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PROCEDURES MANUAL - 1973

LAKE ERIE NUTRIENT CONTROL PROGRAM: AN ASSESSMENT OF ITS EFFECTIVENESS IN CONTROLLING LAKE EUTROPHICATION

PROGRESS REPORT - 1973 FIELD SEASON

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CENTER FOR LAKE ERIE AREA RESEARCH
THE OHIO STATE UNIVERSITY
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TABLE OF CONTENTS

OBSERVED AND/OR ESTIMATED LIMITS OF DETECTION AND PERCENT RELATIVE ERROR OR ACCURACY OF ANALYTICAL PROCEDURES AND MEASUREMENTS DURING YEAR 1973-1974

MANUAL ANALYTICAL PROCEDURES:

Parameter	Method	Detection Limits	Relative Error Range %	Range
Ammonia Nitrogen*	Phenate	3 µg/l	10 to 2	5-100 µg/l
Nitrate plus Nitrite Nitrogen*	Cadmium Reduction	0.5 µg/l	10 to 1	1-1000 µg/l
Reactive Soluble Phosphorus*	Molybdenum-Blue-hydrazine sulfate	1 µg/l	50 to 5	1-50 µg/l
Total Phosphorus*	Molybdenum-Blue-hydrazine sulfate, Persulfate sulfuric acid hydrolysis	1 µg/l	20 to 3	10-100 µg/l
Alkalinity	HCl-titration mixed bromocresol-green-methyl red indicator	1 mg/l	10 to 0.5	10-500 mg/l
Dissolved Oxygen	Winkler-azide modification	0.1 mg/l	10 to 0.7	1-10 mg/l

* Manual methods were used only until October 1973.

OBSERVED AND/OR ESTIMATED LIMITS OF DETECTION AND PERCENT RELATIVE ERROR OR ACCURACY OF ANALYTICAL PROCEDURES AND MEASUREMENTS DURING YEAR 1973-1974

AUTOMATED AND INSTRUMENTAL PROCEDURES:

Parameters	Indophenol	Instrument	Detection Limits	Relative Error Range %	Range
Ammonia Nitrogen	Indophenol	AAII	1 µg/l	10 to 1	2-50 µg/l
Nitrate plus Nitrite Nitrogen	Cadmium Reduction	AAII	0.2 µg/l	10 to 1	1-100 1-1000 µg/l
Reactive Soluble Phosphorus	Molybdenum-blue-$SnCl_2$ reduction	AAII	0.1 µg/l	10 to 1	1-50 µg/l
Total Phosphorus	Molybdenum-blue-$SnCl_2$ reduction, Persulfate sulfuric acid hydrolysis	AAII Autoclave	0.1 µg/l	5 to 1	10-50 µg/l
Particulate Organic Carbon	Combustion	Perkin-Elmer 240	0.5 µg	1 to 0.3*	150-2000 µg/l
Particulate Organic Nitrogen	Combustion	Perkin-Elmer 240	0.5 µg	1 to 0.5*	50-300 µg/l
Chlorophyl a**	Colorimetric	Varian spectrophotometer, programmed wave length setting, computer output. Cells 10 mm and 100 mm	0.001 absorbance	~1	0.01-1.5 absorbance

* Depending on the concentration range the blank correction increases the relative error up to 5 to 7%.

** The overall error increases by calculation of the chlorophyl a and the overall error depends on the equations used for chlorophyl a calculation.

OBSERVED AND/OR ESTIMATED LIMITS OF DETECTION
AND PERCENT RELATIVE ERROR OR ACCURACY
OF ANALYTICAL PROCEDURES AND MEASUREMENTS
DURING YEAR 1973-1974

AUTOMATED AND INSTRUMENTAL PROCEDURES (Continued):

Parameter	Method	Instrument	Accuracy	Range
Depth	Pressure Transducer	Martek	± 4% for full scale	0-30 metres
Temperature	Thermistor temperature transducer	Martek	± 0.5° C	0°-40° C
Conductivity	Platinized conductivity cell	Martek	± 2% of full scale	Scales: (micromhos/cm) 0-1000 0-500 0-250 0-100 0-50 0-25
Dissolved oxygen	Gold/silver polarographic teflon membrane temperature compensated 0-40° C	Martek	± 1% of full scale	Scales: (mg/l) 0-2 0-10 0-20
pH	Glass pH electrode Ag/AgCl reference electrode Thermo-compensator	Martek	Resolution: 0.01 pH Accuracy: ± 0.05 pH	3-10.2 pH in 1.20 pH full scale increments
Transparency	Photometer with one metre folded path length	Martek	± 1.0%	Scales: (% Transmitance) 0-10 0-25 0-100
Bathythermograph	Temperature Depth		Temp: ± 0.25°C Depth: ± 0.10 metres	

PROCEDURES

PHYSICOCHEMICAL FIELD MEASUREMENTS IN THE CENTRAL AND WESTERN BASINS OF LAKE ERIE

Objectives

The Field Operations Segment of the project consists of a series of cruises in the central and western basins of Lake Erie designed to characterize the temperature, dissolved oxygen and suspended solids regime of the lake as a function of depth. The spacial distribution of these parameters and their interrelationships are important determinations of this study.

Methods

Nine major cruises were accomplished during the period June 28, 1973 through December 4, 1973. Fifty (50) stations were established in the central and western basin (Tables 1 and 2 and Figure 1) and at the same time 25 stations were set in the eastern basin as part of a companion study being performed by the New York State University College at Buffalo, Great Lakes Laboratory. In addition several short cruises (6 stations per cruise) were made in the vicinity of the Bass Islands and extending 25 km into the central basin. Five vessels were used for the survey: R. V. Dambach (Great Lake Laboratory, NYSUC, Buffalo), Bluewater (USEPA, Grosse Ile Laboratory), R. V. Maple (Great Lakes Research Division, University of Michigan), Bio-Lab (F. T. Stone Laboratory, Ohio State University), and R. V. Hydra (Center for Lake Erie Area Research, Ohio State University).

The cruises were of two types: (1) general and (2) "DO". General cruises were constituted by those in which all parameters (i.e. physicochemical measurements, nutrient samples, methane production, plankton and benthos, etc.) were included, while "DO" cruises were more abbreviated in the data collected (i.e. direct physicochemical measurements).

Stations were plotted on Lake Survey Center (NOAA) charts of Lake Erie, nos. 3 and 39. The same charts were used to plot and contour data generated by these studies. The ships were positioned by compass and/or radar. When a thermocline was present in the central basin, the cruise track was accomplished by making north-south transects and usually required 5 days of running time. Stations in the western basin were usually processed over a period of 2-3 days in the following manner; stations 52, 54, 65, 67, 68 and 74 were all worked on the same day and the remainder in a clockwise direction around the basin. The stations were visited only during the daylight hours.

TABLE 1

LAKE ERIE NUTRIENT CONTROL STUDY

STATION LOCATIONS

STATION NO.	LATITUDE N.	LONGITUDE W.	BASIN	OSU	NY
1	42° 47' 24"	79° 12' 06"	E		X
2	42° 50' 36"	78° 57' 30"	E		X
3	42° 46' 54"	78° 57' 36"	E		X
4	42° 39' 06"	79° 08' 00"	E		X
5	42° 38' 30"	79° 16' 18"	E		X
6	42° 37' 54"	79° 24' 00"	E		X
7	42° 30' 48"	79° 28' 42"	E		X
8	42° 23' 54"	79° 32' 48"	E		X
9	42° 32' 18"	79° 37' 00"	E		X
10	42° 40' 48"	79° 41' 30"	E		X
11	42° 48' 12"	79° 33' 30"	E		X
12	42° 48' 12"	79° 47' 30"	E		X
13	42° 45' 12"	80° 00' 48"	E		X
14	42° 38' 30"	79° 56' 00"	E		X
15	42° 31' 00"	79° 53' 36"	E		X
16	42° 20' 00"	79° 45' 30"	E		X
17	42° 19' 48"	80° 00' 00"	E		X
18	42° 25' 18"	80° 04' 48"	E		X
19	42° 30' 54"	80° 09' 12"	E		X
20	42° 29' 06"	80° 18' 18"	E		X
21	42° 20' 18"	80° 12' 48"	E		X
22	42° 12'48"	80° 07' 42"	E		X
23	42° 02' 48"	80° 27' 06"	C	X	
24	42° 05' 54"	80° 29' 00"	C	X	
25	42° 14' 54"	80° 33' 36"	C	X	
26	42° 24' 00"	80° 38' 12"	C	X	
27	42° 32' 54"	80° 45' 30"	C	X	
28	42° 35' 30"	81° 01' 00"	C	X	
29	42° 36' 18"	81° 17' 54"	C	X	
30	42° 25' 48"	81° 12' 18"	C	X	

TABLE 1 (Con't.)

STATION NO.	LATITUDE N.	LONGITUDE W.	BASIN	OSU	NY
31	42° 15' 12"	81° 06' 24"	C	X	
32	42° 04' 54"	81° 00' 42"	C	X	
33	41° 55' 54"	80° 55' 00"	C	X	
34	41° 50' 00"	81° 08' 54"	C	X	
35	41° 45' 48"	81° 23' 00"	C	X	
36	41° 56' 06"	81° 28' 42"	C	X	
37	42° 06' 36"	81° 34' 30"	C	X	
38	42° 16' 54"	81° 40' 18"	C	X	
39	42° 21' 30"	81° 42' 24"	C	X	
40	42° 11' 30"	81° 55' 18"	C	X	
41	42° 08' 06"	82° 08' 24"	C	X	
42	41° 57' 54"	82° 02' 30"	C	X	
43	41° 47' 18"	81° 56' 42"	C	X	
44	41° 31' 48"	81° 42' 30"	C	X	
45	41° 36' 24"	81° 53' 48"	C	X	
46	41° 40' 54"	82° 05' 12"	C	X	
47	41° 50' 18"	82° 12' 48"	C	X	
48	42° 02' 48"	82° 21' 54"	C	X	
49	41° 55' 54"	82° 24' 30"	C	X	
50	41° 48' 48"	82° 30' 06"	C	X	
51	41° 38' 30"	82° 24' 12"	C	X	
52	41° 31' 54"	82° 27' 12"	C	X	
53	41° 25' 12"	82° 30' 12"	C	X	
54	41° 34' 00"	82° 38' 08"	C	X	
55	41° 44' 18"	82° 44' 00"	W	X	
56	41° 54' 42"	82° 50' 24"	W	X	
57	41° 49' 54"	83° 01' 06"	W	X	
58	41° 41' 06"	82° 56' 00"	W	X	
59	41° 43' 36"	83° 09' 00"	W	X	
60	41° 53' 30"	83° 11' 48"	W	X	
61	41° 56' 48"	83° 02' 42"	W	X	
62	42° 51' 00"	79° 54' 00"	E		X

TABLE 1 (Con't.)

STATION NO.	LATITUDE N.	LONGITUDE W.	BASIN	OSU	NY
63	42° 25' 00"	79° 48' 00"	E		X
64	42° 12' 00"	80° 03' 00"	E		X
65	41° 39' 00"	82° 44' 00"	W	X	
66	41° 58' 00"	82° 40' 00"	W	X	
67	41° 40' 00"	82° 52' 00"	W	X	
68	41° 45' 00"	82° 51' 00"	W	X	
69	41° 33' 00"	82° 55' 00"	W	X	
70	41° 46' 00"	83° 20' 00"	W	X	
71	42° 18' 00"	81° 22' 20"	C	X	
72	41° 57' 50"	81° 11' 00"	C	X	
73	41° 58' 40"	81° 45' 25"	C	X	
74	41° 40' 00"	82° 35' 00"	C	X	
75	41° 54' 00"	83° 19' 00"	W	X	
76	41° 36' 30"	83° 04' 00"	W	X	
77	41° 39' 30"	82° 49' 96"	W	X	

W = Western Basin
C = Central Basin
E = Eastern Basin

TABLE 2

STATIONS OCCUPIED IN THE CENTRAL AND WESTERN BASINS IN LAKE ERIE IN 1973

STA	DATE AND CRUISE								
	28 June–12 July (179–193)	17 July–23 July (198–204)	25 July–2 Aug (206–214)	7 Aug–11 Aug (219–223)	29 Aug–4 Sept (241–247)	19 Sept–29 Sept (262–272)	14 Oct–24 Oct (287–297)	7 Nov–15 Nov (311–319)	4 Dec (338)
	1	2	3	4	5	6	7	8	9
23	X	X	X	X	X	X	X	X	
24		X		X	X	X	X	X	
25		X	X	X	X	X	X	X	
26		X	X	X	X	X	X	X	
27		X	X	X	X	X	X	X	
28		X	X	X	X	X	X	X	
29		X	X	X	X	X	X	X	
30		X	X	X	X	X	X		
31		X	X	X	X	X	X		
32		X	X	X	X	X	X	X	
33		X	X	X	X	X	X	X	
34		X		X		X	X	X	
35		X		X	X	X	X	X	
36		X		X	X	X	X	X	
37		X		X	X	X	X		
38		X		X	X	X	X		
39		X		X	X	X	X		
40	X	X			X	X	X		
41	X	X		X	X	X	X		
42	X	X		X	X	X	X	X	
43	X	X		X	X	X	X	X	
44	X	X		X	X		X	X	
45	X	X	X	X	X	X	X	X	

TABLE 2 (Con't.)

STA	DATE AND CRUISE								
	28 June–12 July (179–193)	17 July–23 July (198–204)	25 July–2 Aug (206–214)	7 Aug–11 Aug (219–223)	29 Aug–4 Sept (241–247)	19 Sept–29 Sept (262–272)	14 Oct–24 Oct (287–297)	7 Nov–15 Nov (311–319)	4 Dec (338)
	1	2	3	4	5	6	7	8	9
46		X		X	X	X	X	X	
47	X	X		X	X	X			
48	X	X		X	X		X		
49	X	X		X	X		X		
50	X	X		X	X	X	X	X	
51	X	X		X	X	X	X		
52	X	X	X		X	X	X	X	X
53	X	X		X	X	X	X	X	
54	X	X	X		X	X		X	X
55			X		X		X	X	
56	X		X		X	X	X	X	
57	X		X		X	X	X	X	
58	X		X		X	X	X	X	
59	X		X		X	X	X	X	
60	X		X		X	X	X	X	
61	X		X		X	X	X	X	
65	X		X		X	X	X	X	X
66	X		X		X		X	X	
67	X		X		X	X	X	X	X
68	X		X		X	X	X	X	X
69	X		X		X	X		X	
70	X		X		X	X	X	X	
71		X		X	X		X		
72		X			X	X	X	X	
73		X		X	X			X	
74		X	X		X	X	X	X	X
75	X		X		X	X	X	X	

At each station the following meteorological conditions were recorded: weather, air temperature (by means of a mercury thermometer 10' above the water surface), wave direction and height, wind direction and speed (via an anemometer), and cloud cover. Transparency was measured by either a 20 cm diameter, black and white Secchi disk or a 30 cm, all white Secchi disk and marked line. Whenever possible an effort was made to read this parameter to the nearest 0.1 meter. Also, transmittance was measured on several cruises by a Martek Model 96 MS Transmissometer. This instrument gives a measure of turbidity by determining the percent transmission of a light beam through a known path length of water. The external optical parts were cleaned, and when necessary the instrument was zeroed and calibrated prior to use on each station.

