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IOC/INF-978
Paris, 5 January 1995
English only

INTERGOVERNMENTAL OCEANOGRAPHIC COMMISSION
(of UNESCO)

REPORT OF THE 1993 IOC/WESTPAC NUTRIENT INTERCALIBRATION EXERCISE

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1. INTRODUCTION

1 During the IOC/WESTPAC Workshop on River Input of Nutrients to the Marine Environment in the Western Pacific (Penang, Malaysia 26-29 November 1991) it was recommended that a nutrient intercalibration exercise be conducted, by correspondence, for scientists in the WESTPAC region with a view to enhancing national capabilities in the region in the assessment of river inputs to the seas. This recommendation was approved by IOC and during 1993 a program was designed. Drs. Manuwadi Hungspreungs of Chulalongkorn University, Bangkok, Thailand and Herbert L. Windom, Skidaway Institute of Oceanography, Savannah, USA, agreed to coordinate the exercise.

2 As a first step in the planning of the exercise, scientists in the WESTPAC Region who might be interested in participating were identified. Letters (example given in Annex I) were sent to them to solicit their interest and, based on their response, fifteen participants (Annex II) were chosen to receive intercalibration samples.

3 During the Fall of 1993, two intercalibration samples for ammonia, nitrate and orthophosphate were sent to each selected participant. The selection of the easier nutrient species to be included in this exercise was made to maximize the likelihood of success.

4 The intercalibration samples were obtained through the U.S. Environmental Protection Agency with the kind assistance of John A. Winter, Director of the Quality Assurance Research Division. The participants were sent a detailed set of instructions (Annex III) requesting that results be returned to H. Windom by 1 January 1994. Although some participants returned their results by the deadline, many results were delayed considerably. The first draft of this report was completed and submitted to the IOC Secretariat on 23 February 1994 and included results returned as late as 17 February. Subsequently three additional sets of data were received in April so this present, second, draft of the report includes these results.

2. RESULTS

5 The true values along with the performance evaluation criteria for the two intercalibration standards are provided in Table 1. Twelve of the fifteen selected participants (as indicated in Annex II) returned data. These results, shown in Table 2, indicate that the overall ability of the participants to obtain values within the performance criteria is poor. Only one laboratory (Lab No. 14) was able to produce valid results for all analyses. Results for Laboratory 2 are considerably higher than the rest and probably reflect the fact that the participant did not follow directions and calculate the concentration on the diluted basis. If this had been done, these results (Lab No. 2) should be a factor of 100 lower.

6 The analysis of nitrate appears to be the most difficult for the participants with only 30% of the results falling within acceptable values. This is followed by ammonia (38% acceptable) and phosphate (50% acceptable).

7 To assess the reasons for poor results, the data for the two intercalibration samples were plotted on Youdan diagrams (Figures 1-3). This presentation of the data allows for the identification of systematic versus random errors in the analyses. For example, results for ammonia (Figure 1) suggest that errors are mostly systematic. This is evident by the fairly linear distribution of the data through the intersection of the perpendicular lines representing the true

value. It is likely that the data point in the bottom left quadrant of the figure is due to consistently low recovery for both samples or perhaps systematically high standards. The two data points in the upper right quadrant may reflect systematic errors in sample dilution and/or the use of low (perhaps old) standards.

8 The presentation of results shown in Figures 2 and 3 can be used in a similar way to interpret overall results, but, in general, results show systematic errors to be more common than random errors. It is, however, clear that random errors are more frequent in the analysis of nitrate and phosphate then they are in the analysis of ammonia.

3. RECOMMENDATION

9 It is clear from the results given above that there is considerable need for improvement in nutrient analyses for the laboratories participating in this exercise, particularly when one considers that the more difficult analyses (i.e., particulate and organic species) were not included. It is, therefore, highly recommended that a regional training exercise for nutrient analysis of river samples be conducted followed by an additional intercalibration exercise to assess improvements.

