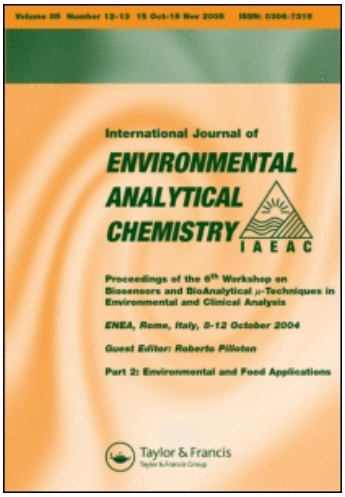

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Evaluation of the Procedure for Determining Low Levels of Phosphorus in Fresh Water

Shirley J. Mackay[a]
[a] Chemical Branch, Metropolitan Water, Sewerage and Drainage Board, Sydney South, Australia

To cite this Article Mackay, Shirley J.(1975) 'Evaluation of the Procedure for Determining Low Levels of Phosphorus in Fresh Water', International Journal of Environmental Analytical Chemistry, 4: 1, 33 — 46
To link to this Article: DOI: 10.1080/03067317508071099
URL: http://dx.doi.org/10.1080/03067317508071099

Intern. J. Environ. Anal. Chem., 1975, Vol. 4, pp. 33–46

Printed in Great Britain

Evaluation of the Procedure for Determining Low Levels of Phosphorus in Fresh Water

SHIRLEY J. MACKAY

Chemical Branch, Metropolitan Water, Sewerage and Drainage Board, Sydney, P.O. Box A53, Sydney South, 2001, Australia

(*Received April 4, 1973*)

Low levels of phosphorus in fresh water were determined by a variant of the heteropoly-blue method using extraction with isobutanol to bring the detection limit to 0.001 mg phosphorus/litre. The estimation was accurate and reliable under controlled conditions and a number of internal errors were eliminated. Although filtration of the sample at the site was desirable it was sometimes impracticable, so satisfactory alternative ways of maintaining the total phosphorus/dissolved phosphorus ratio were found. Samples could be stored for no longer than three days at 5°C or at ambient room temperature. A modification of the method excluded the additive effect of arsenic.

KEY WORDS: Phosphorus, fresh water, heteropoly-blue method.

INTRODUCTION

Phosphorus was estimated both as total and as filterable phosphorus.

Total phosphorus (T.P.): phosphorus contained in both filterable and suspended organic compounds, acid hydrolyzable compounds and orthophosphate.

Total filterable phosphorus (T.F.P.): as for T.P. but without suspended matter. The separation was made with a 0.45-micron pore diameter membrane filter.

All results are expressed in milligrammes phosphorus per litre (mg P/l). The upper detection limit from the calibration curve for a 2-cm cell was 0.107 mg P/l and for a 5-cm cell, 0.042 mg P/l, whereas the lower limits were 0.002 mg P/l and 0.001 mg P/l. Measurements were made at the wavelength of maximum absorption of the complex, 700 nm.

Review of published data

Various methods have been reported for the estimation of reactive phosphorus

in sea water and fresh water in the past twenty years. Most were variants of the heteropoly-blue method in which the yellow molybdophosphoric acid was reduced to a blue complex whose optical density was measured. The sensitivity was increased when the blue complex was extracted into an organic solvent. One of the most reliable methods of converting all forms of phosphorus to the orthoform used persulphate oxidation with acid hydrolysis.[1-3] Stannous chloride reduced molybdophosphoric acid[4] and a mixture of benzene isobutanol extracted it from aqueous solution. Benzene, however, is highly toxic, can cause chronic poisoning and has insidious effects on the bone marrow,[5] so that an alternative procedure is desirable. Other methods[2,6] were developed from publications in 1958 and 1962[7] by Murphy and Riley. These used ascorbic acid to reduce the phosphomolybdate complex and isobutanol or *n*-hexanol to extract the blue complex which was formed.[8,9] The method detailed in this article was based on procedures set out in Standard Methods[4] and in the Canadian Fisheries Research Board's Manual of Sea Water Analysis.[9]

Scope of the investigation

As higher results occurred occasionally and at random for T.F.P. than for T.P. from the one sample the whole procedure was examined minutely. The stability of the calibration graph, the accuracy and precision of the analytical method and the recovery of a standard addition of phosphorus were checked. Possible sources of errors were investigated, such as oxidation time variation, extraneous phosphorus and arsenic interferences and adsorption on to the plastic containers and glass beakers. Other problems as sampling and sample storage were followed through also.