A Martek Mark II, Model A monitoring system (an integrated multi-parameter instrument for simultaneous measurements) was utilized for measures of temperature, conductivity, dissolved oxygen, pH and depth. The sensor unit and an attached weight were lowered to the bottom and read at one meter intervals to the surface, thus giving a profile for each station. This profile was then used for the accurate location of the thermocline and the subsequent collection of water samples. The pH and conductivity meter were checked against reference solutions prior to each cruise and immediately after battery replacement. The temperature meter was checked daily against a mercury thermometer. Prior to daily use the dissolved oxygen sensor was visually examined and then placed into air saturated lake water for standardization. This reading was compared to the values obtained via a YSI instrument, a table of oxygen solubilities, and the Winkler method for dissolved oxygen. In addition, 2-4 water samples were drawn from known depths in the epilimnion and hypolimnion for comparison to the Martek Mark II readings. All meters were checked prior to use on each station and zeroed if necessary. The sensor portion of the instrument was stored in lake water.

Methods proposed for the 1974 field season are consistant with those described above. In addition to these techniques, we plan to use a standard Woods Hole-type bathythermograph (BT) during periods of stratification to obtain temperature profiles. The Canada Centre for Inland Waters has made available to the Ohio State University an electric bathythermograph (EBT) and accompanying water pump. We intend to experiment with this device and compare the results with those obtained with our present methods.

WATER QUALITY OF THE CENTRAL AND WESTERN BASINS OF LAKE ERIE

Objectives

The main objective of the Water Quality Analysis Segment, in close cooperation with the Field Operations Segment, is to analyze the water samples of Lake Erie taken by the Field Operations Segment and to participate in the data analysis process.

It is anticipated in the 1974 cruise season to determine the chemical and physical parameters of the Western and Central Lake Erie water at selected 50 to 52 locations (stations) at various depths. The chemical and physical parameters to be determined were selected such as to facilitate the evaluation of prevalent trophic conditions of the lake and to be useful for comparison with the previous and future conditions, and leading to the evaluation of the trend of trophic conditions in the lake and the effectiveness of the nutrient control program over extended periods of time.

The selected chemical and physical parameters to be determined in field and laboratory are as follows:

1. Depth
2. Temperature
3. Conductivity
4. Dissolved Oxygen
5. pH
6. Transparency
7. Alkalinity
8. Reactive soluble phosphorus
9. Total phosphorus
10. Nitrate plus nitrite nitrogen
11. Ammonia nitrogen
12. Particulate organic nitrogen
13. Particulate organic carbon
14. Chlorophyll a

The depth, temperature, conductivity, dissolved oxygen (polarographic method), pH, and transparency will be measured in situ, at depths of one meter intervals at every station, from board of the boat. It is expected to conduct seven cruises in 1974 (mid-June - November) visiting all 50 stations. Taking measurements at one meter depth intervals the total number of measurements, to determine the six parameters mentioned above, will amount to more than 15,000.

The alkalinity, reactive soluble phosphorus, total phosphorus, nitrate plus nitrite nitrogen, and ammonia nitrogen will be determined in the laboratory located on the boat. The analysis of the water samples for these five parameters will require more than 4,500 determinations.

The analysis for particulate nitrogen and carbon and chlorophyll a will be performed on samples shipped to the Chemical Engineering Department Laboratory at OSU in Columbus. To accomplish the task more than 2,700 determinations will be required.

Considering the economical factors only, it is obvious that the use of automated and instrumental analytical procedures is a necessity.

Methods

The detailed description of the methods used in determinations of the chemical and physical parameters of Lake Erie water already mentioned, are attached and presented in form of Xerox copies or transcripts from standard methods, publications, instruction and operating manuals. The modifications, alterations, and deviations used in our laboratories are explicitly stated at the corresponding place.

The in situ determinations of depth, temperature, conductivity, dissolved oxygen, pH, and transparency using "Martek" instruments were generally satisfactory. Minor troubles were caused by faulty performance of pressure transducers for depth measurement. The cause of trouble will be eliminated before the 1974 season. Concurrent with the determination of dissolved oxygen with "Martek" instruments the dissolved oxygen was also determined by the modified Winkler method on samples taken for analysis of nutrients, the performance was good. The Water Quality Analysis Segment and the Field Operations Segment are now equipped with two "Martek" TDC-DOA-HMS monitoring systems, one is in use and the other is a stand-by instrument ready for operation at any time. This arrangement is considered adequate and no changes are anticipated during 1974-1975 study.

The results of determination of alkalinity on unfiltered samples showed scatter, as earlier observed by CCIW researchers, and therefore the procedure will be changed. During 1974-1975 study the alkalinity will be determined on filtered samples.

The "manual" procedures used in determining the soluble reactive phosphorus, total phosphorus, nitrate nitrogen, nitrite nitrogen, and ammonia nitrogen showed the desired accuracy, sensitivity and reproducibility, but required too much manual work and therefore were time consuming.

Particularly the time factor turned out to have a disastrous effect on the separate determinations of reactive soluble phosphorus, nitrate nitrogen, nitrite nitrogen, and ammonia nitrogen due to unduly prolonged storage of water samples. In June, 1973, just after the funds for this study became available, it was expected to receive the least required automated equipment during the following month. It turned out differently. The automated equipment was received after more than three months from the date of issue of purchase orders. To do the analytical work manually during permissable time periods would require much more skilled manpower which was not available on short notice. The work was accomplished with the available personnel in much longer periods of time than anticipated which in turn affected considerably the accuracy of the results and the financial requirements for this study.

As already mentioned in the description of the methods used in this study, the samples immediately after filtration on Whatman GF/C filter were refrigerated and then shipped in ice boxes to OSU in Columbus where they were stored in refrigerators (at $\approx +4^{o}C$).

During prolonged storage of the water samples in a refrigerator, the nitrification microorganisms oxidize ammonia and nitrite to nitrate thus changing the ratio of the nitrogen compounds originally present in the sample. A very small quantity of nitrogen and phosphorus is also stored in the microorganisms. Some researchers recommend freezing the samples during storage but others mention that the freezing is effective only for short periods of storage. It is obvious that reliable results are only obtained when the determination of nitrate plus nitrite and ammonia is made at once after sampling. Considering the factors mentioned above, we anticipate changes in the analytical procedures for 1974-1975 accordingly.

Reasonably accurate results are obtained when the sum of nitrate nitrogen, nitrite nitrogen and ammonia nitrogen (total inorganically bound nitrogen) is considered. Therefore the total inorganically bound nitrogen will be reported in the final study report for the 1973 field season.

Determination of soluble reactive phosphorus is even more strongly affected by the storage of samples. Besides the microorganism activity already mentioned, also the hydrolysis of polyphosphates and some organic phosphates affects the results but to a much greater extent the access of oxygen to the samples, refrigerated or frozen, affects the results of reactive phosphorus determinations. In water samples taken from places where anoxic conditions prevail, phosphorus is frequently in solution as ferrous phosphate which on access of air oxygen is oxidized to insoluble ferric phosphate which forms a precipitate. Analyzing such a sample result showed a near zero phosphorus content but on dissolving the precipitate considerable phosphorus content was found. Considering the above information, there is no way to store the samples without changes of forms of phosphorus compounds in the sample and therefore only the immediate analysis of the filtered sample is the correct approach to the problem.

Upon the receipt of the Technicon one channel AutoAnalizer II (AAII) in the laboratory of the Chemical Engineering Department of OSU the power requirements were greatly reduced and working conditions and obtained results considerably improved. AAII performed very well, particularly after the modifications in methodology suggested by CCIW workers were implemented.

Still the problems associated with the sample shipment and storage were not eliminated and therefore considerable changes are anticipated for 1974-1975. As discussed above, the correct approach to the problem is to analize the water samples at once after sampling in a laboratory aboard the boat. To do this it is absolutely necessary to convert the one channel AAII to a three channel instrument by purchasing two photometer units and one two-pen recorder and to install the three channel AAII in the lab on the boat. The boat will then be properly outfitted for the 1974-1975 cruise season.

Results of the determination of total phosphorus obtained by the "manual" procedure are considered satisfactory and the results obtained by AAII are good. No changes are anticipated in the methodology for determination of total phosphorus by the AAII. The procedure used to hydrolize the various phosphorus compounds to orthophosphate was found too time consuming and consequently limited the number of samples to be treated per day. In 1974-1975 it is anticipated to use an autoclave located in the lab on the boat.

Autoclaving reduces considerably the time for hydrolysis and the number of manpower hours required to perform the operations involved.

The determination of particulate nitrogen and carbon is being performed only on samples taken during the last cruise in 1973. The delay was caused by receiving the Perkin-Elmer Elemental Analyzer late in the season. At present, the instrument performs well and shows an excellent reproducibility and accuracy. We are handicapped by the use of the old type of microbalance which is required for frequent calibrations of the Elemental Analyzer. The weighing on this old type balance is tedious and time consuming. Considering the economic factors only, it is practical to purchase a Perkin-Elmer Model AD-1 Precision Electronic Autobalance. The use of AD-1 eliminates the errors frequently observed in use of older types of microbalances, reduces the manpower hour requirement, and considering particularly the Biological Analysis Segment proposal to determine the biomass on Lake Erie water samples. The purchase of a AD-1 autobalance appears highly justified.

The sampling procedure, shipment and storage of samples for particulate carbon and nitrogen determination appears adequate and no changes are anticipated for the 1974-1975 season.

The procedures used in sampling, storage, shipment, and determination of chlorophyll a appears to be adequate and no changes are anticipated for the 1974-1975 study.

Short summary of changes anticipated for 1974-1975:

(1) The alkalinity will be determined on filtered samples.
(2) The determination of soluble reactive phosphorus, nitrate plus nitrite nitrogen, and ammonia nitrogen will be performed immediately and concurrently on filtered samples in the lab on the boat using the three channel AutoAnalyzer II.
(3) The total phosphorus will be determined on unfiltered samples collected during one day's cruise after hydrolysis in the autoclave in the lab on the boat. AAII will be used in the final step of the determination.

MANUAL PROCEDURES FOR THE CHEMICAL ANALYSIS OF LAKE ERIE WATER

DETERMINATION OF TEMPERATURE, CONDUCTIVITY, DISSOLVED OXYGEN AND pH

Determination of the depth at which determinations of the temperature, the conductivity, the dissolved oxygen and the pH *in situ* were made using "Martek II" Water Quality Analyzer from the board of the boat.

The "Martek II" Water Quality Analyzer utilizes: a diaphragm pressure transducer for depth measurement, a thermistor temperature transducer for temperature measurement, and platinized conductivity cell for electrical conductivity measurement, a polarographic (silver anode and gold cathode with a teflon membrane) sensor for dissolved oxygen measurement, and a glass electrode and reference electrode assembly for pH measurement. The solid-state readout module is located on the board of the boat and the instrument readings are recorded.

Concurrently with the dissolved oxygen determination with "Martek II", frequent dissolved oxygen determinations by the Winkler modified azide method were also performed ("Standard Methods for the Examination of Water and Wastewater", Thirteenth Edition, Prepared by APHA, AWWA and WPCF, 1971, p. 477-481).

DETERMINATION OF ALKALINITY

The determination of alkalinity was performed by the method given in "Standard Methods for the Examination of Water and Wastewater", Thirteenth Edition, Prepared by: APHA, AWWA, and WPCF, 1971, p. 52-56, with minor changes as indicated.

The determination was made at once on obtaining the samples, unfiltered, on the board of the boat. The samples were titrated with hydrochloric acid using phenolphthalein and mixed bromeresol green-methyl-red indicators. The hydrochloric acid was standardized with certified tris-hydroxymethylaminomethane standard solution, end point pH = 4.70. 100 ml of sample was used for each determination using 25 ml burett.

DETERMINATION OF SOLUBLE PHOSPHORUS

The determination of the soluble phosphate in water samples filtered on 0.45 u millipore filter, was made by the Molybdenum Blue-Hydrazine Sulfate Photometric Method adapted for water analysis from the 1964 Book of A.S.T.M. Standards, Part 32, Chemical Analysis of Metals, pp. 97-100. This method of analysis offers the least interference from iron and is suitable for determination of soluble phosphate at concentrations as low as 0.002 mg/l.

Summary of Method

Phosphorus reacts with ammonium molybdate to form a phosphomolybdenum complex. The latter is reduced by hydrazine sulfate to form the molybdenum blue complex which is suitable for photometric measurements. The water sample is treated with $HClO_4$ sodium sulfite and ammonium molybdate-hydrazine sulfate solution to develop a colored complex. The phosphate forms the heteropolyphosphomolybdate which is reduced by the hydrazine sulfate to form the strongly colored "molybdenum blue" complex of uncertain composition. Photometric measurement is made at 830 mμ.

Concentration Range

The recommended concentration ranges, in milligrams per 1000 ml, are 0.02 to 0.5 (for measurements at 830 mμ) in 1 cm cells and 0.002 to 0.05 mg/l in 10. cm cells.

Stability of Color

The heteropoly molybdenum blue color is stable for at least 2 hours after development of color.

Interferences

None of the elements usually present in waters except arsenic, which must be removed.

Apparatus

Glassware

Freedom of the glassware from phosphorus is of primary importance. The commonly used detergents contain phosphorus and should be avoided. Treatment of glassware with concentrated HCl followed by rinsing with distilled and double distilled demineralized water is one of the best means of cleansing glassware.

Photometers and Photometric Practice

Beckman Spectrophotometer, Model DU with 1, 5 and 10 cm cells was used for photometric measurements.

Reagents

Ammonium Molybdate Solution (20 g per liter)

Add 300 ml of H_2SO_4 concentrated to 500 ml of water (water means double distilled demineralized) and cool. Add 20 g of ammonium heptamolybdate ($(NH_2)_6$ Mo_7 O_{24} · $4H_2O$) and dilute to 1 liter.

Ammonium Molybdate-Hydrazine Sulfate Solution

Dilute 250 ml of the ammonium molybdate solution to 600 ml, add 100 ml of the $(NH_2)_2$ · H_2SO_4 solution and dilute to 1 liter. Prepare immediately before use.

Hydrazine Sulfate (1.5 g/l)

Dissolve 1.5 g hydrazine sulfate ($(NH_2)_2$ · H_2SO_4) in water and dilute to 1 liter.

Phosphorus, Standard Solution (1 ml = 0.05 mg P)

Dissolve 0.2292 g of sodium monohydrogen phoshpate (Na_2HPO_4) in about 200 ml of water. Add 100 ml $HClO_4$ (1:5) and dilute to 1 liter in a volumetric flask.

Sodium Sulfite Solution (100 g per liter)

Dissolve 100 g of sodium sulfite (Na_2SO_3) in water and dilute to 1 liter. Prepare as needed.

Preparation of Calibration Curve

Transfer 10.0 ml of the standard phosphorus solution (1 ml = 0.05 mg P) to a 100 ml volumetric flask. Add 1 ml $HClO_4$ and dilute to the mark. Use seven 100 ml volumetric flasks. Transfer to

flasks No. 2, 3, 4, 5, 6, and 7 - 1, 2, 4, 6, 8 and 10 ml of the diluted phosphorus standard solution (1 ml = 0.05 mg P) respectively and dilute to 25 ml with water. To flask 1 add only 25 ml of water. Add 1 ml of $HClO_4$ concentrated and 15 ml of Na_2SO_3 solution to each flask. Boil gently for 30 sec., and immediately add 50 ml of the freshly prepared ammonium molybdate-hydrazine sulfate solution. Heat on a steam bath at 85 - 90°C for 20 min. and then quickly cool to 20°C. Dilute to the mark and mix. (The color is stable for at least 2 hours.)