Table 1

PERFORMANCE EVALUATION CRITERIA

(mg/l)

Analytes	Sample Number	True Value	Acceptance Limits
Ammonia-Nitrogen	1	5.50	4.35 - 6.65
	2	9.80	7.80 - 11.6
Nitrate-Nitrogen	1	34.0	27.5 - 40.2
	2	7.10	5.70 - 8.43
Orthophosphate	1	0.830	0.692 - 0.961
	2	0.092	.0617 - 0.122

Table 2

INTERCALIBRATION RESULTS

(mg/l)

Laboratory	Ammonia		Nitrate		Phosphate	
Number	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
2	543.00	1280.00	3400.00	845.00	80.5	10.2
4	6.71	12.3	34.4	6.18	0.83	0.09
8	nil	nil	5.62	0.76	1.0	0.11
9	6.22	10.92	6306	399	0.827	0.089
10	7.0	7.0	33.6	7.4	0.86	0.09
11	0.26	0.45	14.00	2.80	0.62	0.16
12	5.29	10.60	36.00	10.30	0.11	0.89
14	6.34	10.70	34.90	7.25	0.82	0.09
15	342.00	464.00	1.71	54.20	71.00	9.30
17	5.6	10.0	22.0	5.6	1.15	0.64
18	8.82	8.22	24.15	5.52	0.821	0.09
19	9.8	12.92	23.87	16.10	0.80	0.09