EXPERIMENTAL PROCEDURE

Apparatus

a) Spectrophotometer for use at approx. 700 nm, providing a light path up to 5 cm.

b) Optical cells with covers—2 cm and 5 cm.

c) Hot plate—a 30 × 45 cm heating surface.

d) Filter holder—all glass membrane filter apparatus with a 1 l suction flask.

e) Membrane filters with an average pore diameter of 0.45 micron.

f) Suction apparatus.

g) Analytical balance capable of weighing to 0.1 mg.

h) Dessicator provided with dessicant containing a colour indicator of moisture content.

i) Polyethylene screw-cap bottles—1 l.

j) Surgical cotton wool.

k) Glassware: Borosilicate glassware was used throughout; it was cleaned with sulphuric acid (Reagent d).

Reagents

Distilled water was used for dilutions and the reagents were analytical reagent grade.

a) *Standard phosphorus solution.* 0.2195 g of oven dried potassium dihydrogen phosphate was dissolved in water and diluted to 1 l. With a 100-fold dilution 1 ml = 0.0005 mg phosphorus.

b) *Standard arsenic (III) solution.* 0.660 g of arsenic trioxide (As_2O_3) was dissolved in 5 ml distilled water containing 2 g sodium hydroxide and diluted to 500 ml. After a 200-fold dilution 1.00 ml = 0.005 mg arsenic.

c) *Standard arsenic (V) solution.* 0.1041 g of $Na_2HAsO_4.7H_2O$ was dissolved in 500 ml of distilled water. With a 10-fold dilution, 1.00 ml contained 0.005 mg As.

d) *Sulphuric acid.* A 7N concentration was used for cleaning the glassware.

e) *Ammonium molybdate solution* $(NH_4)_6\ Mo_7\ O_{24}.4H_2O$. 30 g was dissolved in 1 l of distilled water and stored in a plastic bottle out of direct sunlight. This solution was stable indefinitely.

f) *Sulphuric acid.* 140 ml sulphuric acid, arsenic free, S.G.1.84, was added to 900 ml distilled water.

g) *Potassium antimonyl tartrate solution.* 0.68 g salt was dissolved in 500 ml of water. The solution was stable for a few months in a plastic bottle.

h) *Ascorbic acid (L+) solution.* A 5.4% aqueous solution was prepared on the day of use.

i) *Mixed reagent.* The following were mixed together in the listed order and used within 6 hr:

ammonium molybdate	(e)	100 ml
sulphuric acid	(f)	250 ml
ascorbic acid	(h)	100 ml
potassium antimonyl tartrate	(g)	50 ml

j) *Isobutanol.* A commercial grade, boiling range 106.5–109.5°C.

k) *Ethanol.* Methylated spirits denatured with 2% methanol.

l) *Potassium persulphate solution.* A 5% aqueous solution was prepared on the day of use.

m) *Sulphuric acid.* 31 ml concentrated acid (S.G. 1.84) was added to 69 ml distilled water.

n) Mixed reagent for arsenomolybdate reduction.[10] The following were mixed together on the day of use in the listed order.

sodium metabisulphite	14%	100 ml
sulphuric acid	3.6N	50 ml
sodium thiosulphate	1.4%	100 ml

o) *Mercuric chloride solution*—1%.

p) *Copper sulphate–citric acid solution.* A 0.25 mg/l solution of each salt.

q) *Iodine.* Solid, laboratory reagent grade.

Preparation of sample bottles

One labelled polyethylene screw-cap bottle was kept for each sampling site. So as to discourage bacterial action the dried bottles were treated with iodine vapour (q), about 0.5 g, for two days at about 28°C. The iodine crystals were washed away and then the bottles were filled with distilled water for a few days to remove excess iodine, as algal cells could be ruptured easily. Acid treatment was unsatisfactory, as prolonged washing with distilled water failed to remove the last traces of acid.

Storage of samples

Samples were stored either on ice during transportation back to the laboratory or were kept at 25°C. Thereafter they were kept at 5°C or at ambient room temperature. It was inadvisable to store samples for more than three days.