Photometry

Transfer a portion of the reference (blank, flask 1) solution to an absorption cell and adjust the photometer to the initial setting (100% transmission) using a light band centered at approximately 830 mu. While maintaining the photometric adjustment, take the photometric readings of the calibration solutions.

Calibration Curve

Plot the photometric readings of the calibration solutions (% transmission on log scale and concentration on arithmetic scale) against milligrams of phosphorus per 100 ml of solution (final volume).

For 5 cm and 10 cm cells use smaller volumes of the diluted standard phosphorus solution (1 ml = 0.005 mg P) in the low phosphorus concentration range or use lower concentration standard phosphorus solution. Plot separate calibration curves for 5 and 10 cm cells.

Procedure

Transfer 25.0 ml of water sample to a 100 ml volumetric flask (25 ml of distilled water for blank). Add to each flask 1 ml $HClO_4$ and 15 ml of Na_2SO_3 solution. Boil gently for 30 sec., and immediately add 50 ml of the freshly prepared ammonium molybdate-hydrazine sulfate solution. Heat on a steam bath at 85 - 90°C for 20 min. and then quickly cool to 20°C. Dilute to the mark and mix. Proceed as directed in preparation of calibration curve under photometry using blank solution as reference. Read from plot the P content. When the same reagents are used the calibration curve is good for long periods of time provided the same photometer and cells at the same photometer dial settings are used.

DETERMINATION OF TOTAL PHOSPHORUS

The Persulfate Acid Digestion Method as evaluated by D. E. Sanning, Water & Sewage Works 114(4):131-133, 1967, was used for total phosphorus determination in unfiltered water samples.

Procedure

A. To 100 ml of a water sample in a 250 ml erlenmeyer flask, add 1 ml of concentrated sulfuric acid followed by 8 g of $K_2S_2O_8$ and some glass beads.

B. Boil gently on a hot plate for 1 1/2 hours. If necessary, add distilled water to maintain the volume between 10 to 20 ml.

C. Remove from heat, cool somewhat and transfer to a 50 ml volumetric flask using a funnel with small quantity of Pyrex glass wool in the neck to retain the glass beads and facilitate the transfer process. Rinse the erlenmeyer flask, beads, and the funnel with distilled water and collect the washings in the volumetric flask. Cool the solution to room temperature and dilute to the mark. Note that the volume in the volumetric flask is only 50.0 ml but not 100.0 ml as the original sample.

If the solution after digestion is turbid it should be filtered on Whatman No. 42 paper or membrane filter (0.45 μ). Lake Erie water sample, until now, did not show any turbidity.

D. Transfer a 25.0 ml aliquot to a 100 ml volumetric flask and proceed as directed in the procedure for soluble phosphorus determination only omitting the addition of 1 ml $HClO_4$. A blank is carried throughout the procedure and used as a reference solution in the photometric procedure.

Calibration curves for this procedure should be prepared carrying the standard phosphate solutions through the entire procedure.

DETERMINATION OF NITROGEN

The determination of nitrite nitrogen was made on filtered samples, 0.45 μ millipore filters, according to "Standard Methods for the Examination of Water and Wastewater," Thirteenth Edition, APHA, AWWA, and WPCF (1971) as follows:

NITROGEN (NITRITE)

Nitrite, an intermediate stage in the nitrogen cycle, may occur in water as a result of the biological decomposition of proteinaceous materials. When correlated with the concentration of other nitrogen forms, trace amounts of nitrite may indicate organic pollution. Nitrite may also be produced in water treatment plants or in the distribution system through the action of bacteria or other organisms on ammonia nitrogen fed at elevated temperatures in the combined residual chlorination of water. Nitrite can likewise enter a water supply through its use as a corrosion inhibitor in industrial process water. The nitrite concentration of a drinking water rarely exceeds 0.1 mg/l.

1. General Discussion

a. Principle: The nitrite concentration is determined through the formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by the coupling of diazotized sulfanilic acid with naphthylamine hydrochloride. The diazotization method is suitable for the visual determination of nitrite nitrogen in the range 1-25 μg/l N. Photometric measurements can be performed in the range 5-50 μg/l if a 5-cm light path and a green color filter are available. The color system obeys Beer's law up to 180 μg/l N or 600 μg/l NO_2, with a 1-cm light path at 520 mμ.

b. Interference: Chemical incompatibility makes it unlikely that nitrite, free available chlorine, and nitrogen trichloride will coexist in a sample. Nitrogen trichloride imparts a false red color when the normal order of reagent addition is followed. Although this effect may be minimized somewhat by adding the naphthylamine hydrochloride reagent first and then the sulfanilic acid reagent, an orange color may still result when a substantial nitrogen trichloride concentration is present. A check for a free available chlorine and nitrogen trichloride residual is advisable under such circumstances. The following ions interfere due to precipitation under the conditions of the test and therefore should be absent: antimonous, auric, bismuth, ferric, lead, mercurous, silver, chloroplatinate and metavanadate. Cupric ion may cause low results by catalyzing the decomposition of the diazonium salt. Colored ions which alter the color system should likewise be absent.

When small amounts of suspended solids seriously impair nitrite recovery, a sample may be passed through a membrane filter (0.45 μ pore size) to achieve the necessary clarification before color development is undertaken.

c. Minimum detectable concentration: In the absence of interference, the minimum nitrite nitrogen concentration detectable in a 50-ml nessler tube is 1 μg/l.

d. Storage of Sample: The determination should be made promptly on fresh samples to prevent bacterial conversion of the nitrite to nitrate or ammonia.

In no case should acid preservation be used for samples to be analyzed for nitrite. Short-term preservation for 1 to 2 days is possible by deep-freezing (-20°C), or by the addition of 40 mg mercuric ion (as $HgCl_2$) with storage at 4°C.

2. Apparatus

COLORIMETRIC EQUIPMENT - One of the following is required:

a. Spectrophotometer, for use at 520 mμ, providing a light path of 1 cm or longer.

b. Filter photometer, providing a light path of 1 cm or longer and equipped with a green color filter having maximum transmittance near 520 mμ.

c. Nessler tubes, matched, 50-ml, tall form.

3. Reagents

Prepare all reagents from chemicals which are white in color.

a. Nitrite-free water: Prepare nitrite-free water by either of the following methods -

1) Add to 1 liter distilled water one small crystal each of potassium permanganate and an alkali such as barium or calcium hydroxide. Redistill in an all-pyrex apparatus, discarding the initial 50 ml of distillate. Collect that fraction of the distillate which is free of permanganate.

2) Add 1 ml conc H_2SO_4 and 0.2 ml manganese sulfate solution (36.4 g $MnSO_4 \cdot H_2O$ per 100 ml aqueous solution) to each 1 liter distilled water, and make pink with 1 to 3 ml potassium permanganate solution (400 mg $KMnO_4$ per liter aqueous solution). After 15 min, decolorize with ammonium oxalate solution [900 mg $(NH_4)_2-C_2O_4 \cdot H_2O$ per liter aqueous solution].

b. EDTA solution: Dissolve 500 mg disodium ethylenediamine tetraacetate dihydrate, also called (ethylenedinitrilo) tetraacetic acid sodium salt, in nitrite-free water and dilute to 100 ml.

c. Sulfanilic acid reagent: Completely dissolve 600 mg sulfanilic acid in 70 ml hot distilled water, cool, add 20 ml conc HCl, dilute to 100 ml with distilled water, and mix thoroughly.

d. Naphthylamine hydrochloride reagent: Dissolve 600 mg of 1-naphthylamine hydrochloride in distilled water to which 1.0 ml conc HCl has been added. Dilute to 100 ml with distilled water and mix thoroughly. The reagent becomes discolored and a precipitate may form after 1 week, but it is still usable. Discard when sensitivity or reproducibility is affected. Store in a refrigerator to prolong the useful life of the reagent. Filter before using. (CAUTION: Handle this chemical with extreme care. Never use a mouth pipet for dispensing this reagent but rely on an automatic, dropping or safety pipet to measure the necessary volumes. Avoid inhalation or exposure to the skin.)

e. Sodium acetate buffer solution, 2M: Dissolve 16.4 g $NaC_2H_3O_2$ or 27.2 g $NaC_2H_3O_2 \cdot 3H_2O$ in nitrite-free water and dilute to 100 ml. Filter if necessary.

f. Stock nitrite solution: The reagent grade of sodium nitrite available commercially assays at less than 99%. Since nitrite is readily oxidized in the presence of moisture, fresh bottles of reagent are desirable for the preparation of the stock solution. The preferred approach is to determine the sodium nitrite content immediately before preparation of the stock solution and to keep bottles tightly stoppered against the free access of air when not in use. The sodium nitrite content may be determined by adding an excess of standard potassium permanganate solution, discharging the permanganate color with a standard reductant such as sodium oxalate or ferrous ammonium sulfate solution, and finally back-titrating with standard permanganate solution.

1) PREPARATION OF STOCK SOLUTION - Dissolve 1.232 g sodium nitrite, $NaNO_2$, in nitrite-free water and dilute to 1,000 ml; 1.00 ml = 250 µg N. Preserve with 1 ml chloroform.

2) STANDARDIZATION OF STOCK SOLUTION - Pipet, in order, 50.00 ml standard 0.05N$KMnO_4$, 5 ml conc sulfuric acid, and 50.00 ml stock nitrite solution into a glass-stoppered flask or bottle. Submerge the tip of the nitrite pipet well below the surface of the permanganate acid solution. Shake the stoppered flask gently. Warm the flask contents to 70-80°C on a hot plate. Discharge the permanganate color by adding sufficient standard 0.05N sodium oxalate (3.350 g $Na_2C_2O_4$ primary standard grade, per 1,000 ml solution) in 10.00-ml portions. Titrate the excess sodium oxalate with standard 0.05N $KMnO_4$ to the faint pink end point. Carry a nitrite-free water blank through the entire procedure and make the necessary corrections in the final calculation.

When standard 0.05N ferrous ammonium sulfate solution is substituted for sodium oxalate, omit the heating to 70-80°C and instead extend the reaction period between the permanganate and ferrous ions to 5 min before the final $KMnO_4$ titration is undertaken. This standard 0.05N ferrous solution contains 19.607 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 20 ml conc H_2SO_4 per 1,000 ml solution.

Calculate the nitrite nitrogen content of the stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where A = mg/ml nitrite nitrogen in stock nitrite solution, B = total ml standard $KMnO_4$ used, C = normality of standard $KMnO_4$, D = total ml standard reductant added, E = normality of standard reductant, and F = ml stock $NaNO_2$ solution taken for titration. Each 1.00 ml 0.05N$KMnO_4$ consumed by the nitrite corresponds to 1,725 µg $NaNO_2$, or 350 µg N.

3) TITRATION OF HIGH-NITRITE SAMPLES - The standardization procedure can also be used for the titration of water samples containing nitrite nitrogen concentrations in excess of 2 mg/l. In such a case, the water sample volume is substituted for F in the previous equation and the factor 7 is increased to 7,000 for the purpose of calculating the nitrite nitrogen value in terms of mg/l.

g. Intermediate nitrite solution: Calculate the volume, G, of stock nitrite solution required for the intermediate nitrite solution by means of the following equation: $G = 12.5/A$. Dilute to 250 ml the calculated volume, G (Approximately 50 ml), of the stock nitrite solution with nitrite-free water; 1.00 ml = 50.0 µgN. Prepare daily.

h. Standard nitrite solution: Dilute 10.00 ml intermediate nitrite solution to 1,000 ml with nitrite-free water; 1.00 ml = 0.500 µg N. Prepare daily.

i. Aluminum hydroxide suspension: Prepare as directed in Nitrogen (Nitrate).

4. Procedure

a. Removal of turbidity and color: If the sample contains suspended solids and color, add 2 ml aluminum hydroxide suspension to 100 ml sample, stir thoroughly, allow to stand for a few minutes, and filter, discarding the first portion of the filtrate.

b. Color development: To 50.0 ml clear sample which has been neutralized to pH 7, or to an aliquot diluted to 50.0 ml, add 1.0 ml EDTA solution and 1.0 ml sulfanilic acid reagent. Mix thoroughly. At this point, the pH of the solution should be about 1.4. After the reaction has proceeded for 3 to 10 min, add 1.0 ml naphthylamine hydrochloride reagent and 1.0 ml sodium acetate buffer solution; mix well. At this point, the pH of the solution should be 2.0 to 2.5. Measure the reddish purple color after 10 to 30 min.

c. Photometric measurement: Measure the absorbance at or near 520 mµ against a reagent blank and run parallel checks frequently against known nitrite standards, preferably in the nitrogen range of the samples. Redetermine complete calibration curves following the preparation of new reagents.

d. Color standards for visual comparison: Prepare a suitably spaced series of visual color standards in nessler tubes by adding the following volumes of standard sodium nitrite solution and diluting to 50 ml with nitrite-free water: 0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0 and 2.5 ml.

5. Calculation

$$\text{mg/l nitriteN} = \frac{\mu\text{g nitrite N}}{\text{ml sample}}$$

$$\text{mg/l } NO_2 = \text{mg/l nitrite N} \times 3.29$$

6. Precision and Accuracy

A synthetic unknown sample containing 250 µg/l nitrite N, 108 mg/l Ca, 82 mg/l Mg, 3.1 mg/l K, 19.9 mg/l Na, 241 mg/l chloride, 1.1 mg/l nitrate N, 259 mg/l sulfate, and 42.5 mg/l total alkalinity (contributed by $NaHCO_3$) was determined by the diazotization method, with a relative standard deviation of 21.4% and a relative error of 12.0% in 49 laboratories.

7. References

1. AMERICAN CHEMICAL SOCIETY. 1961. Reagent Chemicals - American Chemical Society Specifications, 1960. ACS, Washington, D. C., p. 474.

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BARNES, H. & A. R. FOLKARD. 1951. The determination of nitrites. Analyst 76:599.

BOLTZ D. F., Ed. 1958. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, pp. 124-132.

9. Note

Demineralized double distilled water as supplied by the Reagent Laboratory of The Ohio State University was used, no nitrate was found in it. The water samples were stored in refrigerator for prolonged time so the results were used mainly for nitrate correction and for total nitrate + nitrite values.

The determination of nitrate nitrogen was made on filtered samples, 0.45 μ millipore filter discs, according to "STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTE-WATER," Thirteenth Edition, APHA, AWWA, and WPCF (1971), as follows:

NITROGEN (NITRATE)

Cadmium Reduction Method (TENTATIVE)

1. General Discussion

Nitrate is reduced almost quantitatively to nitrite when a sample is run through a column containing amalgamated cadmium fillings. The nitrite thus produced is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine to form a highly colored azo dye which is measured colorimetrically. A correction may be made for any nitrite initially present in the sample.