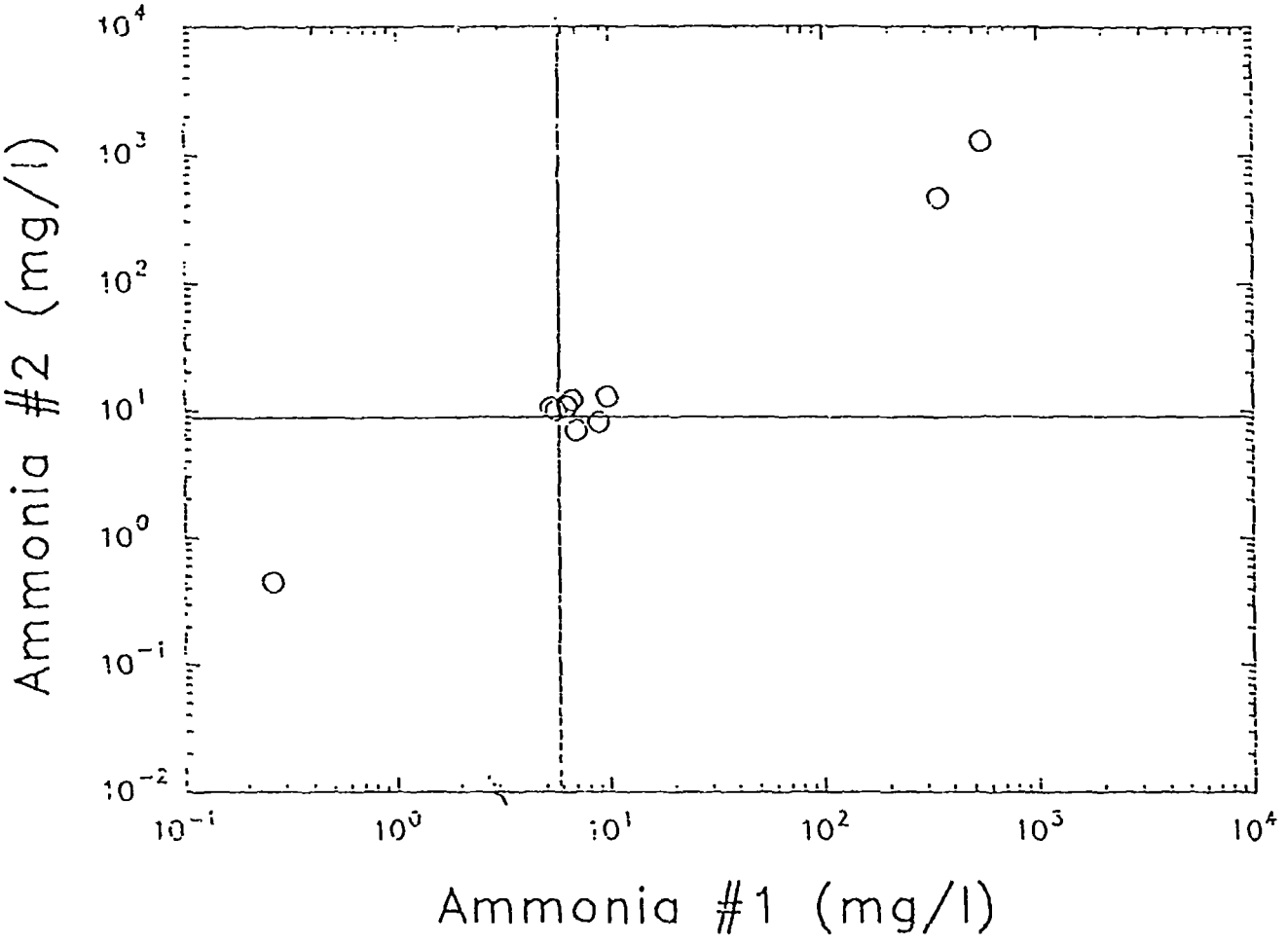

Figure 1. Youdan plot for ammonia results. Perpendicular lines represent true values.