METHOD

Preparation of the sample

Two distilled water blanks were run parallel with each set of samples. The importance of thoroughly mixing any large volume of water before sampling is obvious but vigorous shaking is best avoided as the algae can be damaged or ruptured. Sample volumes of 200 ± 2 ml were required. Samples for T.P. and T.F.P. were processed alike after that for T.F.P. was filtered through a membrane filter. Each was evaporated to 50 ± 10 ml in a conical beaker. 1 ml sulphuric acid (m) and 15 ml potassium persulphate (l) were added and the sample boiled for $1 - 1\frac{1}{2}$ hr on a hot plate. Distilled water was added to maintain the volume.

Estimation of the orthophosphate

When step (b) was included a reduction of the arsenomolybdate complex

occurred. This procedure was referred to as (R.P.). When step (b) was excluded no correction for arsenic was made. This was the usual procedure (U.P.).

a) About 50 ml distilled water was added to the 500-ml separatory funnel, followed by the oxidized sample and three distilled water rinsings, each about 5 ml. The volume was adjusted to 200 ± 10 ml. The temperature of the sample was kept between 15 and 30°C.

b) 20 ± 1 ml mixed reagent (n) was added and the sample was shaken vigorously, immediately.

c) After 15 min, 20 ± 1 ml. of mixed reagent (i) was added and the sample was shaken immediately to mix reagents.

d) After 10 min for the U.P. or $1\frac{1}{2}$ hr for the R.P., 40 ± 1 ml. isobutanol, (j), was added. The funnel was shaken gently for 1 min and the aqueous layer was rejected after 5 min settling. The extracted coloured complex was stable in the alcoholic phase for 2–$2\frac{1}{2}$ hr.

e) The alcoholic phase was run into a dry 25-ml volumetric flask using a small glass funnel lightly plugged with cotton wool and made up to volume with ethanol (k).

f) The optical density was read at 700-nm wavelength, using 2-cm or 5-cm cells.

RESULTS

1. Calibration curve

The calibration curve repeated after an interval of two years was found to be identical.

2. Precision and accuracy

The number of samples tested, containing standard phosphorus, was 18, rejected no. Nil, mean 0.025 mg P/l, the S.D. was 0.00074 and the rel. S.D. was 3%. This was very satisfactory.

3. Recovery of added inorganic phosphorus

Phosphorus at a concentration of 0.025 mg P/l was added to well-mixed tap water. The number of samples tested was 19, rejected no. 1, mean 0.0539 mg P/l, the S.D. 0.00163 and the rel. S.D. was 3%.

Using the same well-mixed tap water the number of samples tested was 19, rejected samples nil, mean 0.0286 mg P/l, the S.D. 0.0012 and the rel. S.D. was 4.2%. This recovery of standard added phosphorus was satisfactory.

4. Alteration of the time of oxidation of organic matter.

The effects of this alteration are summarized in Table I, in which Ref. 13 mentioned in Table I, has been mentioned between refs 10 (on p. 4) and 11 (on p. 7). There are four sets of results obtained over a period. The same volume of tap water was used throughout but inorganic phosphorus was added to sets 2–4. On all occasions comparative results were obtained using oxidation periods of ½ hr versus 1½ hr. Contrary to fact, results for two sets indicated that the identical samples of each set were from different sites. As this indicated incomplete oxidation, a 1-hr versus 1½-hr oxidation period was tried and more reliable results followed. A minimum oxidation time of 1 hr was adopted, as 1½ hr was sometimes impracticable.

5. Sources of interference

Membrane filters Two sets of five membrane filters were each soaked in 200 ml distilled water for 24 hr. They yielded 0.008 and 0.005 mg P/l to the water. Subsequently, all filters were presoaked for 4 hr, a handy method of removing available phosphorus.

Rubber fittings A rubber bung sealed the glass millipore filter base to the filter flask. Two similar new bungs were soaked separately for 18 hr in 200 ml distilled water and the leached phosphorus was 0.058 mg/l and 0.069 mg/l. Consequently, a glass joint was substituted for the rubber bung.

