sharma S.K.	SP09or
Schulin R.	SP07p	Sharshenova A.	SP09p
Schulin R.	SP11or	Shaw G.	SYM06or
Schulin R.	SP11p	Shaw G.	SYM06p
Schulin R.	SYM02p	Shi Z.	SYM03p

Shieffler A.	SP11p	Smith J.	SP11or
Shin K.	SYM05or	Smith J.	SP11p
Shin-ichi Y.	SP02p	Smolders A.J.P.	SP02p
Shipigina E.A.	SP02p	Smolders E.	SP02or
Shrivass K.	SP02p	Smolders E.	SP02p
Shrivass K.	SP03p	Smolders E.	SYM03or
Shtangeeva I.	SP06p	Smolders E.	SYM03p
Shtangeeva I.	SP14p	Smolders E.	SYM04or
Shtangeeva I.	SYM06or	Smolders E.	SYM06or
Sidoli-O'Connor C.	SP07or	Sokolik G.	SP11p
Sidoli-O'Connor C.	SYM05p	Sokolov V.	SP02p
Siebielec G.	SP07or	Solano Marín A.M.	SP01p
Siebielec G.	SP07p	Solda P.	SYM05or
Siewers U.	SYM07or	Solinas V.	SYM02or
Sigg L.	SP11or	Solinas V.	SYM02p
Sigg L.	SP11p	Solovitch N.	SYM09p
Sigg Laura	SP05p	Sommer P.	SP06or
Sikder A.M.	SYM01p	Sommer P.	SYM02or
Silva D.C.	SP02p	Song J.	SP09or
Silva I.	SP07p	Rodrigues S.M.	SP10p
Silva M.R.S.	SP02p	Soubrand M.	SP05p
Silva M.R.S.	SP03p	Souta Ikuo	SP09or
Silva S.	SP10p	Spadini L.	SYM09p
Silva Y.	SP11or	Sparks D.L.	SYM01or
Simini M.	SYM03or	Speicher J.A.	SYM03or
Simmons J.S.	SYM10or	Speir T.	SYM03p
Simmons R.W.	SP02p	Spezia S.	SP10p
Simmons R.W.	SP09or	Spiegel H.	SP01p
Simon L.	SP03p	Spiers G.A.	SYM03or
Simon L.	SP06p	Sprenger M.	SYM03or
Šimonek P.	SYM06p	Sprenger M.	SYM03p
Sinaj S.	SP11or	Srivastava P.	SYM09p
Sinaj S.	SYM04or	Stadler S.	SYM08or
Singh B.	SYM05p	Stahl P.D.	SP06p
Singh B.	SYM09p	Stahr K.	SP07p
Singh K.	SP02p	Stanisawska-Glubiak E.J.	SP11or
Sinha Ray S.P.	SYM08or	Stark K.	SP02p
Siobhán S.	SP05p	Stárková A.	SP05p
Siroos J.	SP02p	Statovoi A.	SP05p
Sitandi I.W.	SP06p	Staunton S.	SYM06or
Skaggs R.	SYM10or	Staunton S.	SYM06p
Skinner Q.D.	SYM05p	Stefaniak S.	SP02p
Skipperud L.	SP14p	Steinnes E.	SP11p
Skipperud L.	SYM06p	Steinnes E.	SYM06p
Skyllberg U.	SP05p	Steinnes E.	SYM07p
Skyllberg U.	SP05or	Steinnes E.	SP01or
Slaveykova V.I.	SP11or	Stemmer M.	SYM02p
Smiljanic Z.	SP03p	Stephan C.	SP05or
Smith E.	SP11or	Sterckemant T.	SYM07or
Smith E.	SP11p	Stingeder G.J.	SP11p
Smith E.	SYM01or		

