On the Amount of Phosphoric Acid in the Sea-Water off Plymouth Sound.

By

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In spite of its biological importance, only a few investigators have turned their attention to the content of the sea in phosphoric acid. The older analyses by C. Schmidt (1) and Forchammer are quoted by K. Brandt (2). Schmidt found from 2·8 mg. to 5·5 mg. of P_2O_5 per 1000 grms. of water in the colder seas, and from 0·76 mg. to 1·8 mg. in warmer regions, while Forchammer found from 4·6 mg. to 12·5 mg. in the Gulf of Finland. Krümmel (4) refers to these as inaccurate, and quotes the results of analyses made much more recently by E. Raben, in connection with the International Fishery Investigations, as alone reliable. K. Brandt (3) also refers to Raben's work in various papers, but neither the present writer nor Professor Martin Knudsen, of Copenhagen, who has very kindly made a search also, has been able to find any original paper by Raben on the subject, so that we unfortunately know nothing of his methods beyond the fact, given by Brandt, that the samples were filtered immediately after collection through Schleicher and Schull's hardened paper. This is a very necessary precaution, as there would be danger of an increase in the phosphates owing to the decomposition of suspended organic matter by bacteria. The present writer has found that even a filtered sample cannot be put aside in safety for any length of time unless previously sterilised, as the whole of the phosphoric acid may be removed by the growth of moulds.

Krümmel mentions also some analyses by Sir John Murray as equally defective.

Raben's samples were collected in the Baltic and North Sea ; he found as a rule less than one milligram of P_2O_5 per litre, with a minimum of 0·14 mg. to 0·25 mg. in February and May, and a maximum of 1·46 mg. in the autumn.

The present writer, in sea-water taken half a mile outside the Breakwater at Plymouth, has found as a maximum 0·1 mg. per litre, less than the lowest result given by any of the workers mentioned above.

The determination of phosphoric acid in sea-water falls into two parts, first concentration in small bulk, and then determination of the amount. Concentration may be effected either by precipitating, by the addition of ammonia, a portion of the alkaline earths, which carries down the phosphoric acid, or by adding an iron salt, ammonium chloride, and ammonia and precipitating a mixture of ferric phosphate and hydroxide. The estimation may be carried out either gravimetrically as ammonium phospho-molybdate, or colorimetrically by the very sensitive reaction with nitro-molybdate of strychnine described by Pouget and Chouchak (5). The writer finally adopted the colorimetric method following concentration with iron, but all four processes have been used.

From April 21 to September 13, 1915, the method was as follows: About 1500 ccm. of filtered water was precipitated, after making slightly acid and heating, with iron and ammonium chloride and ammonia. The precipitate was filtered off, dissolved in warm dilute nitric acid, and evaporated to dryness several times with further addition of acid to remove silica. The phosphorus was determined by precipitating with ammonium molybdate and weighing as anhydride. For such small quantities, the amount of anhydride being only a few milligrams, the method cannot be considered satisfactory, as there is danger of loss through some of the precipitate dissolving in the wash-waters or being carried through the asbestos of the Gooch crucible, and on the other hand there is the risk of the results being too high owing to the simultaneous precipitation of a trace of molybdic acid. With one exception the figures obtained were much higher than most of those by the colorimetric method. From April 21 to July 14 about 0·07 mg. per litre was found gravimetrically; there was then a break in the series and the next analysis, made on September 13, showed a considerable fall to 0·04 mg. by the gravimetric method, in good agreement with that for September 21, when 0·046 mg. was found by the colorimetric method, which was adopted for all following work.

Samples for the colorimetric method were at first concentrated by precipitating 500 ccm. of filtered sea-water with 3 ccm. of pure concentrated ammonia, heating, and filtering off the voluminous precipitate of hydroxides, dissolving it in nitric acid, and evaporating on the water-bath to dryness, after which it was treated as described later. The method had the advantage that only one reagent was used for precipitation, and as a rule the solution filtered quickly and duplicate analyses agreed well, the average difference being 0·0029 mg. per litre. Its accuracy, however, was difficult to establish without making up an artificial sea-water free, or nearly so, from phosphates, and this was found to be impossible with the purest chemicals of Merck and Kahlbaum. As an example, the artificial sea-water was found to contain 0·0143 mg. of P_2O_5 in 500 ccm.; 0·0234 mg. was added to 500 ccm. and 0·0328 mg. found, a loss of 0·0049 mg. In another experiment made on 250 ccm. the loss was only 0·0007 mg. These differences are both of about the same magnitude as those found between duplicate analyses of sea-water, so the experiments are not conclusive, though it shows that the method is at any rate approximately accurate. Comparison was also made with the method finally used; simple precipitation by ammonia gave 0·0312 mg. per litre, against 0·0318 mg. and 0·0316 mg. by the final method. Again, precipitation by ammonia gave 0·057 mg. in duplicate analyses; treatment of the filtrate from one of these by ammonia gave a further voluminous precipitate which contained no recognisable phosphates. The method is therefore probably accurate to about 0·003 mg.

In the end, concentration by iron was found to be the most satisfactory and quickest method, duplicate determinations taking five hours or less when the water had been previously filtered. The requisites are:—

Ferric nitrate or chloride solution, nearly neutral, containing 5 to 6 mg. of iron in 1 ccm.

Nitric acid, strong and 25 per cent by volume.

Ammonia, dilute; 2 N is a convenient strength.

Ammonium chloride; about 2 N.