Arsenic Golterman[11] reported that arsenate formed a similar complex with molybdate to that of phosphorus but Stephens[8] found that normal concentrations of arsenate did not interfere in phosphorus determinations. Johnson[10] observed that as arsenate formed a blue complex with molybdate and arsenite did not, the reduction of arsenate (As V) to arsenite (As III) eliminated its absorbance contribution in phosphorus measurements. Pentavalent arsenic is the stable form in water under aerobic conditions.[12] The reproducibility of the phosphorus graph with the extra R.P. was checked. Reduction to the blue complex was permitted to proceed for 1½ hr (see estimation, (d)) in both cases, U.P. and R.P., as the optical densities had to be read successively after identical time lapses. The slope of the curve for phosphorus standards by the R.P. was different from that obtained for phosphorus standards by the U.P. Differing quantitites of arsenate were added to samples containing phosphorus and the complete procedure under the previous heading, Method (R.P. and U.P.), was repeated eight times. The optical density for phosphorus and arsenic V was read as phosphorus. The R.P. curves were identical, both for added arsenate and for phosphorus alone, showing that pentavalent arsenic did not then interfere. There was no shift in the absorption maxima because of the addition of the reducing agent (n).

TABLE I

Alteration of the Oxidation Time

Test	No. of samples	Mean	Standard deviation	Dixon test[13] No. of samples rejected	F test[13]	T test[13]	Interpretation of F and T tests results
1. Variation of oxidation time							
1½ hr. reference	12	0.0161	0.00068	Nil	Table 2.9	Table 2.2	The reference and the ½ hr. samples are significantly different and came from different origins.
½ hr.	12	0.0153	0.00044	1	found 2.4	found 4.6	
1½ hr. reference	10	0.0466	0.00302	Nil			The reference and the ½ hr. samples are significantly different and came from different origins.
½ hr.	12	0.0503	0.00227	Nil			
1½ hr. reference	12	0.0299	0.00079	Nil	Table 2.8	Table 2.2	Both came from the same origin.
½ hr.	12	0.0307	0.00120	1	found 2.2	found 1.8	
2. 1½ hr. reference	10	0.0466	0.00302	Nil	Table 3.0	Table 2.2	Both came from the same origin.
1 hr.	12	0.0457	0.00242	1	found 1.5	found 1.0	The precision was the same for both reference and test group.

TABLE II

The optical density of differing quantities of pentavalent arsenic added to the same concentration of phosphorus (data from curves).

As V mg/l	P mg/l	Optical density R.P.	Optical density U.P.
0.025	0.050	0.36	0.45
0.050	0.050	0.37	0.51
0.100	0.050	0.36	0.61

The lowest standard addition of As V used was 0.025 mg/l and this had a marked effect on the optical density, U.P. From the method outlined, it seemed likely that trivalent arsenic would be oxidized to the pentavalent form by persulphate. Accordingly, arsenite (As III) at 0.050 mg/l was added to a solution containing As V and P. The same additive effect was evident.

As V mg/l	P mg/l	As III mg/l	Optical density U.P.
0.050	0.050	0.050	0.63

Some interference, probably by arsenic, appears in samples of untreated water from the Hawkesbury River at Windsor, N.S.W. The means of five samples were

mg P/l U.P.	mg P/l R.P.
0.0379	0.0344

No result was rejected by the Dixon Test.[13] The difference could be attributed, perhaps, to drainage from agricultural land alongside the river.

6. Adsorption of phosphorus on to

a) *Glass beakers* The effects of various delays in completing the analysis are set out in Table III. The samples used were of tap water, nos. 1, 2, 4 and tap water and added inorganic phosphorus for 3. All samples remained in glass beakers after oxidation, but the reference ones were processed im-

TABLE III

Delay in the completion of analysis

Test	No. of samples	Mean	Standard deviation	Relative standard deviation	Dixon test[13] no. of samples rejected	F test[13]	T test[13]	Interpretation of F and T test results
1. Baking the oxidized sample								
—reference	13	0.0161	0.00068	4.2%	Nil	Table 2.9	Table 2.2	Precision the same, but reference and baked samples are significantly different.
—baked	11	0.0145	0.00101	7.0%	Nil	found 2.2	found 5.0	
2. A two day delay after oxidation								
—reference	12	0.0161	0.00068	4.2%	Nil	Means close, thus no calculation		
—2 days	11	0.0159	0.00037	2.3%	Nil			
3. A three day delay after oxidation								
—reference	12	0.0299	0.00079	2.6%	Nil	Table 2.8	Table 2.2	Precision the same but two sets of figures are from the same sample.
—3 days	12	0.0299	0.00049	1.6%	1	found 2.4	found 1.9	
4. A five day delay after oxidation								
—reference	12	0.0161	0.00068	4.2%	Nil	Table 2.8	Table 2.2	Precision the same, but the reference and 5 day samples are significantly different.
—5 days	13	0.0148	0.00055	3.7%	1	found 1.4	found 3.3	

mediately. For the first set, the liquid was evaporated off and the solid residue was baked hard. In the other three sets further processing of the samples was delayed by 2, 3 and 5 days. From the results of 24 samples it was apparent that baking of the oxidised and hydrolyzed residue invalidated the results as adsorption had occurred. Inaccuracy also occurred when liquid samples remained in beakers for more than three days after oxidation. Thus the maximum break permitted was three days.