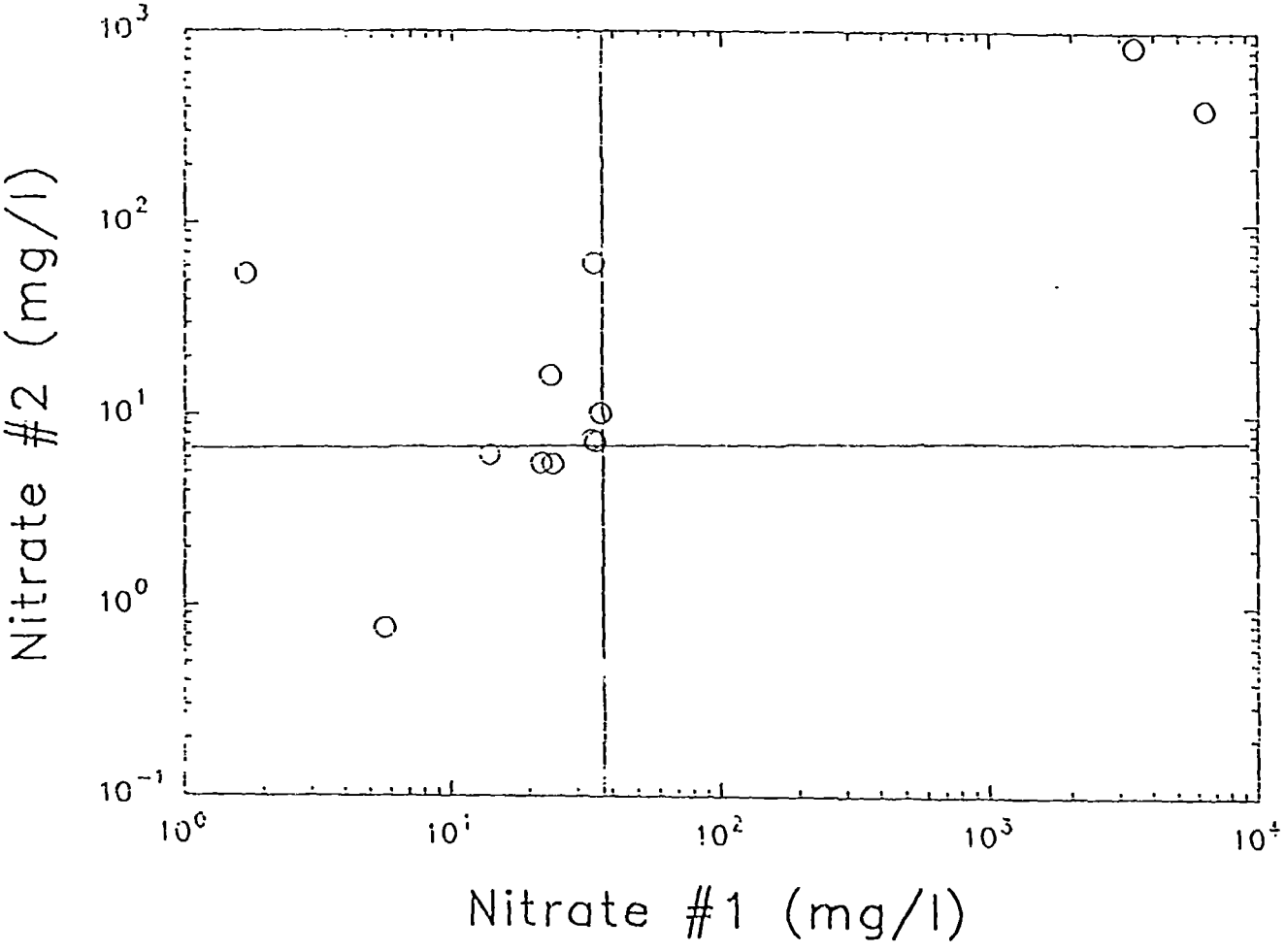

Figure 2. Youdan plot for nitrate results. Perpendicular lines represent the true values.