2. Apparatus

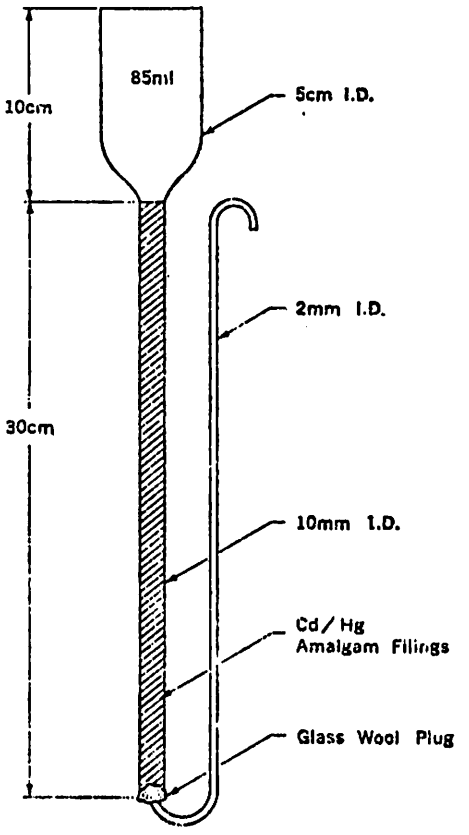
a. Reduction columns (see illustration) constructed from three pieces of glass tubing joined end to end: 10 cm of 5-cm I.D. tubing is joined to 30 cm of tubing, 10-mm I.D., which in turn is joined to 35 cm of 2-mm I.D. tubing. The last tube is bent just below the joint into a U, so that it runs up parallel to the 10-mm diameter tube; its upper end is bent over to form an inverted U-siphon. This last bend should be just level with the top of the 10-mm diameter tube. Using this arrangement, liquid placed in the top reservoir should flow out of the system and stop when the level of the liquid just covers the metal filings (see below). Place a mark on the upper wide portion of the column to indicate the height of an additional 80 ml of liquid.

b. Colorimetric equipment:

1) SPECTROPHOTOMETER, Beckman DU, for use at 543 mμ, providing a light path of 1 cm or longer.

3. Reagents

a. Distilled water: Demineralized double distilled water should be of the highest purity, preferably prepared by mixed-bed ion-exchange deionization of ordinary distilled water.



REDUCTION COLUMN

b. Ammonium chloride solution, conc: Dissolve 100 g NH_4Cl in 500 ml distilled water and store in a glass or plastic bottle.

c. Dilute ammonium chloride solution: Dilute 50 ml conc ammonium chloride solution to 2,000 ml with distilled water. Store in a glass or plastic bottle.

d. Amalgamated cadmium filings: File sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction which passes a sieve with 2-mm openings and is retained on a sieve with 0.5-mm openings. Stir about 300 g of filings with 300 ml mercuric chloride solution (1 g $HgCl_2$/100 ml) for 3 min. (This amount suffices for six reduction columns.) Allow the metal particles to settle and decant off the liquid. Wash the amalgamated filings several times with distilled water, then briefly in 1 + 99 nitric acid. Wash several times with 1 + 99 HCl. Wash copiously with distilled water until no nitrite

can be detected in the supernatant fluid. Store the filings in the dark under dilute ammonium chloride solution.

e. Sulfanilamide reagent: Dissolve 5 g sulfanilamide in a mixture of 50 ml conc HCl (sp gr 1.18) and about 300 ml distilled water. Dilute to 500 ml with distilled water. The solution is stable for many months.

f. N-(1-naphthyl)-ethylenediamine dihydrochloride solution: Dissolve 500 mg dihydrochloride in 500 ml distilled water. Store the solution in a dark bottle. Renew the solution once a month, or immediately when it develops a strong brown coloration.

g. Stock nitrate solution: Dissolve 721.8 mg anhydrous KNO_3 in distilled water and dilute to 1,000 ml. This solution contains 100 mg/l N.

h. Standard nitrate solution: Dilute 4.00 ml stock standard nitrate to 2,000 ml with distilled water and use immediately; 1.0 ml = 0.20 μg NO_3-N.

4. Procedure

a. Preparation of reduction column: Pack a plug of glass wool in the bottom of a reduction column and fill the column with distilled water. Pour in sufficient amalgamated cadmium filings to produce a column 30 cm in length. Use the column size specified, because columns of lesser diameter and length give erratic results and show rapid deterioration. Wash the column thoroughly with dilute ammonium chloride solution. Use a flow rate no greater than 8 ml per min. If the rate is too fast, slow it by constricting the end of the outlet siphon or by packing more glass wool at the base of the column. Flow rates less than 5 ml per min unnecessarily increase the time for an analysis and may give low results. When not in use, cover the metal in the column with dilute ammonium chloride solution.

b. Treatment of sample:

1) TURBIDITY REMOVAL - If turbidity or suspended solids are present, clarify the sample by membrane filtration.

2) pH ADJUSTMENT - If the pH of the sample is above 9, adjust to between 8 and 9, using a pH meter and dilute HCl.

3) REDUCTION OF NITRATE - Place 80 to 90 ml sample in a 125-ml erlenmeyer flask and add 2.0 ml conc ammonium chloride solution. Mix, and pour the sample from the flask onto the column

until the marked level in the top part of the column is reached. Place a 50-ml graduated cylinder under the outlet to collect the effluent and discard any sample left in the erlenmeyer flask. Shake the flask as dry as possible and retain it for collecting the main portion of the effluent.

Allow between 25 and 30 ml effluent to collect in the cylinder and then replace it with the original erlenmeyer flask which contained the sample. Discard the contents of the cylinder. (The passage of 25-30 ml of solution removes the ammonium chloride solution or a previous sample from the voids in the reduction column. The volume flushed through is not critical provided that it exceeds 25 ml, but sufficient sample should be left in the column so that 50 ml more can be reduced. The flushing effluent should therefore not exceed about 30 ml. A maximum of about eight columns can be handled conveniently at one time. The operator should experiment to find a suitable short delay between adding the samples to successive columns so that there will be time to reject the flushing liquid from one column and replace the cylinder by the erlenmeyer flask before too much flushing liquid has escaped from the next column in line. In the case where a sample having a very low concentration of nitrate is followed by a sample with a high nitrate concentration, or vice versa, a full 30-ml volume should be used to flush the column.)

Allow the remainder of the sample to collect in the flask. When the flow of sample from the column has ceased, pour exactly 50 ml from the flask into the 50-ml measuring cylinder. Drain and discard the remaining effluent from the erlenmeyer flask. Shake the flask as dry as possible and then pour back into it the 50 ml of reduced sample from the measuring cylinder.

There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour 50 ml dilute ammonium chloride solution onto the top and allow it to pass through the system. Store the cadmium filings in this ammonium chloride solution and never allow them to go dry. If there are indications that the columns are becoming inactivated, as shown by a significant decrease in the color intensity produced per μg NO_3-N in the standards, empty the columns, wash the cadmium filings briefly with 1 + 99 HNO_3, and copiously, with distilled water, air-dry at about 60 C, resieve and reamalgamate. Well-prepared columns of the correct size should be stable for many weeks before such reactivation becomes necessary.

4) COLOR DEVELOPMENT AND MEASUREMENT - As soon as possible, and in any case no longer than 15 min after reduction, add to the sample 1.0 ml sulfanilamide solution from an automatic pipet. Allow the reagent to react for a period longer than 2 min but not exceeding 8 min. Add 1.0 ml 1-naphthyl-ethylenediamine solulion and mix immediately. Between 10 min and 2 hr afterward, measure the absorbance of the solution against a distilled-water reagent blank, using a wavelength of 543 mμ. As a guide, use the following recommended light paths for the indicated NO_3-N concentration ranges:

Light Path Length (cm)	NO_3-N Concentration μg/l
1	2-20
5	2-6
10	less than 2

c. Standards: Use the standards both to obtain a calibration curve and to check the efficiency of the reduction columns.

Add about 110 ml dilute standard nitrate solution to clean dry 125-ml erlenmeyer flasks, and carry out the determination exactly as described for the samples, except that the volume of flushing liquid collected from each column should be 50 ml. Measure the absorbance in a cell which provides a suitable light path. Perform the determination initially in triplicate for each column and correct the mean of the three absorbances thus obtained by the absorbance of a distilled water reagent blank. Subsequently check each column once a day.

1) DETERMINATION OF F FACTOR - Determine the factor F relating absorbance in a 1-cm cell to NO_3-N concentration (mg/l) from the following equation:

$$F = \frac{0.2}{A}$$

where A is the absorbance at 543 mμ.

Reactivate the column when the value of F consistently increases beyond 0.33.

5. Calculation

$$\text{mg } NO_3\text{-N/l} = (A_s - A_b) \times \frac{F}{L} - C$$

$$\text{mg } NO_3\text{/l} = \text{mg } NO_3\text{-N/l} \times 4.43$$

where A_s = absorbance of sample
A_b = absorbance of reagent blank
F is defined in paragraph 4c(1) above.
L - light path length, cm
C = concentration of NO_2-N (separately determined).

DETERMINATION OF AMMONIA NITROGEN

The determination of ammonia nitrogen was performed in our laboratory manually by the PHENATE METHOD. A procedure was adopted for our laboratory utilizing the results of research done by:

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7. FWPCA Methods of Chem. Anal. of Water & Wastewater, November, 1966, p. 137.
8. Technicon, Auto Analyzer II, Industrial Method No. 154-71W (Tentative) Date released Feb. 1973.

and the method:

9. Standard Methods for the Examination of Water and Wastewater, Thirteenth Edition Prepared by A.P.H.A., A.W.W.A., and W.P.C.F., 1971, pp. 232. 132c phenate method (tentative).

1. General Discussion

a. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite and phenol catalized by the sodium nitroprusside.

b. Interference: None expected in Lake Erie waters.

2. Apparatus

COLORIMETER - Beckman DU.

3. Reagents

a. Ammonia-free water: Demineralized double distilled water supplied by the reagent laboratory of The Ohio State University was satisfactory.

b. EDTA (6%) - Dissolve 6.0 g of EDTA in water and dilute to 100 ml.

c. Phenol Reagent - Add 45 ml of 20% NaOH - soln. to 20.75 g of reagent grade phenol; dilute to 250 ml. Prepare fresh daily.

d. Sodium Hypochlorite Solution - Dilute 200 ml of "Clorox" to 1000 ml.

e. Sodium Nitroprusside reagent - Dissolve 0.50 g of sodium nitroferricyanide in water and dilute to 1000 ml.

f. Stock Ammonium Solution - Dissolve 3.819 g of anhydrous ammonium chloride in water and dilute to 1000 ml.

g. Standard Solution A - Dilute 10.00 ml of stock ammonium solution to 1000. ml.

h. Standard Solution B - Dilute 25.00 ml of Standard Solution A to 1000 ml:

ml of Std. Sol. B. in 50 ml vol. flask	2.00	4.00	6.00	10.00	14.00	20.00
ppB N_{NH4}	10	20	30	50	70	100

4. Procedure

1. Pipet 25.0 ml of sample, filtered on 0.45 μ millipore, into 50 ml vol. flask.

2. Pipet appropriate quantity of std. soln. B into 50 ml volumetric flask.

3. Pipet 1.0 ml of 6% EDTA solution into each flask and mix thoroughly.

4. Taking each flask separately, add 10.0 ml of Phenol reagent with a fast-running pipet while mixing. Mix briefly (2-3 seconds)

5. Immediately add 5.0 ml of Sodium Hypochlorite solution by forcing from pipet with "Propipet". Mix thoroughly, for about 10 seconds.

6. Add 5.0 ml of nitroprusside reagent at a normal rate by pipet with mixing.

7. Fill volumetric flask to 50.0 ml mark with demineralized double distilled water and invert 10 times to mix thoroughly.

8. Place in dark for 60 minutes

9. Repeat steps 4 to 8 with each sample

10. Take absorbance readings after one hour on the Beckman DU with 1.00 cm cells at λ = 630 mm and slit width - 0.5 mm. Read all samples versus a distilled water blank.

5. Washing Instructions

After analysis has been completed, rinse each flask with distilled water four times, fill with double distilled demineralized water and let stand until next analysis.

6. Detection limits: $\approx$0.003 ppm of ammonia nitrogen.

DETERMINATION OF PARTICULATE CARBON AND NITROGEN

Determination of particulate C and N was made by using Perkin-Elmer Model 240 Elemental Analyzer.

Water samples were filtered on Whatman glass fiber paper FG/C 4.25 cm diameter (ignited for one hour at 500° C) immediately after sampling. The filters were washed with I N HCl and distilled water. The filters were placed in open plastic petri dishes and dryed in a vacuum drying cabinet on the board of the boat. After dry, the dishes were closed with the tightly fitting lid and shipped to the laboratory where they were stored in a desiccator until analyzed. The glass fiber papers were handled according to procedures used in microchemical analysis. The determination of C and N were performed according to the procedures suggested by Perkin-Elmer Company Model 240 Elemental Analyzer manual. The quartz ladle was modified according to the suggestions by Dr. Robert F. Culmo, Perkin-Elmer Corporation, and by research staff members of CCIW.

Pigment Analysis

Water samples for pigment analysis were taken one meter below the surface, one meter above the thermocline, one meter below the thermocline and one meter above the bottom. If no thermocline existed, samples were taken from one meter below the surface, mid-depth and one meter above the bottom. Samples of water were obtained from each depth with a six liter, clear, plastic, non-toxic Van Dorn sampler.

1. Two liters of water from each depth were filtered using Millipore filter equipment fitted with 4.25 cm GF/C glass filter paper. Filtering was carried out at a vacuum of 1/3 atm. Approximately five drops of a 1% suspension of $MgCO_3$ were added when 100 ml of sample remained to be filtered.

2. When filtration was completed, each glass filter paper was placed in a 50 X 12 mm tight lid petri dish which was then wrapped in aluminum foil and immediately frozen.

3. After storage, the glass filter pads were placed in a Thomas Tissue Grinder equipped with a teflon pestal. Four ml of 90% acetone (reagent grade acetone was mixed with double distilled water have a pH greater than 7) were added to the tissue grinder, and the samples were ground for 3-4 minutes.

4. Contents from the tissue grinder were transferred to 15 ml centrifuge tubes, and 90% acetone was added to make a total volume of 12 ml. Samples were then refrigerated in darkness for 4-6 hours to allow for complete extraction.

5. Following extraction, samples were then centrifuged for 10 minutes at 4-5000g using a M.S.E. FT 4 swing-out centrifuge.

6. The supernatant liquid was decanted into 1 cm path length spectrophotometer cells, and extinctions of the extract were measured at 7300, 6630, 6450, and 6300A. Spec. Specifications.

7. Determination of phaeo-pigments was made by adding 2 drops of I N HCl to the extracted solution, and extinctions were again measured at 7300 and 6630 A.

Concentrations of pigments were calculated using SCOR/UNESCO formulas for chlorophylls a, b, c and phaeo-pigments.

References

Determination of photosynthetic pigments. 1966. Report of SCOR-UNESCO Working Group 17. Monographs on Oceanographic Methodology. Publ. UNESCO.

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Strickland, J. D. H. and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fisheries Research Board of Canada. Bulletin 167. Ottawa. p. 185-206.

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AUTOMATED PROCEDURES FOR THE CHEMICAL ANALYSIS OF LAKE ERIE WATER

AUTOMATED ANALYTICAL PROCEDURES

Determination of reactive (soluble) phosphate ($\underline{P}_s$), total phosphorus ($\underline{P}_T$), nitrate plus nitrite nitrogen ($N_{NO_3+NO_2}$), and ammonia nitrogen (N_{NH_3}) in Lake Erie water samples, beginning September, 1973, were made by use of "Technicon Auto Analyzer II" (AAII).

The water samples for determination of $\underline{P}_s$, $^{N}NO_3 + NO_2$, and N_{NH_3}, were filtered immediately (on the board of the boat) on millipore 0.45u membranes and the filtered samples, packed in ice in icechests, were shipped to the laboratory and stored in refrigerator. Next season the AAII will be on the board of the boat and the samples will be analyzed immediately after taking. Unfiltered samples were used for the determination of $\underline{P}_T$.

1. The automated procedure for the determination of reactive (soluble) orthophosphate ($\underline{P}_s$) in water depends on the formation of phosphomolybdenum blue complex which is read colorimetrically. Ortho phosphate reacts with ammonium molybdate to form a phosphomolybdenum complex. The latter is reduced by stannous chloride to form the molybdenum blue complex.