b) *Plastic bottles* Sampling was done at Ingleburn Dam and half the volume was transferred from the mixing container into stoppered borosilicate flasks. The mean value of total phosphorus from five samples was 0.0261 mg P/l. This reference estimation was commenced on the date of sampling. The 95% confidence level was 0.0261 ± 0.0012.

The other half of the sample was transferred from the mixing container into treated polyethylene bottles marked Ingleburn Total. Samples were received in the laboratory within 2 hr and were stored at 5°C for six days. The mean of five samples for total phosphorus was 0.0242 mg/l. Thus phosphorus was adsorbed on to plastic bottles during storage.

7. Means of preservation

Fitzgerald and Faust[14] found that copper sulphate and citric acid (1:1) inhibited some algae but did not kill them, whereas treatment with chloroform resulted in the release of significant amounts of phosphorus. They also noted the value of refrigeration at 3–5°C in retarding phosphorus release and the advisability of filtration on site. Standard Methods[4] mentioned that the best single procedure was preservation by freezing at or below −10°C either in the presence or absence of 40 mg $HgCl_2$/l.

A freezing cabinet was unavailable so an alternative method was sought. As power is not available at some sampling sites in the Board's extensive catchment area and as immediate filtration to separate particulate matter was advisable but impracticable, other means were sought to prolong the status quo of the T.P./T.F.P. ratio. As some of the sites involve a day's travelling time back to the laboratory a closer site at Ingleburn Dam was chosen. Water was drawn from 0.30–0.45 m below the surface for the 134 samples. Samples were collected into treated plastic bottles, marked Ingleburn Total or Ingleburn Dissolved, and the 134 were well mixed in a clean plastic can. Seven samples were filtered on the spot for T.F.P. and seven others were put aside for T.P., all as reference samples (R.S.). They were put into stoppered borosilicate flasks. The remaining 120 samples were distributed into 24 plastic bottles. Those samples kept at ambient room temperature for one day were then refrigerated at 3–4°C. Those returned in ice were refrigerated immediately and the ones treated with mercuric chloride

and with the copper sulphate–citric acid were refrigerated after one day. A concentration of 0.25 mg/l of copper sulphate was chosen because the effective range for killing was 0.5–2.0 mg/l. Some algae, especially blue greens, are susceptible at this concentration. Algae were numerous at this time. The effects of the various preservation methods examined are summarized in Tables IV, V, VI.

TABLE IV

Results obtained using various methods of preservation. The means are listed.

	Total phosphorus mg/l			
	Ambient temp.	On ice	Mercuric chloride	Copper sulphate
Reference (R.S.)	0.0217			
Analyzed after 5 hr.	0.0205	0.0210	0.0226	0.0226
1 day	0.0208	0.0215	0.0220	0.0215
4 days	0.0213	0.0207	0.0215	0.0193

TABLE V

Results obtained using various methods of preservation. The means are listed.

		Dissolved phosphorus mg/l			
		Ambient temp.	On ice	Mercuric chloride	Copper sulphate
Reference (R.S.)		0.0049			
Stored in plastic	5 hr	0.0044	0.0044	0.0099	0.0071
bottles, filtered	1 day	0.0041	0.0046	0.0111	0.0072
and analyzed.	4 days	0.0082	0.0054	0.0088	0.0080

See Table IV The 95% confidence limits placed the value of the reference sample (R.S.) at 0.0217 ± 0.005 and the S.D. was 0.0005. The results are generally variable but it seemed that some loss of phosphorus from solution occurred after four days from samples treated with copper sulphate. The higher results after 5 hr storage for samples treated with copper sulphate or mercuric chloride were difficult to explain but slight variations could occur owing to the difficulty in obtaining identical individual samples from the bulk one. Some adsorption on to the wall of the plastic bottles probably