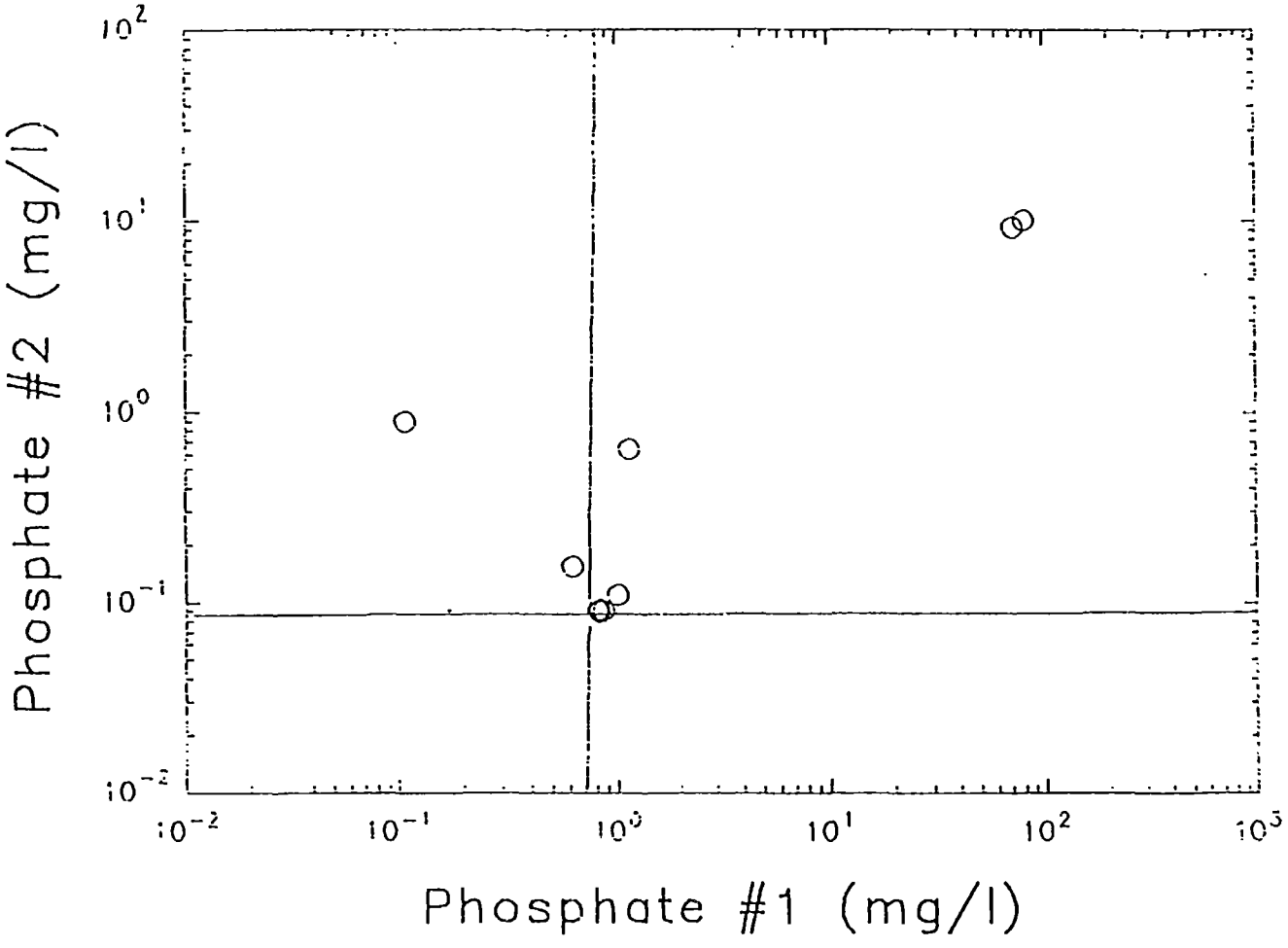

Figure 3. Youdan plot for phosphate results. The perpendicular lines represent the true values.

ANNEX I

SKIDAWAY INSTITUTE OF OCEANOGRAPHY
UNIVERSITY SYSTEM OF GEORGIA
P O BOX 13687
SAVANNAH, GEORGIA 31416

July 12, 1993

Drs. Ong Jin Eong and Gong Wooi Khoon
Center for Marine and Coastal Studies
Universiti Sains Malaysia
11800 Penang
MALAYSIA

Dear Drs. Ong and Gong:

You have been selected to participate in an IOC/WESTPAC Nutrient Intercalibration Exercise. You will receive a package containing a set of two samples along with instructions regarding participation. The standardized samples were prepared by the US EPA Environmental Monitoring Systems Laboratory. These packages will be sent to you from Bangkok during the next two months by Dr. Manuwadi Hungspreugs, Faculty of Science, Chulalongkorn University, Bangkok 10500, THAILAND.

You will be expected to return results by 1 January 1994. If for any reason you cannot participate, please contact Dr. Hungspreugs by letter, with a copy to me indicating such before 15 August 1993. Please also indicate an alternative or additional person or laboratory that would be willing to participate from your country.

The results of the intercalibration will be reported by laboratory number (not name) and each participant will receive a copy.

I hope we can count on your participation.

Sincerely,

Herbert L. Windom
Professor

HLW/ll

ANNEX II

Laboratories Wishing to Participate in "IOC/WESTPAC Intercalibration Exercises on Nutrient"

*DR. MASARU MAEDA	Tokyo University of Fisheries Department of Marine Science and Technology 5-7 Konan 4, Minatoku, Tokyo 108 JAPAN
DR. ONG JIN EONG DR. GONG WOOI KHOON	Centre for Marine and Coastal Studies Universiti Sains Malaysia 11800 Penang, MALAYSIA Phone: 604/877888 ext. 3511. FAX: 604/871526
*DR. TIANBAO FU	Third Institute of Oceanography, SOA P.O. Box 0570, Xiamen, Fujian, 361005 P.R. CHINA
*DR. LAW AH THEEM	Faculty of Fisheries and Marine Science Universiti Pertanian Malaysia 43400 UPM, Serdans, elangor, MALAYSIA
*DR. EN ISMAIL ISHAK DR. SHAKUNTHALA DEVI	Fisheries Research Institute, Jln. Akudium, Glugor, 11700 Glugor, Penang, MALAYSIA
*DR. SOO HYUNG LEE	Chemical Oceanography Laboratory Korea Ocean Research and Development Institute Ansan P.O. Box 29, Seoul 425-600 KOREA
**DR. GAO SHANGQUAN	Second Institute of Oceanography, SOA. P.O. Box 1207, Hangzhou, 310012 P.R. CHINA
*DR. EVGUENY SHUMILIN	Pacific OceanoENDFIELD logical Institute. 7 Radio Street, Vladivostok, 690032 RUSSIA
DR. JING ZHANG	Dept. of Marine Chemistry University Qingdao 5 Yushans Rd. Qingdao 266003 P.R. CHINA