Xerox copy of the "Technicon Industrial Method No. 155-71W/ tentative" followed by a modified procedure used in our laboratory is attached. Modification of the procedure was done by Canadian Centre for Inland Waters, Burlington, Ontario (CCIW) researchers and kindly made available to us for use in our laboratory. The modified procedure is highly sensitive and accurate.

2. The total phosphorus was determined similarly as the $\underline{P}_s$. The unfiltered samples were hydrolized according to the procedure given by "Standard Methods for the Examination of Water and Wastewater", Thirteenth Edition, APHA, AWWA, and WPCF (1971) as follows:

Persulfate Digestion Method

a. Apparatus
   1) Hot plate: A 30x50 cm heating surface is adequate.
   2) Autoclave: An autoclave or pressure cooker of developing 15-20 psi may be used in place of a hot plate.

b. Reagents

1) Phenouphthalein indicator solution.

2) Sulfuric acid solution: Carefully add 300 ml conc H_2SO_4 to approximately 600 ml distilled water and then dilute to 1 liter with distilled.

3) Potassium persulfate solution: Dissolve 5g $K_2S_2O_8$ in 100 ml distilled water. Prepare daily.

4) Sodium hydroxide, 1 N.

c. Procedure

1) Take 100 ml or a suitable aliguot of thoroughly mixed sample. To each 100-ml sample or aliguot diluted to 100 ml, add 1 drop (0.05 ml) phenolphtalein indicator solution. If a red color develops, add sulfuric acid solution dropwise to just discharge the color. Then add 1 ml sulfuric acid solution and 15 ml potassium persulfate solution.

2) Boil gently for at least 90 min., adding distilled water to keep the volume between 25 and 50 ml. Alternatively heat for 30 min. in an autoclave or pressure cooker at 15-20 psi. Cool, add 1 drop (0.05 ml) phenolphthalein indicator solution, and neutralize to a faint pink color with sodium hydroxide solution. Restore the volume to 100 ml with distilled water. Determine the phosphorus present by AAII. Carry the standards through the persulfate digestion procedure.

3. This automated procedure for determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitratrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye which is read photometrically.

Xerox copy of the "Technicon Industrial Method No. 100-70W/ Preliminary" followed by a slightly altered procedure according to CCIW is attached.

4. The automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue colored compound believed to be closely related to indophenol occurs

when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate and sodium citrate is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium and a solution of sodium nitroprusside to catalize the reaction.

Xerox copy of the "Technicon Industrial Method No. 154-71W/ Tentative" followed by a modified procedure, according to CCIW used in our laboratory is attached.

Technicon AutoAnalyzer II

INDUSTRIAL METHOD No. 155-71W/TENTATIVE DATE RELEASED: JAN. 1973

ORTHO PHOSPHATE IN WATER AND SEAWATER

RANGE: 0-4 µgat P/l
0-124 µg P/l (ppb)

GENERAL DESCRIPTION

The automated procedure for the determination of ortho phosphate in seawater depends on the formation of a phosphomolybdenum blue complex which is read colorimetrically at 880 nm.[1]

A single reagent solution is used consisting of an acidified solution of ammonium molybdate containing ascorbic acid and a small amount of antimony.

Interference from copper and iron is insignificant. Silicon at a level of 100 µgat Si/l causes an interference equivalent to approximately 0.04 µgat P/l.

Although arsenate produces a similar color to phosphate, sea water rarely contains arsenate in concentrations high enough to interfere. The salt error has been found to be less than 1%.

PERFORMANCE AT 30 SAMPLES PER HOUR USING AQUEOUS STANDARDS

Sensitivity at 4.0 µgat P/l (124 µg P/l)	0.15 absorbance units
Coefficient of Variation at 2 µgat P/l (62 µg P/l)	1.98%
Detection Limit	0.08 µgat P/l (24 µg P/l)

REAGENTS

SULFURIC ACID, 4.9N

Sulfuric Acid, concentrated (sp. gr. 1.84) (H_2SO_4)	136 ml
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Add 136 ml of concentrated sulfuric acid to 800 ml of deionized,distilled water while cooling. After this solution has cooled, dilute to one liter with deionized, distilled water.

AMMONIUM MOLYBDATE

Ammonium Molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$)	40 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 40 g of ammonium molybdate in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

ASCORBIC ACID

Ascorbic Acid, U.S.P. (Technicon No. T11-5070) ($C_6H_8O_6$)	18 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 18 g of U.S.P. quality ascorbic acid in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

ANTIMONY POTASSIUM TARTRATE

Antimony Potassium Tartrate $[K(SbO)C_4H_4O_6 \cdot 1/2H_2O]$	3.0 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 3.0 g of antimony potassium tartrate in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

COMBINED WORKING REAGENT

Sulfuric Acid, 4.9N	50 ml
Ammonium Molybdate	15 ml
Ascorbic Acid	30 ml
Antimony Potassium Tartrate	5 ml

Preparation:

Combine reagents together in the order listed above: 50 ml of sulfuric acid, 15 ml of ammonium molybdate, 30 ml of ascorbic acid and 5 ml of antimony potassium tartrate. This reagent is stable for about eight hours.

[1] Murphy, J., and Riley, J.P., A Modified Single Solution Method for the Determination of Phosphate in Natural Waters, Anal. Chim. Acta, 27, p. 30, 1962.

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WATER DILUENT

To deionized, distilled water, add 2.0 ml of Levor IV* per liter.

STANDARDS

STOCK STANDARD A, 1000 µgat P/l (31,000 µg P/l)

Anhydrous Potassium Dihydrogen Phosphate (Technicon No. T13-5069) (KH_2PO_4)	0.136	g
Deionized, Distilled Water, q.s.	1000	ml
Chloroform	1	ml

Preparation:

Dissolve the potassium dihydrogen phosphate in 500 ml of deionized, distilled water in a volumetric flask. Dilute to one liter with deionized, distilled water. Add 1 ml of chloroform as a preservative.

STOCK STANDARD B, 40 µgat P/l (1240 µg P/l)

Stock Standard A	4 ml
Deionized, Distilled Water, q.s.	100 ml

Preparation:

Dilute 4 ml of stock standard A in a volumetric flask to 100 ml with deionized, distilled water. Prepare fresh daily.

WORKING STANDARDS

ml Stock B	µgat P/l	µg P/l
0.20	0.08	9.9
2.0	0.8	24.8
4.0	1.6	49.6
6.0	2.4	74.4
8.0	3.2	99.2
10.0	4.0	124

Preparation:

Pipette stock B into a 100 ml volumetric flask. Dilute to 100 ml with deionized, distilled water. Prepare fresh daily.

OPERATING NOTES

1. When analyzing seawater, a blank reading for the particular seawater of interest should be determined by sampling the seawater while running distilled water only through the reagent lines. The blank reading obtained should then be subtracted from the readings the unknowns.

2. Glassware for the preparation of reagents and standards should be washed with one normal hydrochloric acid and rinsed thoroughly with deionized, distilled water in order to remove any traces of phosphate. Sample cups should be treated in a similar manner and then rinsed with the solution to be measured.

3. The ascorbic acid solution is stable for about two months if kept in a freezer or refrigerator. It is stable for about two weeks if not refrigerated. However, the container must be kept well stoppered.

4. Samples which are not run immediately should be preserved with 1 ml/l of chloroform.

5. The reagent baseline absorbance with reference to water should be approximately 0.015 absorbance units.

6. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.

7. Before running the method, position the controls of the Modular Printer

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	30
RANGE Switch	400
DECIMAL Switch	0.00

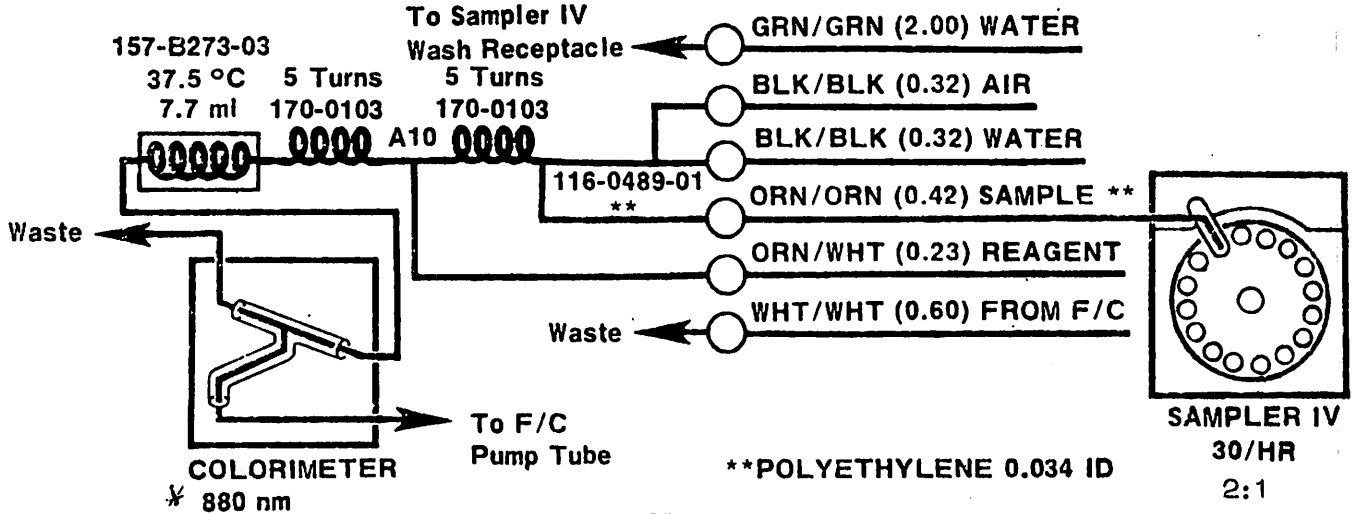
Details of Modular Printer Operation are provided in Technical Publication No. TA1-0278-10.

8. The Colorimeter should be operated in the Damp 1 mode.

9. The use of multiple working standards is only to establish linearity. For day-to-day operation, the 2.4 µgat P/l standard is recommended for instrument calibration.

ORTHO PHOSPHATE IN WATER AND SEAWATER

RANGE: 0-4 μgat P/l
0-124 μg P/l (ppb)
MANIFOLD NO. 116-D221-01



**POLYETHYLENE 0.034 ID

NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

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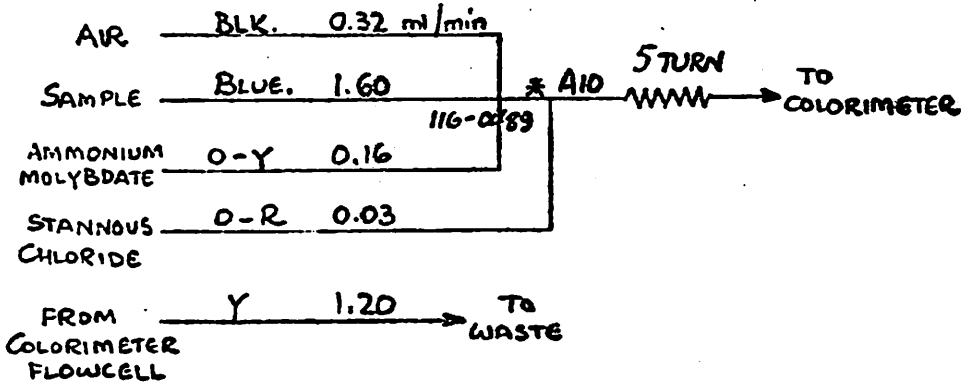
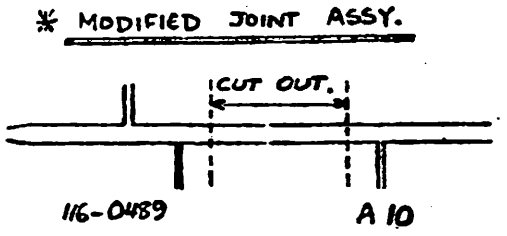
AUTOANALYSER II PHOSPHORUS (P)

RANGE : 0 - 50 ppb
FLOWCELL : 50 mm x 1.5 ID mm.
FILTERS : 660 nm.





AMMONIUM MOLYBDATE (230 ml/Day)

a) 11.9g Ammonium Molybdate in 500 mls H_2O

b) 73.8 ml ¢ H_2SO_4 in 500 mls H_2O

cool solutions and mix

STANNOUS CHLORIDE STOCK

1.2 g $SnCl_2$ $2H_2O$ Dissolved in 100 ml ¢ HCl

Stable for 2 weeks at 4°C.

STANNOUS CHLORDIE WORKING SOLN.

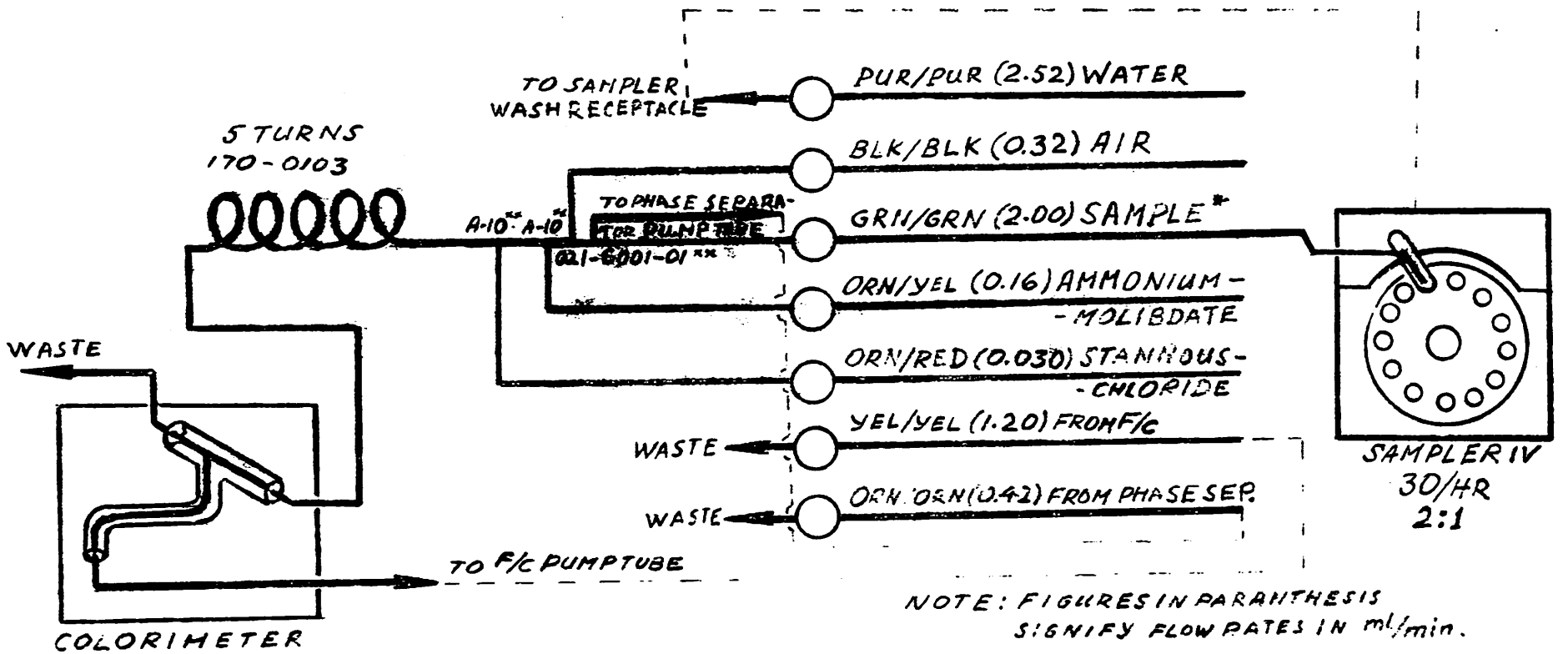
To 80 ml of H_2O add 10 ml saturated hydrazine sulfate solution (2g/100 ml) and mix, add 0.7 ml Levor IV and mix, add 4 ml (3.8 ml) Stannous Chloride stock solution in 0.5 ml portions and mix thoroughly after each addition. To be prepared fresh daily.