TABLE VI

Effect of storage

Test	No. of samples	Mean	Standard deviation	Relative standard deviation	Dixon test[13] no. of samples rejected	F test[13]	T test[13]	Interpretation of F and T test results
1. Total phosphorus 1 day room temp. +3 day at 3–5°C								
—reference	10	0.0466	0.00302	6.5%	Nil	Table 3.1	Table 2.3	Precision the same, the samples were from the same population.
—stored	13	0.0433	0.00140	3.2%	1	found 2.3	found 1.7	
2. Dissolved phosphorus 1 day room temp. +3 day at 3–5°C								
—reference	9	0.0389	0.00032	0.8%	Nil	No values calculated as the means were close.		
—stored	12	0.0385	0.00050	1.3%	1			

occurred after four days. Methods of preservation have little effect on total phosphorus results.

See Table V The 95% confidence limits placed the value of the reference sample (R.S.) at 0.0049 ± 0.0003 and the S.D. was 0.0003. The use of mercuric chloride and of copper sulphate–citric acid mixture resulted in a large increase in dissolved phosphorus. It seems likely that algal cells were damaged and that phosphorus was released. With the other two methods it seems that some phosphorus was taken up by the cells during the first hours of storage and released later.

The total organic carbon of this water sampled was 3.5 mg/l and the plate count on standard yeast extract agar was 1300/ml for 3 days at 22°C.[15]

See Table VI Tap water was spiked with inorganic phosphorus and stored in treated plastic bottles for 1 day at 25°C and then 3 days at 3–5°C. The reference samples were analyzed directly after sampling. Total phosphorus results showed no significant loss compared with the reference ones. The reference samples filtered after collection gave readings almost identical to those of the stored ones. This reticulated water contained few algae and had a low biological activity.

During this study the main variable in a sample was the susceptibility of algae to unintentional damage by known lytic agents such as preservatives and also to unknown ones as viruses. This susceptibility could depend upon their age, fragility, the water temperature and its composition. If it is practicable, it is advisable to commence analysis within two days of sampling. It was simple to estimate phosphorus precisely, but accuracy was affected by many variables, e.g., the most fundamental being that of obtaining a representative sample. This figure may be influenced if repetitive sampling is done at different times of the day.

Acknowledgement

The author wishes to thank the Metropolitan Water, Sewerage and Drainage Board, Sydney, for permission to publish this paper.

References

1. M. E. Gales, E. C. Julian, and R. C. Kroner, *Journal of American Water Works Association* **58**, 1363–1368 (1966).
2. J. E. Harwood, R. A. van Steenderen, and A. L. Kühn, *Water Research* **3**, 425–432 (1969).
3. D. E. Sanning, *Water and Sewage Works* **114**, 131–133 (1967).
4. A.W.W.A., A.P.H.A., and W.P.C.F., Standard Methods for the Examination of Water and Waste Water (American Public Health Association, Washington, 1971) 13th Edition, pp. 518–534.

5. K. P. Dubois and E. M. K. Geiling, *Textbook of Toxicology* (Oxford University Press, New York, 1959) pp. 198–199.
6. J. E. Harwood, R. A. van Steenderen, and A. L. Kühn, *Water Research* **3**, 417–423 (1969).
7. J. Murphy and J. P. Riley, *Anal. Chim. Acta.* **27**, 31 (1962).
8. K. Stephens, *Limnology and Oceanography* **8**, 361 (1963).
9. J. D. H. Strickland and T. R. Parsons, *A Manual for Sea Water Analysis* (Fisheries Research Board of Canada, Ottawa 1965) Bulletin No. 125, 2nd Edition, pp. 43–65.
10. D. L. Johnson, *Environment Science and Technology* **5**, 411 (1971).
11. H. L. Golterman and R. S. Clymo (ed.), *Methods for Chemical Analysis of Fresh Waters* (Blackwell Scientific Publications, Oxford, 1970) IBP Handbook No. 8, p. 78.
12. J. F. Ferguson and J. Gavis, *Water Research* **6**, pp. 1259 (1972).
13. G. H. Brown and E. M. Sallee, *Quantitative Chemistry* (Prentice Hall Inc., Englewood Cliffs, N. Jersey, 1963) pp. 103.
14. G. P. Fitzgerald and S. L. Faust, *Limnology and Oceanography* **12**, 332 (1967).
15. Reports on Public Health and Medical Subjects No. 71, *The Bacteriological Examination of Water Supplies* (Her Majesty's Stationery Office, London, 1969) 4th ed., pp. 7, 34, 45.