Štipek K.	SP11p	Takeda A.	SYM06p
Stockley C.S.	SP03p	Takenaka C.	SP06p
Stollenwerk K.G.	SYM01or	Takenaka C.	SP11p
Stoltz E.	SP06p	Takenaka C.	SP14p
Stornioło A.R.	SYM01or	Takenaka C.	SYM02p
Strauß J.	SP06or	Takenaka C.	SP01p
Strzyszcz Z.	SP02p	Tamm A.	SP06p
Stubblefield W.	SYM03or	Tampali C.S.	SP11p
Stuczynski T.I.	SP07or	Tandukar N.	SYM01or
Stuczynski T.I.	SP07p	Tandy S.	SP07or
Studer B.	SYM09or	Tanji K.K.	SYM01p
Stukin E.D.	SYM09p	Tanskanen H.	SYM01p
Stukin E.D.	SYM08p	Tao S.	SYM02or
Stüben D.	SYM01or	Tarvainen T.	SYM07or
Su C.	SYM08or	Teien H.C.	SYM06p
Su J.	SYM01or	Teixeira S.	SP07p
Su J.	SYM01p	Temminghoff E.J.M.	SYM09or
Subburam V.	SP11p	Temminghoff E.J.M.	SYM09p
Subramanian V.	SP09or	Ten Bookum W.M.	SP06p
Subramanian V.	SYM01p	Terekhina N.V.	SP02p
Sudre D.	SYM03p	Terelak H.	SP01or
Sugahara K.	SP10p	Terry N.	SP06p
Sukhorukov F.V.	SP14p	Tervahauta A.	SP06or
Sultana S.	SYM01p	Terytze K.	SP01or
Sunderland G.	SP07or	Tezuka T.	SP01p
Sunderland G.	SYM05p	Thabet A.G.	SP06p
Sunnerdahl I.	SP01p	Thabet A.G.	SP07p
Sunyer J.	SP10p	Thangavel P.	SP11p
Swanwick S.	SYM01or	Thiry Y.	SYM02or
Sverdrup H.	SP01or	Thiry Y.	SYM06p
Swindoll M.C.	SYM03or	Thompson I.A.	SYM02p
Sylvie T.	SYM02p	Thukral A.K.	SP02p
Sysoeva A.A.	SYM06p	Thørring H.	SYM06p
Száková J.	SP11or	Tibbett M.	SP11p
Szakova J.	SP06or	Tiemann K.J.	SYM06p
Szakova J.	SP11p	Tiffreau C.	SYM06p
Száková J.	SP05p	Tijhuis L.	SYM01p
Száková J.	SP06p	Timbal J.	SP11p
		Timbang R.N.	SP03p
T		Tipping E.	SYM09or
Taboada M.A.	SP03p	Tlustoš P.	SP11or
Tack F.	SP07p	Tlustoš P.	SP06or
Tack F.M.G.	SP11p	Tlustoš P.	SP05p
Tack F.M.G.	SP05p	Tlustoš P.	SP06p
Taggart M.A.	SYM01or	Tlustoš P.	SP11p
Taghavi S.	SYM05p	Tolrà R.P.	SYM02p
Tagmount A.	SP06p	Toma S.	SYM02p
Takahashi T.	SYM06p	Tomasoni C.	SP11p
Takaku Y.	SP11p	Tomassone R.	SP02p
Takeda A.	SP11p	Tomaszewska B.	SP11p
		Tomiooka R.	SYM02p

Tom-Petersen A. SP11or
 Tonin C. SYM02or
 Topoyan E. SP09p
 Torres J.C. SP11or
 Torri S.I. SP11p
 Tóth A. SYM05p
 Tournassat C. SYM09or
 Tovar Frutos P.J. SP01p
 Tran H.C. SYM01or
 Traoré M. SP09p
 Tretner A. SYM08or
 Trevisan M. SYM03p
 Tripathy S. SP11p
 Tripathy S. SYM10p
 Troiani H. SYM06p
 Tropp M. SP09or
 Truccolo E.C. SP01p
 Truu M. SYM02or
 Tsadilas C. SP11p
 Tsadilas C.D. SP05or
 Tsitsias K. SP05or
 Tsukada H. SP11p
 Tsukada H. SYM06p
 Tu C. SP06or
 Tu M.S. SYM01or
 Tu S. SP06or
 Tuomainen M. SP06or
 Turkan I. SP03p
 Turnau K. SP06or
 Turner B.D. SYM08p
 Twardowska I. SP02p
 Twardowska I. SP07or
 Twardowska I. SYM10or
 Tynybekov A. SP09p