STANDAR STOCK SOLUTION

0.4394g K H_2PO_4 per liter = 100 ppm P

AUTOANALIZER II. PHOSPHORUS (P)

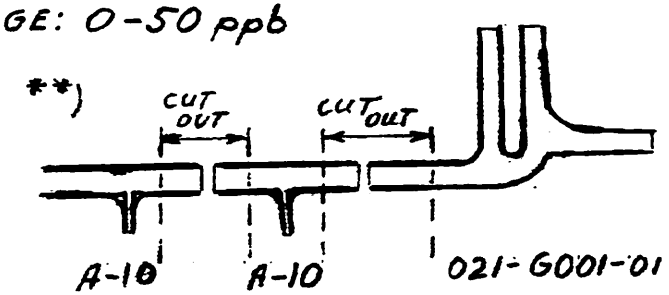
MOST RESENT MODIFICATION



NOTE: FIGURES IN PARANTHESIS SIGNIFY FLOW RATES IN ml/min.

*) 0.034 POLYETHYLENE SAMPLE TUBE

**)



Technicon AutoAnalyzer II

INDUSTRIAL METHOD No. 100-70W/PRELIMINARY DATE RELEASED: JAN. 1973

NITRATE AND NITRITE IN WATER AND WASTEWATER
(RANGE: 0-2.0 mg N/l)

GENERAL DESCRIPTION

This automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column. [1,2] The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye.

In surface waters normally encountered in surveillance studies, the concentration of oxidizing or reducing agents and potentially interfering metal ions are well below the limits causing interferences. When present in sufficient concentration, metal ions may produce a positive error, i.e., divalent mercury and divalent copper may form colored complex ions having absorption bands in the region of color measurement.[3]

PERFORMANCE AT 40 SAMPLES PER HOUR

USING AQUEOUS STANDARDS

Sensitivity at 2.0 mg N/l	0.72 absorbance units
Coefficient of Variation at 1.0 mg N/l	0.31%
Detection Limit	0.04 mg N/l

REAGENT

AMMONIUM CHLORIDE REAGENT (Technicon No. T01-5064)

Ammonium Chloride (NH_4Cl)	10 g
Alkaline Water q.s.	1000 ml
Brij-35* (Technicon No. T21-0110)	0.5 ml

Preparation:

Dissolve 10 g of ammonium chloride in alkaline water and dilute to one liter. Add 0.5 ml of Brij-35 per liter.

NOTE: Alkaline water is prepared by adding just enough ammonium hydroxide to distilled water to attain a pH of 8.5.

COLOR REAGENT (Technicon Nos. T11-5065, T01-5017)

Sulfanilamide ($C_6H_8N_2O_2S$)	20 g
Concentrated Phosphoric Acid (H_3PO_4)	200 ml
N-1-Naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)	1.0 g
Distilled Water, q.s.	2000 ml
Brij-35 (Technicon No. T21-0110)	1.0 ml

Preparation:

To approximately 1500 ml of distilled water add 200 ml concentrated phosphoric acid and 20 g of sulfanilamide. Dissolve completely. (*Heat if necessary.*) Add 1.0 g of N-1-naphthylethylenediamine dihydrochloride, and dissolve. Dilute to two liters. Add 1.0 ml Brij-35 (Technicon No. T21-0110). Store in a cold, dark place. STABILITY: one month.

CADMIUM, POWDER (Technicon No. T11-5063)

Use coarse cadmium powder (99% pure). Rinse the powder once or twice with a little clean diethyl ether or 1*N* HCl followed by distilled water to remove grease and dirt. Allow the metal to air-dry and store in a well-stoppered bottle.

Preparation of Reductor Column:

The reductor column tube is a U-shaped fourteen inch length of 2.0 mm I.D. glass tubing (Technicon No. 189-0000). Before filling the column, prepare the cadmium in the following manner.

Wash 10 g of previously cleaned cadmium with 50 ml of 2% w/v copper sulfate ($CuSO_4 \cdot 5H_2O$) (Technicon No. T01-5068) until no blue color remains in the solution and semi-colloidal copper particles begin to enter the supernatent liquid. Wash thoroughly with distilled water to remove all of the colloidal copper which is present. A minimum of ten washings is usually required.

Fill the reductor column tube with water to prevent

[1] Armstrong, F.A.J., Sterns, C.R. and Strickland, J.D.H., 1967 Deep-Sea Res. 14, pp. 381-389. The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment.

[2] Grasshoff, K., Technicon International Congress, June, 1969.

[3] Federal Water Pollution Control Administration Methods for Chemical Analysis of Water and Wastes, November, 1969.

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the entrapment of air bubbles during the filling operation. Transfer the prepared cadmium granules to the column using a Pasteur pipette. When the entire column is filled with granules, insert glass wool** in both ends of the tube. Sleeve both ends with 0.090 I.D. Tygon tubing and insert an N5 nipple on one side of the tube. Connect the other side of the tube directly to the A_2 debubbler by means of the 0.090 I.D. Tygon.

Start pumping reagents. When the pump tubes are filled with reagents and all air is removed from transmission lines, attach the distal end of the tube to the injection fitting (116-0489) using a short length of 0.034 I.D. Polyethylene tubing.

Preparing the column in this fashion keeps it effective for hundreds of samples.

STANDARDS

STOCK STANDARD A, 100 mg N/l

Potassium Nitrate (KNO_3) (Technicon No. T13-5074)	0.72 g
Distilled Water, q.s.	1000 ml
Chloroform ($CHCl_3$)	1 ml

Preparation:

Dissolve 0.72 g of potassium nitrate in distilled water and dilute to one liter. Store in a dark bottle. Add 1 ml of chloroform as a preservative.

STOCK STANDARD B, 20 mg N/l

Stock Standard A	20 ml
Distilled Water, q.s.	100 ml

Preparation:

Dilute 20 ml of stock standard A in a volumetric flask to 100 ml with distilled water. Store in a dark bottle. Prepare fresh daily.

WORKING STANDARDS

ml Stock B	mg N/l
0.2	0.04
2.0	0.40
4.0	0.80
6.0	1.20
8.0	1.60
10.0	2.00

Preparation:

Pipette stock B into a 100 ml volumetric flask. Dilute to 100 ml with distilled water. Store in a dark bottle. Prepare fresh daily.

OPERATING NOTES

1. Samples should be processed and analyzed as soon as possible. If this cannot be done immediately, they should be refrigerated at 5-10 °C or preserved with 1 drop of chloroform per 100 ml sample.

2. Where particulate matter is present, the solution must be filtered prior to the determination. This can be accomplished by using the Technicon Continuous Filter as an integral part of the system if the sample is such that Whatman #4 or equivalent filter paper is satisfactory. (See Continuous Filter Manual, No. CFO-1.)

3. It is of the utmost importance that the water used in preparing reagents and standards be completely free of contaminants. Reagents should be stored in glass bottles and contact with air should be avoided.

4. In order to determine nitrate levels, the nitrite alone must be subtracted from the total (nitrate and nitrite). The nitrite value can be determined by eliminating the reductor column from the manifold, or by using the Technicon Methodology for Nitrite (102-70W).

5. The reductor column must be clean and have good flow characteristics for the system to operate satisfactorily. Colloidal copper is the primary contaminant

6. For initial activation of the reductor column, a midscale standard should be pumped through the syst for about one hour.

7. The efficiency of the reductor column has been found to be 99%.

8. Before running the method, position the controls of the Modular Printer as follows:

CONTROLS	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	40
RANGE Switch	200
DECIMAL Switch	0.00

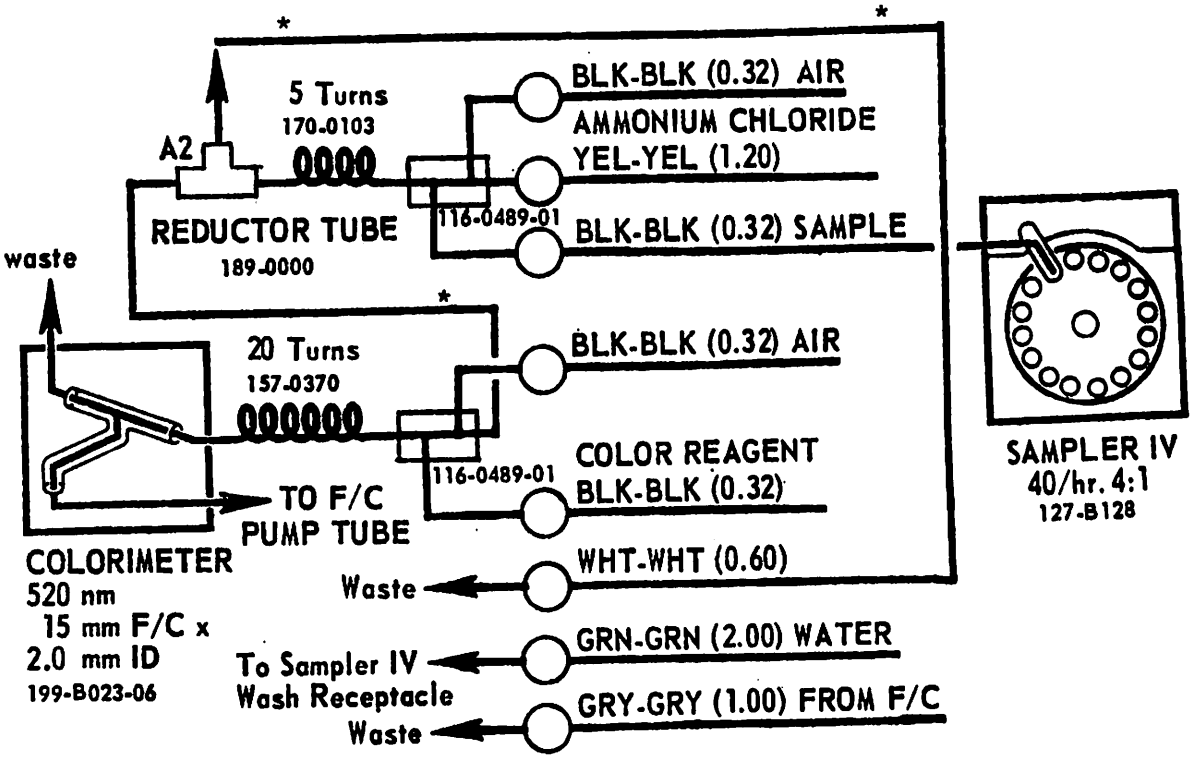
Details of Modular Printer Operation are provided in the Technical Publication TA1-0278-10.

9. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.

10. The use of multiple working standards is only to establish linearity. For day-to-day operation, the 1.2 mg/l standard is recommended for instrument calibration.

** Use Pyrex Glass Wool Corning No. 3950.

NITRATE AND NITRITE IN WATER AND WASTEWATER
(RANGE: 0-2.0 mg N/l)
MANIFOLD NO. 116-D049-01



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

* 0.034 POLYETHYLENE

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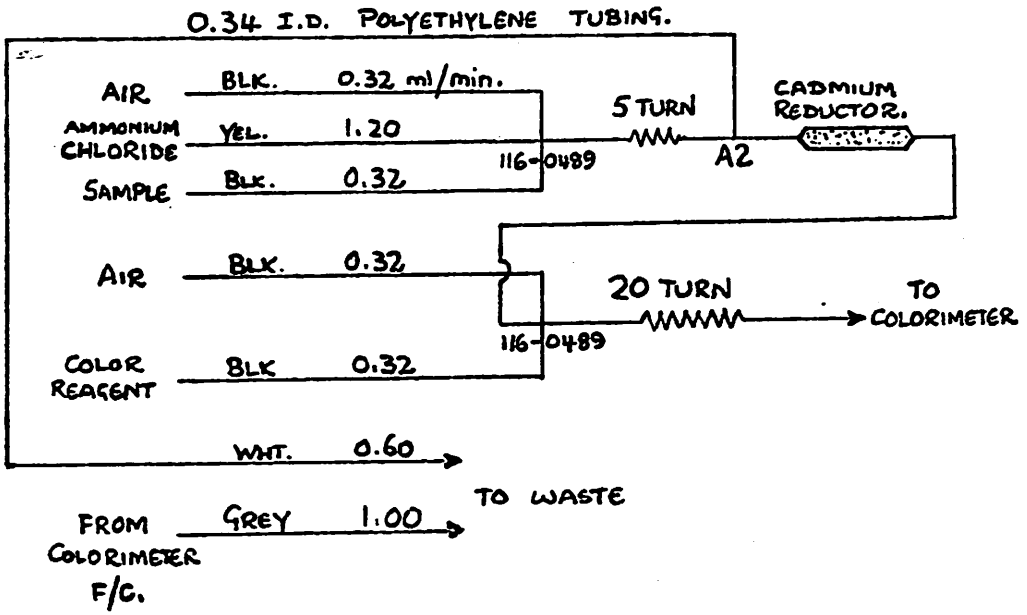
AUTOANALYSER II NITRATE + NITRITE (N)

RANGE : 0 – 1 PPM.
FLOWCELL : 50 mm. x 1.5 mm I.D.
FILTERS : 520mm.



Sampler:
20/HR
2:1

AMMONIUM CHLORIDE. (1728 mls/DAY)

10.0 g/L NH_4Cl. ADJUST PH TO 8.5.
ADD 0.5 ml BRIJ-35 PER LITER.

COLOR REAGENT. (460 mls/DAY)

20 g SULPHANILAMIDE + 1.0 g NAPHTHYL-ETHYLENE DIAMINE DIHYDROCHLORIDE + 200 ml PHOSPHORIC ACID PER 2 LT.
STABLE IN DARK FOR 4 WEEKS.

CADMIUM REDUCTOR.

a) WASH CADMIUM WITH 1N-HCl.
b) WASH 10 g WITH 50 ml ALIQUOTS OF 2% w/v $CuSO_4$ UNTIL NO BLUE COLOR REMAINS.
c) WASH WITH DEIONISED WATER TO REMOVE COLLOIDAL COPPER.
d) FILL PURPLE PUMP TUBE WITH ABOVE CADMIUM AND STORE UNDER WATER.

N.B. DO NOT ALLOW AIR TO ENTER COIL.
REACTIVATE WITH APPROX 20% HCl IN NH_4Cl line for about 5 mins.

STOCK SOLUTION.

7.2200 g KNO_3 / LT ≡ 1000 PPM. N.