*DR. MARIA CONSOLACION
DR. N. CAPINO
Department of Environment and Natural Resources
National Capital Region Laboratory Services
100 El Al Bldg.
Quezon Avenue, Quezon City
PHILIPPINES

*DR. PHILOMENA GANGAIYA
Institute of Natural Resources
University of the South Pacific
P.O. Box 1168
Suva, FIJI

*DR. PHAM VAN NINH
Research Centre for River and Sea Dynamics
NCSR VIETNAM
208 D, Doican, Hanoi, VIETNAM
Telephone: 2 54976
Telex: 411525 NCSR VT
FAX: 84 42 52 483

*DR. W. UTOOMPRURKPORN
Marine Science Department
Chulalongkorn University
Bangkok 10330
THAILAND
FAX: 66 2 2511951

*DR. A.V. TKALIN
Far Eastern Regional Hydrometreological Research Institute
24 Kzerzhinsky St.
Vladivostok 690600
RUSSIA

*DR. C. LANCASTER
DR. J. MORRISON
BACAS
University of Wollogong
Wollongong, NSW 2522
AUSTRALIA

* Returned Data
** Could not participate because of cruise commitment

ANNEX III

GENERAL NOTICE

PLEASE READ THIS IMPORTANT NOTICE FOR IOC/WESTPAC NUTRIENT INTERCALIBRATION PARTICIPANTS:

1. Please read ALL instruction sheets and General Reporting Instructions carefully BEFORE analyzing any samples.

2. Use ONLY the Data Report Form provided with this instruction package.

3. To replace broken ampuls contact: Dr. Manuwadi Hungspreugs
   Faculty of Science
   Chulalongkorn University
   Bangkok, THAILAND
   Telephone: 2524949, 2527984
   FAX: 662 2511951, 662 2550780

4. The lab identification number indicated at the top left of the Data Report Form will be used to identify your results in the final report rather than the name of the laboratory.

5. Appropriate analytical techniques are provided but it is recognized that other methods are suitable. If you use a method other than the one provided, briefly describe it on the Data Report Form.

6. Make 2 copies of your results, keep one for your files and return the original and one copy to the address listed on your cover memo.

TO: Participants of the IOC/WESTPAC Nutrient Intercalibration:

Included in this package are the following:

1. Two sample ampuls labeled "1" and "2". These samples were prepared and supplied by the U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

2. A GENERAL NOTICE sheet

3. INSTRUCTIONS FOR NUTRIENT ANALYSES

4, A set of analytical procedures as follows:

    Ammonia determination
    Nitrate determination
    Phosphate determination

5. A DATA REPORT FORM

Read all instructions carefully before starting analyses.

When analyses are complete send the DATA REPORT FORM to:

Dr. Herbert L. Windom
Skidaway Institute of Oceanography
P.O. Box 13687
Savannah, GA 31416

The deadline for receiving results is 1 January 1994.

LAB NUMBER:

DATA REPORT FORM

IOC/WESTPAC NUTRIENT INTERCALIBRATION EXERCISE

Participant: Name: _____

Organization: _____

Address: _____

_____

Country: _____

FAX No.: _____

RESULTS: Provide results in mg/L

	Sample 1	Sample 2
Ammonia-Nitrogen as N	____.____	____.____
Nitrate-Nitrogen as N	____.____	____.____
Orthophosphate as P	____.____	____.____

DESCRIPTION OF ANALYTICAL TECHNIQUE:

RETURN THIS REPORT FORM TO:

Dr. Herbert L. Windom
Skidaway Institute of Oceanography
P.O. Box 13687
Savannah, GA 31416
USA
Phone: 912/598-2490; FAX: 912/598-2310

Instructions for NUTRIENT Analyses

IOC/WESTPAC NUTRIENT INTERCALIBRATION EXERCISE

CAUTION: Read Instructions Carefully Before Opening Ampuls.