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Uddin M.K. SP09p
 Uddin M.N. SYM01or
 Uemura T. SP11p
 Ufimtseva M.D. SP02p
 Ugo P. SP01or
 Ugo P. SP07p
 Ulrich K-U. SYM09or
 Unamuno V.I.R. SP05p
 Underhill R. SP06or
 Unterbrunner R. SYM02or
 Uri Z. SP03p
 Usman A.R.A. SP07p
 Utermann J. SYM07or
 Utermann J. SYM07p

Utermann J. SP10p

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Vácha R. SP05p
 Waegeneers N. SYM06or
 Wagner F. SYM01or
 Vaiskunaite R. SP07p
 Valarezo C. SP01or
 Vale C. SP02p
 Vale C. SP03p
 Valero A.A. SYM01or
 Wallberg P. SP02p
 Wallschläger D. SYM01or
 Valsami-Jones E. SYM01p
 Walter J. SYM03or
 Van de Werf H. SP09p
 van der Lelie D. SP02p
 van der Lelie D. SP06p
 van der Lelie D. SYM05p
 Van der Vliet L. SP02p
 van der Zee S. E.A.T.M. SYM09p
 Van Gestel C.A.M. SP11p
 Van Gestel C.A.M. SYM03or
 van Gestel K. SP11p
 Van Hees M. SYM06or
 Van Hees M. SYM06p
 van Lagen B. SP05p
 van Leeuwen Herman P. SP05p
 van Oort F. SP12p
 Van Oort F. SYM03or
 van Oort F. SP01p
 Van Pelt R.S. SP14p
 van Riemsdijk W.H. SYM09or
 van Riemsdijk W.H. SP05or
 van Schaik A. SYM03p
 van Sprang P.A. SP12p
 Van Straalen N.M. SP03p
 Vancluysen J. SP14or
 Vandenhover H. SYM06p
 Vandenhover H. SYM06or
 Vanderdeelen J. SP05p
 Wang G. SYM06p
 Wang G. SP05p
 Wang J.P. SP02p
 Wang J.R. SP06p
 Wang L. SYM06or
 Wang M.C. SP14or
 Wang M.K. SYM02p
 Wang Q. SYM10p