Technicon AutoAnalyzer II

INDUSTRIAL METHOD No. 154-71W/TENTATIVE

DATE RELEASED: FEB. 1973

AMMONIA IN WATER AND SEAWATER

RANGE: 0-10 μgat N/l
0-140 μg N/l (ppb)

GENERAL DESCRIPTION

The automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate and sodium citrate is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium.[1,2,3,4,5,6]

PERFORMANCE AT 60 SAMPLES PER HOUR

USING AQUEOUS STANDARDS

Sensitivity at 10 μgat N/l (140 μg N/l)	0.15 absorbance units
Coefficient of Variation at 8.0 μgat N/l (112 μg N/l)	0.31%
Detection Limit	0.2 μgat N/l (2.8 μg N/l)

REAGENTS

COMPLEXING REAGENT

Potassium Sodium Tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)	33 g
Sodium Citrate ($HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$)	24 g
Distilled Water, q.s.	1000 ml
Brij-35* (Technicon No. T21-0110)	0.5 ml

Preparation:

Dissolve 33 g of potassium sodium tartrate and 24 g of sodium citrate in 950 ml of distilled water. Adjust the pH of this solution to 5.0 with concentrated sulfuric acid. Dilute to one liter with distilled water. Add 0.5 ml of Brij-35.

[1] Van Slyke, D.D. and Hillen, A.J., BioChem., *102*, p. 499. 1933.

[2] Kallman, S., Presentation at Div. I Meeting of ASTM Committee E-3, April, 1967, San Diego, California.

[3] Bolleter, W.T., Bushman, C.J. and Tidwell, P.N., Anal. Chem., *33*, p. 592, 1961.

[4] Tellow, J.A. and Wilson, A.L., Analyst, *89*, p. 453, 1964.

[5] Tarugi, A. and Lenci, F., Boll Chim. Farm., *50*, p. 907, 1912.

[6] FWPCA Methods of Chem. Anal. of Water & Wastewater, November, 1969, p. 137

*Registered trademark of Atlas Chemical Industries, Inc.

ALKALINE PHENOL

Phenol (C_6H_5OH)	83 g
Sodium Hydroxide, 20% w/v (NaOH)	180 ml
Distilled Water, q.s.	1000 ml

Preparation:

Using a one liter Erlenmeyer flask, dissolve 83 g of phenol in 50 ml of distilled water. Cautiously add, while cooling under tap water, in small increments with agitation, 180 ml of 20% NaOH. Dilute to one liter with distilled water.

SODIUM HYPOCHLORITE (STOCK)
(Technicon No. T01-0114)

Any good commercially available household bleach having 5.25% available chlorine may be used.

SODIUM HYPOCHLORITE (WORKING)

Dilute 200 ml of stock sodium hypochlorite to one liter with water.

SODIUM NITROPRUSSIDE

Sodium Nitroprusside ($Na_2Fe(CN)_5NO \cdot 2H_2O$)	0.5 g
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 0.5 g of sodium nitroprosside in 900 ml of distilled water and dilute to one liter.

STANDARDS

STOCK STANDARD A, 5000 μgat N/l (70,000 μg N/l)

Ammonium Sulfate [$(NH_4)_2SO_4$]	0.3310 g
Distilled Water, q.s.	1000 ml
Chloroform	1 ml

Preparation:

In a one liter volumetric flask, dissolve 0.3310 g of ammonium sulfate in 900 ml of distilled water. Dilute to volume with distilled water. Add 1 ml of chloroform as a preservative.

STOCK STANDARD B, 100 μgat N/l (1400 μg N/l)

Stock Standard A	2 ml
Distilled Water, q.s.	100 ml

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A DIVISION OF TECHNICON INSTRUMENTS CORPORATION

Preparation:

Dilute 2 ml of stock standard A in a volumetric flask to 100 ml with distilled water. Prepare fresh daily.

WORKING STANDARDS

ml Stock B	µgat N/l	µg N/l
0.2	0.2	2.8
2.0	2.0	28
4.0	4.0	56
6.0	6.0	84
8.0	8.0	112
10.0	10.0	140

Preparation:

Pipette stock B into a 100 ml volumetric flask. Dilute to 100 ml with distilled water. Prepare fresh daily.

OPERATING NOTES

1. All water used in the preparation of reagents should be deionized acid distilled or distilled water which has been passed through a hydrogen form resin.

2. The alkaline phenol reagent should be filtered through a glass filter prior to use.

3. Where particulate matter is present, the solution must be filtered prior to the determination. This can be accomplished by having the Technicon Continuous Filter as an integral part of the system if the sample is such that Whatman #4 or equivalent filter paper is satisfactory.

4. If the system is being run in an ammonia contaminated environment, the air for segmenting the stream should be scrubbed through acid prior to its introduction into the system.

5. Before running the method, position the controls of the Modular Printer as follows:

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	60
RANGE Switch	100
DECIMAL Switch	00.0

Details of Modular Printer Operation are provided in Technical Publication No. TA1-0278-10.

6. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.

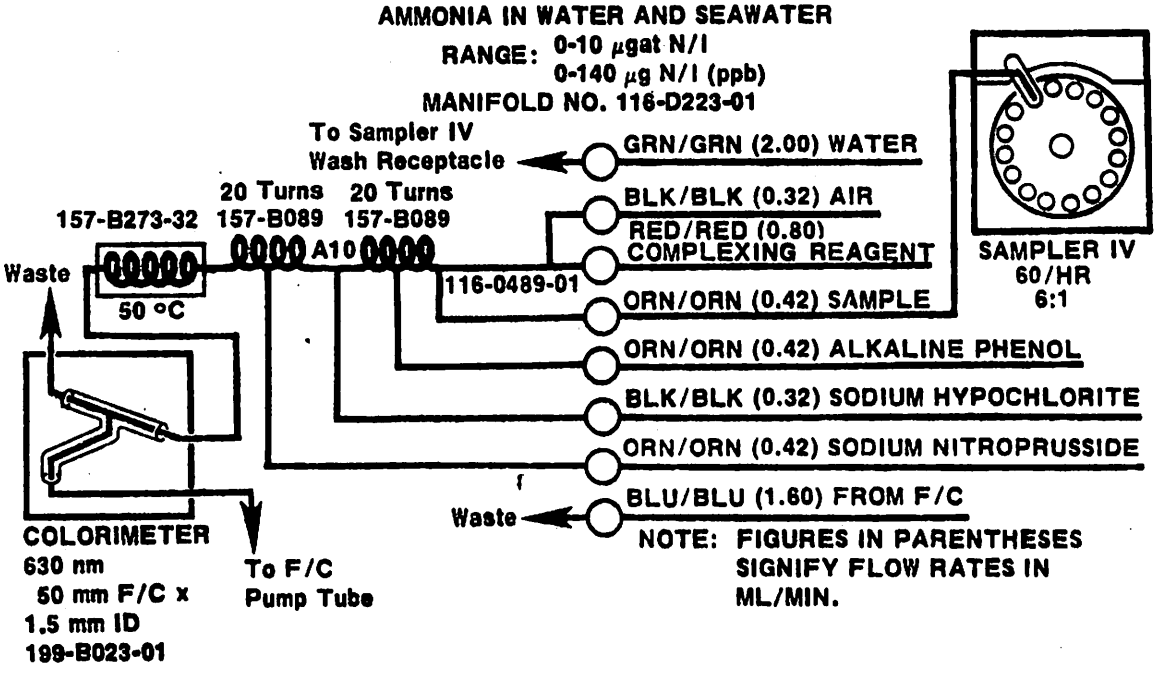
7. The Colorimeter should be operated in the Damp 1 mode.

8. The reagent baseline absorbance with reference to water should be approximately 0.180 absorbance units.

9. Glassware for the preparation of reagents and standards should be washed with one normal hydrochloric acid and rinsed thoroughly with distilled water in order to remove any traces of ammonia. Sample cups should be treated in a similar manner and then rinsed with the solution to be measured.

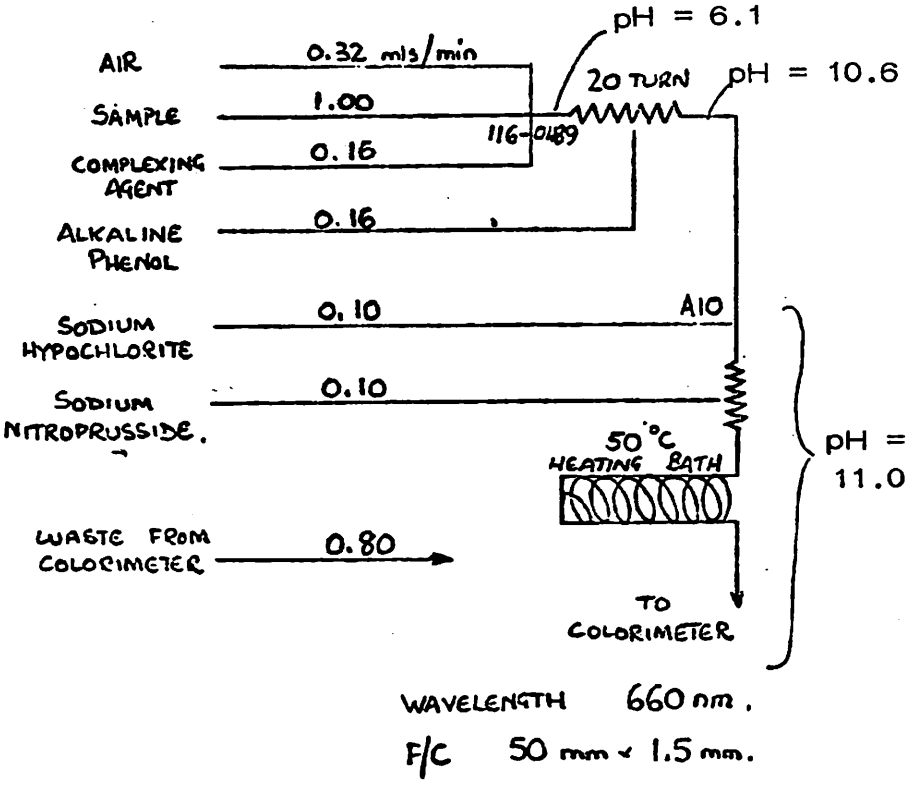
10. When analyzing seawater, a blank reading for the particular seawater of interest should be determined by sampling the seawater while running distilled water only through the system. The blank reading obtained should then be subtracted from the readings of the unknowns.

11. The use of multiple working standards is only to establish linearity. For day-to-day operation, the 6 µgat N/l standard is recommended for instrument calibration.



AMMONIA - INDOPHENOL METHOD

AA II METHODOLOGY.



Sampler:
20/HR
2:1

COMPLEXING AGENT

Dissolve 1.65g Potassium Sodium Tartrate Dihydrate & 1.20g Sodium Citrate Dihydrate in 950 ml deionised water. Adjust pH to 5.0 with conc. H_2SO_4 and make to 1 lt.

ALKALINE PHENOL

Dissolve 21 g Phenol in 100 ml deionised water and cool. Cautiously add 180 ml of 5% w/v NaOH and dilute to 1 lt.

SODIUM HYPOCHLORITE (0.26% av. chlorine)

Dilute 50 ml stock hypochlorite (having 5.25% av. chlorine) to 1 lt. with deionised water.

SODIUM NITROPRUSSIDE

Dissolve 1.00 g sodium nitroprusside and make to 1 lt. with deionised water.

N.B. All reagent lines & air lines are scrubbed with 20% H_2SO_4.

STOCK AMMONIA SOLUTION: 100 ppm N

Dissolve 0.3819g NH_4Cl in 1 lt. deionised water.

NITROGEN FIXATION AND METHANE PRODUCTION IN THE CENTRAL AND WESTERN BASINS OF LAKE ERIE

Objectives

1. To continue analysis of nitrogen fixation in water and sediments by acytelene reduction technique.

2. To continue analysis of methane production of sediments by gas chromatographic techniques.

3. To continue analysis of methane content of water column by gas chromatographic techniques.

4. To attempt to quantitate in situ methane (gas) evolution from sediments by gas trapping and gas chromatographic techniques.

Methods

Objectives in the field are to perform an on board N_2 fixation experiment with water samples and sediment and to obtain a sample of the dissolved gases in the water column.

Water samples are obtained from the water column at the surface, upper hypolimnion and the bottom. The bottom and thermocline are determined using a Martek recorder. Once these depths are determined water samples are obtained at one meter above the bottom sediment, one meter below the thermocline and one meter below the surface. When a thermocline is not present, a middle depth is obtained mid way in the water column. From June 25 - July 21, 1973, a 4.4 liter Van Dorn water sampler was used; after that a modified 2.2 liter Alpha Bottle was used.

The sampler is lowered to the desired depth by an electric winch and then a weighted "messenger" is sent down the line to set off the trigger mechanism. The desired sample is then retrieved and placed on board. Three 50 cc water samples are drawn off using 50 cc plastic syringe. These three samples which are obtained from each of the three depths are injected into pre-evacuated 60 cc serum bottles fitted with air tight stoppers. All the bottles are then injected with 1 cc of acetylene. One sample bottle from each depth is then immediately injected with 1 cc of 50% tri-chloro acetic acid (TCA) for a 0-time control. A second bottle from each depth is incubated in the light for three hours at

ambient temperature prior to the addition of the TCA. The remaining third bottle from each depth is incubated at ambient temperature prior to the addition of the TCA. The remaining third bottle from each depth is incubated at ambient temperature for three hours in the dark prior to addition of TCA. The resulting nine (for each station) acidified samples are stored at ambient temperature until it is possible to do laboratory analysis of the gases present.

To obtain a sample of the dissolved gases in the water one bottom water sample is used. After the 50 cc of water is injected into the bottle it is shaken vigorously and allowed to come to ambient temperature. Five (5) cc of helium are removed from a 15 cc helium purged serum bottle and replaced with a 5 cc gas sample from the dead space over the bottom water sample. One (1) cc of acetylene and one (1) cc of 50% TCA are then injected into the water sample. One (1) cc of acetylene and one (1) cc of 50% TCA are then injected into the water sample and again allowed to equilibrate. Another 5 cc gas sample is then taken and stored in a helium purged 15 cc serum bottle to obtain gases from the water after acidification.

Sediment samples are also obtained for N_2 fixation capacity and gas production. A ponar sediment sampler is lowered to the bottom from the boat on a steel cable with an electric winch. A grab sample of the surface sediments is retrieved and dumped into a pan onboard. The ponar is washed with a hose and set aside for next use. Using a 20 cm piece of glass tubing with a 1 cm I.D. a 15 x 1 cm core of sediment is drawn out of the grab sample and blown out into an empty 30 cc serum bottle. This size core of sediment averages 10 - 15 grams wet weight. Three serum bottles are prepared in such a way and stoppered. Two of the bottles are injected with 1 cc of acetylene and one is left as a control. The sediments are then incubated for 5 days at ambient temperature and then atored at $4^{o}C$ until lab analysis is possible.

Laboratory and Data Analysis

The acquired water and sediment samples are analyzed for two parameters, nitrogen fixation and methane production. A Varian 2700 series gas chromatograph with a flame ionization detector is used in the analysis.

Nitrogen fixation is determined by the reduction of acetylene to ethylene. From the amount of ethylene produced, one may

determine the amount of atmospheric nitrogen that would have been "fixed" by this biological activity. Thus this acetylene reduction is an indirect method for testing for nitrogenase activity. The molar ratios of ethylene: ammonia produced vary, but are usually in the range 1.4-1.8

A 1 cc air sample is taken from the dead space above the water sample and injected into the Varian gas chromatograph. This gas sample is carried through a 6 foot glass column which is packed with silca gel which acts as a physical barrier and separates the various gas molecules according to their molecular size. The detector burns these separated gases as they exit from the column and an electrical impulse corresponding to the quantity of the individual gases is sent to a recorder where a peak is recorded with respect to time. The recorded peaks are identified according to their retention times which are compared to a known standard, i. e. an unknown peak which is suspected to be ethylene is compared to the known retention time of a standard ethylene sample.