Concentrates for two different samples are enclosed. Each sample is prepared by diluting a measured amount of the concentrate from one ampul to volume with reagent-grade water (equivalent to Type II Reagent Water as specified in ANSI/ASTM Standard D 1193-83). Samples 1 and 2 contain inorganic nitrogen and phosphorus and may be analyzed for ammonia-N, nitrate-N, and orthophosphate-P. When diluted according to instructions, the concentrations of these nutrients will be less than 40 milligrams per liter.

The concentrates were prepared by dissolving known amounts of ACS-grade chemicals in reagent-grade water and were preserved by autoclaving the sealed ampuls. However, the preservative treatment is not effective after opening the ampuls; therefore, the samples must be analyzed as soon as possible after opening and dilution. In instances where the analysis will be delayed longer than two days from the sample preparation, a sufficient amount of sample should be preserved with 0.2 mL of concentrated sulfuric acid per 100 mL of sample (pH <2) and stored at 4°C. Analyze the preserved sample as soon as possible within the maximum holding time (28 days).

Please note that approximately 21 mL of each concentrate is supplied. If clean dry pipets are used, this is sufficient to prepare double volumes of sample if one liter is not enough. Since all constituents are present in soluble form, filtration is not necessary.

SAMPLE PREPARATION
(Use only Class A volumetrics)

CAUTION: AVOID INJURY FROM CUTS OR FLYING GLASS BY WRAPPING AMPUL IN CLOTH OR PAPER TOWEL BEFORE OPENING.

Sample 1:

Open Ampul 1 by snapping off the top at the narrow part of the neck. Pipet 10.0 mL of the concentration into a 1000 mL volumetric flask, make up to volume with reagent-grade water near 20°C and mix well. Sample 1 is now ready for analysis.

Sample 2:

To produce Sample 2, repeat the above procedure using Ampul 2.

A reagent-grade water blank should be analyzed concurrently for background correction.

REPORTING RESULTS

Enter the results of each determination and give a brief description of method used (if not the same as the procedure provided) on the attached form. All results should be reported to three significant figures as milligrams per liter (mg/L) of prepared sample.

CAUTION: Report orthophosphate as P.
Report ammonia and nitrate as N.

Nitrate determination

Reagents

1. Imadazole Buffer. Dissolve 6.81 gms of imidazole in approximatewly 900 ml H_2O in a 1 liter beaker. Position the beaker on a magnetic stirrer and insert a pH electrode in the solution. Adjust the pH of the sol. to pH 7.5 using Conc HCL. Pour the pH adjusted solution into a 1 liter volumetric and adjust to volume.
2. Sulphanilamide solution. Dissolve 10 gms of sulphanilamide in 100 ml of conc HCl and dilute to 1000 ml. (stability-months)
3. N-(1-naphyl elthyenediamine dihydrochloride). Dissolve 0.5 gm in 500 ml H2O and store in an amber bottle. (stability- 1 month)
4. Cadmium metal (40-60 mesh)
5. Copper Sulphate. Dissolve 10 gm of CU2SO4.5H2O in 1000 ml of H2O.
6. Nitrate standard. Dissolve 1.011 gm of dry KNO3 in 1000 ml of H2O. (10umol/ml)
7. Reductor. Wash the Cd filings with 2 molar HCl. Shake vigorusly with 100 ml of the copper sol. (5) for 3 minutes. Wash thoroughly with distilled water to remove colloidial copper. Gently fill a 60 cm U-tube (3mm id) with the aid of a funnel. Fill one side of the tube and then the other.