Wang S.	SP01p	Viksna A.	SP02p
Wang Y.D.	SP06p	Vikström A.	SP09or
Vangronsveld J.	SP06p	Wilcke W.	SP01or
Vangronsveld J.	SYM05or	Wilkie J.A.	SYM08or
Vangronsveld J.	SYM05p	Wilkin R.T.	SYM01or
Wannijn J.	SYM06or	Wilkinson K.J.	SP11or
Vanrolleghem P.A.	SP12p	Villamar L.	SP05p
Vansuyt G.	SP06p	William S.P.M.P.	SP06p
Vanthuyne M.	SP05p	Villora G.	SP03p
Vares A.	SYM2or	Wilson M.A.	SYM07or
Vargas Jr E.A.	SP07p	Viñas P.	SP10p
Vartiainen S.	SYM01p	Vinci I.	SYM07p
Vassilieva E.	SP02p	Wingenfelder U.	SP07p
Veado M.A R.V.	SP02p	Vink J.	SP11p
Veado M.A.R.V.	SP03p	Vink J.P.M.	SP02or
Veeresh H.	SP11p	Vink J.P.M.	SYM09or
Veeresh H.	SYM10p	Violante A.	SYM01or
Vega M.	SP05p	Violante A.	SYM02or
Vega M.	SP01p	Violleau D.	SP05or
Weiliang Z.	SP14p	Virta M.	SP02p
Welch A.H.	SYM01or	Virta M.	SP05or
Welp G.	SYM07or	Wolpher H.	SP09or
Wennrich R.	SP02p	Wolthers M.	SYM09or
Wennrich R.	SP11or	Wolthoorn A.	SYM09or
Wentsel R.S.	SYM03or	Wragg J.	SYM01p
Wenzel W.W.	SP06or	Wright K.	SYM01p
Wenzel W.W.	SP11p	Wu L.	SP06p
Wenzel W.W.	SYM02or	Wu L.H.	SP09or
Wenzel W.W.	SYM02p	Vulkan R.	SYM02or
Vera R.	SP02p	Vynogradskaja V.D.	SP14p
Verdonck F.A.M.	SP12p	Vyslouzilova M.	SP06or
Veres S.	SYM05p	Vyslouzilova M.	SP06p
Verkhovceva N.	SP02p	Wängstrand H.	SP03p
Verloo M.	SP07p	Wällstedt T.	SP01p
Verloo M.G.	SP11p		
Verméglio A.	SP11p	X	
Verplanck P.L.	SYM08or	Xiao T.	SP02p
West H.M.	SP06or	Xu J.-R.	SYM02p
Vetrov V.A.	SP11p	Xue Q.	SYM05or
Vetterlein D.	SYM02p		
White N.	SYM01p	Y	
Whitney J.W.	SYM01or	Yamasaki S.	SP02p
Vidal J.	SP01p	Yanai J.	SYM02or
Vidal Otón J.	SP01p	Yang J.L.	SYM02p
Widmer F.	SYM02p	Yermiyahu U.	SYM02or
Vieira e Silva J.M.	SP05p	Yi J.-M.	SP02p
Viers J.	SP05or	Yi J.-M.	SP11p
Wieshammer G.	SP06or	Yli-halla M.	SP10p
Wieshammer G.	SYM02or	Yoshida K.	SP01p
Vijver M.	SP11p		
Vijver M.	SYM03or		

Young C. SP02p
Young S.D. SP06or
Yount J.C. SYM01or
Yu Pei-Fang SP10p
Yu X. SYM02p
Yun S.-G. SP03p

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Zabochnicka-Swiatek M. SYM08p
Zaccheo P. SP02p
Zakaria M. SP02p
Zakharenkov V. SP11p
Zakharenkova N. SP11p
Zanetti M. SP02p
Zanetti M. SP03p
Zarcinas B.A. SP07or
Zaayah S. SP03p
Zdimal K.L. SP06p
Zech W. SP01or
Zevenhoven R. SYM01p
Zhang H. SYM03or
Zhang H. SP11p
Zhang H. SP05p
Zhang W. SYM01or
Zhang W. SYM01p
Zhao F. SYM03p
Zhao F. SYM03or
Zhao F.J. SP06p
Zhao F.J. SYM01or
Zhao F.-J. SYM02or
Zhao L. SYM02p

Zheng B. SP02p
Zheng S.J. SYM02p
Zhilin O.V. SP11p
Zhmelkova T.N. SP02p
Zhukova O. SYM06p
Ziemkiewicz P.F. SYM10or
Zilocchi L. SP01or
Zimmermann K. SYM01p
Zimmermann S. SYM02p
Zimmermann S. SYM07or
Zobeck T.M. SP14p
Zupan M. SP11p
Zuykov M.A. SP11p
Zwank L. SYM08p
Zvonaric T. SP01p

Å

Åkerblom S. SP05p

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Öborn I. SP01p
Öborn I. SP02p
Öborn I. SP03p
Öborn I. SYM03p
Öhlander B. SP14or
Orlander G. SP11p
Österreicher-Cunha P. SP07p
Österås A.H. SP01p
Öztürk M. SP09p