The procedures for methane analysis are identical. For identification of the biologically produced methane a 315 parts per million (315 ppm) standard is used. This method of analysis for methane is direct. Any carbon dioxide which is originally present in the water column and sediments and which is biologically converted to methane is detected directly by use of the Varian gas chromatograph.

As was stated in the field procedures, three different depths were sampled at each station, i.e., one meter below water surface, one meter off the bottom, and one meter below the thermocline, if one was present, or else if no thermocline was observed, simply a middle depth.

The data are summarized in two basic ways. First of all, the concentration gradients of the two parameters are recorded separately for each station, i.e., a profile of the water column and sediment is constructed. Then a summary of all the stations is made and a profile of the entire lake is made graphing pictorially these activities throughout the lake.

BENTHIC COMMUNITIES IN THE CENTRAL AND WESTERN BASINS OF LAKE ERIE

Objectives

Since the early 1930's pronounced changes in the bottom fauna of Lake Erie have been observed and recorded (Wright, 1955; Britt, 1955a; 1955b; Beeton, 1961; Carr and Hiltunen, 1965; Britt et al, 1973). These changes appear to be correlated with observed increases in the nutrient level in the lake water. Among the key elements, or nutrients, that have greatly increased in abundance in the water are phosphorus and nitrogen. The chief source of these is domestic sewage.

In recent years new laws have been enacted regulating the treatment and discharge of sewage with the ultimate goal of reducing the amount of phosphorus and other nutrients that are discharged into the lake and therby slowing down or reversing the eutrophication of the lake.

Since benthic communities are relatively immobile and some members have relatively long life cycles (one year or more), their very existence is dependent upon the quality of the water remaining within their range of tolerance for long periods of time.

The objectives of this study are to analyse the benthic communities in terms of diversity and density and thus to provide baseline data for monitoring changes in the water quality of Lake Erie as the phosphorus input decreases. In addition to baseline data, our information will be used for comparing present-day conditions with conditions in the past (Wright, 1955; Brinkhurst et al, 1968; Britt et al, 1973). By correlating our biological data with chemical and physical parameters and using both to determine the trophic level of the lake, it may be possible to find biological indicators of water quality.

Methods

Benthos samples were taken at each station using a Ponar grab sampler. Two grabs were taken per station. Different-sized Ponars were used on different research vessels; however, the area of all samplers used was between 0.51 and 0.58 m^2 and was recorded in all cases. Samples were sieved through U.S. Soil Series No. 40 mesh (.425 mm) and preserved in 5-10% formalin. In the laboratory samples were stained with Rose Bengal and hand-sorted in white enamel pans under bright light. Organisms were identified as far

as possible (either to genus or species) and counted. Results were expressed as number per m^2.

All results thus far have been expressed as numbers of organisms. In the future it would be desirable to weigh organisms and after correcting for weight loss due to preservation, to express values as biomass as well.

An additional aspect which needs attention is the rearing of aquatic insects from larvae to adults. The Chironomidae make up a major part of the benthic fauna, but in many cases cannot be identified beyond the genus level without the adult form. By rearing known larvae and identifying the adults it may also be possible to find larval characteristics for determining species directly.

Benthos References

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Carr, J.F. and J.K. Hiltunen. 1965. Changes in the bottom fauna of western Lake Erie from 1930 to 1961. Limnol and Oceanogr. 10 (4): 551-569.

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PRIMARY PRODUCTIVITY IN THE CENTRAL AND WESTERN BASINS OF LAKE ERIE

Objectives

1) To determine changes in primary productivity in the central and western basins of Lake Erie that have occurred from 1963 through 1974, by use of C^{14} method.

2) To compare primary productivity rates in the central and western basins and to determine any differences that exist.

3) To integrate primary productivity findings with other investigators' data on standing crops of phytoplankton, chlorophyll and chemical conditions in the lake in order to make an overall statement about the eutrophic conditions of the western and central basins of Lake Erie.

Methods

Stations sampled are selected from a cruise list determined in advance by CLEAR. In addition, a location 250 m offshore, south of The Rattles of Rattlesnake Island, is chosen for in situ measurements. This location is close to CLEAR's Lake Erie base of Put-in-Bay and allows easy access by small boat. Cody (1972) has done a recent investigation of this site, and it is found to be representative of the waters of western Lake Erie based on the current flow patterns in the lake.

Sampling depths vary according to whether the station is located in the western or central basin of the lake. A depth below the compensation point is also included; it is determined by the Secchi disk and photometer reading (3.5X Secchi disk reading) (Cody, 1972). For western basin stations, depths of 0.25 m, 1 m, 2 m and compensation depth were sampled. Central basin stations were sampled at depths of 0.25 m, 1 m, 2 m, 5 m and compensation depth or 0.25 m, 2 m, 5 m, 10 m and compensation depth, depending on station depth and the Secchi disk reading.

Water from the indicated depths is collected in a Van Dorn or Kemmerer water sampler. From here the procedure of Cody (1972) is closely followed, except that samples collected on cruises are incubated in a water bath on board ship. In between cruises, the Rattlesnake Island location mentioned previously is sampled.

The collected water is transferred from water sampler to a clear 300 ml BOD bottle and is allowed to overflow for four seconds. Then 1 or 2 ml $NaH^{14}CO_3$ from a sterile stock solution prepared daily is added by using a 2 ml hypodermic syringe. The syringe needle is then raised to just below the water surface where it is rinsed twice by collecting and gently releasing the water to thoroughly remove all $NaH^{14}CO_3$ from syringe to BOD bottle. The stopper is replaced in the bottle, and the bottle is then inverted four times to mix the contents.

One light bottle per depth in both lake basins is innoculated. Dark bottle sampling depths for the western basin are 1 m and compensation depth; for central basin stations they are 1 m, 5 m, compensation depth or 2 m, 10 m, compensation depth, depending again on Secchi disk and station depth readings. Duplicate analyses are run for both dark and light bottles.

The bottles are then placed in a water bath and are incubated for 3-5 hours at ambient temperature and an illumination of 1000 ft-candles. Foil is placed over the dark bottles to prevent heating. Incubation at the Rattlesnake Island station is in situ; the bottles are innoculated following the procedure for the cruises, except that the bottles are incubated at the depth from which the water sample is collected.

After the incubation period is complete, 10 ml of water is removed from each bottle and 10 ml thiomerosol fixative is added to stop the chemical reactions, and the bottles are shaken. The bottles are then filtered on a 47 mm Gelman GA-6 Metrical membrane filter of pore size 0.45μ; suction pressure is 33 cm Hg. The bottles are rinsed twice with filtered lake water to remove all material. After filtration the filters are rinsed twice with filtered lake water, twice with 0.003 N HCl, and twice again with filtered lake water. The filters are then dissolved in 15 ml scintillation vials containing 10 ml scintillation fluid (7 g 2,5-diphenyloxazole; 100 g naphthalene; dissolved in 1 l dioxane). The vials are stored until time to count. The empty BOD bottles are filled with aqua regia for 30 minutes, drained, rinsed well with distilled water, and drained again until dry.

The activity of the samples is determined using a liquid scintillation counter. Calibration of the device includes corrections for electronic noise, percent efficiency of the counter, quenching, and background. Correction for the noise involves careful setting

of the machine. Quenching and percent efficiency are corrected for by adding a known amount of a C^{14} standard to previously corrected samples, and recounting; the formula

$$\text{corrected cpm}_{\text{sample}} = \frac{\text{known cpm standard}}{\text{recorded cpm standard} + \text{sample} - \text{recorded cpm standard}} \times \text{recorded cpm sample}$$

is used. Finally, subtracting background radiation from the corrected cpm sample gives the actual cpm of the sample. Final activity of the phytoplankton at each depth is found by subtracting the average of the dark bottle activities from the average of the light bottle activities.

The activity transferred from the syringe to the incubation bottles is obtained by adding 1 ml of the sterile stock solution to a 15 ml scintillation vial containing 10 ml scintillation fluid. The solution is then stored until ready to be counted with liquid scintillation counter.

Calculation of the primary productivity using the C^{14} method uses the formula of Saunders et al. (1962):

$$P = \frac{r}{R} \times C \times f$$

P = mg carbon assimilated per liter
R = total cpm added to each bottle
r = corrected cpm retained on the filter
C = total available stable inorganic carbon (mg/l), a factor from a table in Saunders et al. (1962) multiplied by total alkalinity
f = 1.06 isotope correction factor

The resulting productivity values are converted to mg atomic weights of carbon per liter, m^3, or m^2 of water column. This allows comparison with other productivity measurements.

Chemical determinations of pH, dissolved oxygen, and temperature are made by potentiometric methods for both the shipboard analysis and the in situ study. Alkalinity determinations are done manually according to Standard Methods. Data on phytoplankton standing crops and chlorophyll are furnished by other segments of the project.

Primary Productivity References

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Parkos, W. G., T. A. Olson, and T. O. Odlaug. 1969. Water quality studies on the Great Lakes based on carbon fourteen measurements on primary productivity. Water Resources Research Center, University of Minnesota Graduate School Bull. 17, 121 p.

Saunders, G. W., F. B. Trama, and R. W. Bachman. 1962. Evaluation of a modified C^{14} technique for shipboard estimation of photosynthesis in large lakes. University of Michigan, Great Lakes Research Division Pub. 8, 61 p.

PLANKTONIC POPULATIONS OF THE CENTRAL AND WESTERN BASINS OF LAKE ERIE

Objectives

The phytoplankton segment of the Lake Erie Nutrient Study will provide quantitative and qualitative assessments of plankton populations of the central and western basins. The predominance of any algal group or group of groups known to be associated with eutrophication, i. e., Chlorophyta, Cyanophyta, Chrysomonadinea, Diatomeae, Cryptomonadinae, Dinophycinae, will aid in evaluating the state of eutrophication of various segments of the lake. Information derived from cell volume estimations, chlorophyll a measurements and C^{14} studies will provide a basis for assessing standing crop and productivity for the lake.

Phytoplankton Techniques

Water samples for phytoplankton assessments were taken one meter below the surface, one meter above the thermocline and one meter below the thermocline. If no stratification existed, samples were taken from one meter below the surface, mid-depth and one meter above the bottom. Samples of water were obtained with a six liter, clear, plastic, non-toxic Van Dorn sampler. One-half liter of water was secured from each depth and immediately preserved with Lugol's solution (Kling, 1972).

Phytoplankton assessment followed the basic procedure devised by Utermohl (1931, 1936, 1958). The Utermohl inverted microscope technique involves sedimenting exact volumes of water and counting the organisms resulting from sedimentation. The counting of phytoplankton using the Utermohl technique has been thoroughly reviewed by Lund (1951). Samples were sedimented in settling chambers of 2, 10, and 25 ml capacity. Phytoplankton samples were examined using a Leitz Divert inverted microscope equipped with phase contrast illumination. Counts and identifications were made at magnifications of 100x, 200x, 400x and 1000x. Counting procedure was that of Utermohl's technique as modified by Nauwerck (1963). Conversions of cells per liter to biomass values were made by estimating the cell volumes of the various species. Cell volume estimates were made by approximating the geometric shape most closely resembling the shape of the cell (Vollenweider, 1968).

Statistical considerations followed the recommendations and discussions by Lund, et al (1958), Javornicky (1958) and Nauwerck (1963).

Zooplankton Techniques

At each station a vertical plankton tow was made using a 0.5 m, #20 net having an 80 micron mesh size. Samples were immediately preserved with Transeau's solution (6 parts water, 3 parts 95% ethanol, 1 part formalin) and stored for later analysis.

Qualitative analyses were carried out using a Leitz Dialux microscope, and identifications were made at magnifications of 50x and 125x. Duplicates of each sample were observed using a Sedgwick Rafter cell. When greater magnification was necessary for identification wet mounts of the sample were prepared and observed at 500x. Organisms were identified to the species level.

Plankton References

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NITROGEN FIXATION

Nitrogen fixation can occur only in a few genera of bacteria, blue-green algae and possibly some fungi. In the aquatic system, the greatest portion of fixation is performed by the blue-green algae and by two genera of bacteria, Clostridium and Azotobacter. The most impressive point is that these organisms are capable of clearing the relatively stable triple bond of the nitrogen molecule. The energy required to cleave this triple bond has been estimated to be approximately three times that required to cleave a carbon-carbon single bond. The enzyme causing this cleavage is called nitrogenase, an iron protein complex which requires molybdenum.

The exact biochemical reactions occurring during nitrogen fixation have not been completely elucidated but a number of significant properties of the nitrogenase system have been deduced. At this present time only a few introductory remarks will be made concerning fixation.

The first stable product able to be isolated from the nitrogenase system is ammonia (NH_3). Thus a number of intermediates must be formed which are short-lived and unstable. Presently, it is postulated that molecular nitrogen ($N \equiv N$) is reduced to ammonia (NH_3) in a three step process.

1) NITROGENASE ENZYME · $N \equiv N$ complex + 2H ➡ ENZYME·HN=NH

2) ENZYME·HN=NH + 2H ➡ ENZYME·H_2N-NH_2

3) ENZYME·H_2N-NH_2 + 2H ➡ ENZYME + $2NH_3$

Although this postulation is universally accepted, the diimide (HN=NH) and hydrazine (H_2N-NH_2) intermediates have not been isolated.

An interesting feature of this enzyme complex is that it is not specific for N_2 but will also reduce N_2O, CN^-, azide and acetylene (HC=CH). The reduction of these compounds serves no useful purpose to the cell but provides a simple means for one to measure nitrogenase activity. The reduction of acetylene to ethylene, i.e.,

$$HC{=}CH + 2H \rightarrow H_2C{=}CH_2$$

provides the simplest method for detecting and estimating nitrogenase activity. In comparing the reduction of nitrogen to that of acetylene

one sees that 6H (6 reducing units) are required for producing 2 NH_3 (or 3 H/NH_3) while 2H (2 reducing units) are required to reduce acetylene to ethylene. Therefore, a ratio of 1.5:1 (NH_3:ethylene) is obtained.

Fixation Procedures for Nutrient Control Study

Procedures for determining nitrogenase activity are extremely simple. Water samples are obtained and placed in appropriate containers. A constant amount of acetylene is added to each sample and then the resulting samples are incubated for an appropriate time. After incubation the samples are "fixed" with 50% solution of trichloro-acetic acid (TCA). The fixed samples may then be stored at room temperature until analysis is possible. The most accurate analyses are performed with a gas chromatograph equipped with a flame ionization detector (FID). The quantity of ethylene produced can be compared to nitrogenase activity as was demonstrated in previous pages.

Actual Procedures for Nutrient Study

1) 60 cc serum bottles are stoppered and then evacuated with a vacuum pump (Total volume in 60 cc serum bottle is 72 cc.)

2) Water samples are obtained (25 ml) from a submersible pump with the aid of a 50 cc syringe equipped with a locking valve.

3) The collected sample is then placed in evacuated bottle and the remaining vacuum is equilibrated to atmospheric pressure with ultra-high purity helium.

4) A 0.5 cc volume of acetylene is injected (approx. 0.7% of total volume in bottle).

5) Resulting sample is incubated at ambient temperature for 3 hours.

6) After incubation period, a 1.0 cc volume of 50% TCA is added to terminate biological activity. Resulting sample is stored at ambient temperature until analysis.

7) Analysis is completed with the aid of a Varian 2740 series gas chromatograph equipped with a flame ionization detector. A permanent record of the ethylene quanitites are recorded on a Linear Instrument intergrating recorder, model 252A. The quantity of ethylene produced biologically is calculated from a known standard of ethylene.