Procedure

To 10 ml of sample add 10 ml of the buffer solution and mix thouroughly. Using a peristlatic pump the sample is forced through the reduction tube with a flow rate of 5-8 ml/min. The first 10 ml of sample is discarded and the next 5 ml is collected for color development. Two huundred ul of the sulphanilamide is added to the 5.0 ml sample. The sample is shaken and allowed to equilibrate for 1 min. before 200 ul of the N-(1-naphthyl ethylenediamine dihydrochloride) is added. The color is completely developed in 15 min. and the absorbance is read at 540nm.

Recommended Standards

1. Make a 1/10 dilution of the standard stock solution.
2. From this dilution make the following standards.
3. Standard A (10uM)
4. Standard B (5.0 uM)
5. Standard C (1.0 uM)

Phosphate determination

Reagents

1. Sulphuric Acid. Dilute 250 ml of H_2SO_4 to 1000 ml of H_2O. Store in a polyethylene bottle.
2. Ascorbic solution. Dissolve 10 gms of ascorbic acid in 50 ml of H_2O and add 50 ml of the sulphuric acid reagent. Store in an amber glass bottle in the refrigerator. (stability- 1 week)
3. Mixed reagent. Dissolve 12.5 gm of ammonium heptamolybdate tetrahydrate in 125 ml H_2O. Dissolve 0.5 gm of potassium antimony tartrate in 20 ml of H_2O. Add the molybdate solution to 350 ml of the sulphuric acid reagent while stirring. Add the tartrate sol. and mix thoroughly. Stored in a glass bottle the solution is stable for months.
4. Phosphate standard. Dissolve 136.1 mg of oven dried KH_2PO_4 into 0.2 ml of the H_2SO_4 reagent and dilute to 100ml. Store cold in a glass bottle. The concentration is 10umol/ml.

Procedure

To 10 ml of sample add 0.2 ml of the ascorbic acid solution, mix and add 0.2 ml of the mixed reagent. Allow color to develop for at least 10 minutes but not more than 30 minutes. Measure the absorbance at 880 nm.

Recommended Standards

1. Make a 1/10 dilution of the stock standard.
2. From this dilution make the following standards.
3. Standard A (100 ul to 10 ml) 10 uM
4. Standard B (50 ul to 10 ml) 5.0 uM
5. Standard C (10ul to 10 ml) 1.0 uM

Determination of ammonia

Reagents

1. Phenol reagent. Dissolve 80 gms of phenol in 300 ml of ethanol and add 600 ml of H20. Dissolve 600 mg of disodium nitroprusside dihydrate in 100 ml of H20 and add to the solution above. Store in a tightly closed amber bottle in a refrigerator. (Stable for months)
2. Hypochlorite Solution. Dissolve 0.5 gm of dichloroisocyanuric acid (Trione) in 100 ml of 0.8M NaOH. Store cold in an amber glass bottle.
3. Tri-sodium citrate solution. Dissolve 240 gms of trisodium citrate dihydrate in approximately 400 ml H2O and add 10 ml of 0.8M NaOH then dilute to 500 ml. Store in polyethylene bottle.
4. Standard Stock Solution. Dissolve 53.5 mg of NH4Cl in 100 ml H2O. Preserve with 1 drop of chloroform. The standard contains 10um/ml. Store cold in glass. (stability-months)

Procedure

To 10 ml of sample, mix well between additions of 0.4 ml of phenol sol., 0.2 ml of the citrate solution and 0.4 ml of the hypochlorite solution. Allow the sample to equillibrate for 6 hrs at room temperature in the dark. Measure the absorbance at 630 nm. The color is stable for Approximately 30 hours.

Note: This procedure should be conducted in a room free of ammonical solutions and cigarette smoke.

Recommended Standards

1. Make a 1/10 dilution of the stock standard.
2. Standard A (100ul of 1 umole/ml diluted to 10 ml) 10 uM
3. Standard B (50ul of 1 umole/ml diluted to 10 ml) 5.0 uM
4. Standard C (10ul of 1 umole/ml diluted to 10 ml) 1.0 uM