Pouget and Chouchak's reagent; two solutions are required. A: 95 grms. of molybdic acid and 30 grms. of anhydrous carbonate of soda are dissolved in 500–600 ccm. of warm water, and after cooling 141 ccm. of strong nitric acid are added. The solution is made up to 1000 ccm. B: a 2 per cent solution of strychnine sulphate. For use 1 ccm. of B is added to 10 ccm. of A and the mixture filtered and used at once. With 0·03 mg. of P_2O_5 in 50 ccm. of 3·7 per cent nitric acid, this reagent gives a strong opalescence at once, while 0·005 mg. will give the reaction in a few minutes. This opalescence is yellow when examined in the colorimeter; it takes twenty minutes to attain its full strength, and after three or four hours a precipitate is thrown down, so the comparisons should be made as soon as possible after the twenty minutes have elapsed. The colour is proportional to the amount of phosphoric acid when the content in P_2O_5 lies between 0·01 mg. and 0·05 mg. in 50 ccm.; it is affected by variations in the amount of reagent used and by the amount of free nitric acid. Pouget and Chouchak give a number of determinations in the presence of various oxides, and show that the results are very good unless the oxides are present in very large amount; for instance, lime is without influence when there is not more present than 20,000 times the weight of the phosphorus pentoxide, while iron should not exceed 1200 times its weight. They recommend also that for the greatest accuracy two standard solutions should be made up, one containing 0·03 mg. P_2O_5 for use with samples containing this amount or more, and another containing 0·02 mg. for samples containing less than 0·03 mg.

Colorimeter. The writer has used the Dubosq pattern with comparison tubes 5 cm. in height and a swinging shade in front to cut off side light. For the comparison of phosphorus samples the model with 10 cm. tubes would probably have been better.

Filter papers should be washed with dilute nitric acid and hot water. The writer has found traces of phosphoric acid in two of the best-known hydrofluoric-acid washed papers.

Porcelain and *glass* should be tested before use by extraction with hot dilute nitric acid and dilute ammonia. There are some varieties which will give up several milligrams of P_2O_5 during an analysis.

India-rubber should not be allowed to come in contact with the acid or alkaline solutions.

The analyses have been carried out as follows: The samples were taken in glass-stoppered "Winchester quart" bottles, holding about 2700 ccm. As they were collected so near to the Laboratory it was generally possible to begin the filtration within three hours, so that there was no necessity for sterilisation. Filtration was carried out by replacing the glass stopper by one of rubber through which passed two glass tubes, which projected about 6 mm. on the inner side; outside the bottle one projected about 25 or 30 mm., the other a few millimetres less. The bottle, full to the stopper, was quickly inverted on a retort ring with the tubes projecting into the filtering funnel below the upper edge of the filter paper. Filtration then goes on without attention; as a rule it was started in the afternoon and was finished by the following morning. Double papers were always used, sometimes Schleicher and Schull, No. 589, "black band" inside, to catch the coarser particles, with a "blue band" outside; at other times Whatman's papers, No. 1 or No. 40. If there is much sediment No. 40 is almost too slow; one sample took thirty-six hours to filter.

As a rule 500 ccm. was taken. The water was measured into a hard glass beaker, and 10 ccm. of 2 N ammonium chloride and 1 ccm. of the iron mixture were added, with a few drops of dilute nitric acid to dissolve the precipitate. The mixture was heated to 70° or 80° C. on the water-bath and precipitated with the smallest possible quantity of dilute ammonia; the heating was continued until the precipitate had collected together, when it was filtered on a small washed paper and washed twice with hot water. The precipitate on the filter and adhering to the beaker was dissolved in warm dilute nitric acid and evaporated to dryness on the water-bath to remove silica. Seven cubic centimetres of 25% HNO_3 and 20 ccm. of water were added, the dish covered, and the solution heated for twenty minutes, when it was transferred to a 50 ccm. graduated flask. If there is much insoluble residue the solution should be filtered. The bulk was then made up to about 47 ccm., leaving space for 2 ccm. of reagent. The standards were prepared by making up the requisite amounts of P_2O_5 to about 47 ccm. with 7 ccm. of 25% HNO_3 and water; the writer has generally diluted the $\frac{1}{15}$-molecular phosphate solutions used in determining hydrogen-ion concentrations by the Sorensen method. Two cubic centimetres of the strychnine-molybdate reagent were then added to each flask, the bulk completed to the mark, and the whole well shaken. In twenty minutes the solutions are ready for comparison.

The writer has never been able to secure perfect equality of illumination in the two halves of the field of the colorimeter owing to the shape and setting of the window in the Laboratory, so the precaution was always taken of reversing the position of the tubes after six readings and then taking another six. The accuracy with which the readings could be made varied very much. On some days a set of six have been obtained which did not differ by more than 0·2 mm. on 40 mm., while at other times the uncertainty was from five to ten times as great. A large sheet of ground glass between the colorimeter and the window was often of great assistance. Comparisons by artificial light were very difficult and fatiguing, but the results were satisfactory.

To test the accuracy of the method the following experiments were made:—

Part of a standard, containing 0·0237 mg., was analysed against itself. In two experiments the results were too low by 0·0005 mg. and 0·0004 mg.

Three lots of 500 ccm. each of distilled water, to which 0·0237 mg. had been added, were analysed; the errors were, +0·0010 mg., +0·0041 mg., and +0·0001 mg. From another 500 ccm., to which no phosphate had been added, 0·0036 mg. was obtained. The filtrate from this was acidified and analysed again without adding any more ammonium chloride. The amount found was again 0·0036 mg. This value was taken as the blank instead of the mean, 0·0025 mg. The result in which an excess of 0·0001 mg. was found was probably erroneous owing to the evaporation having been carried out on the sand-bath, which might give rise to overheating and formation of pyrophosphates. An artificial sea-water was made up and found to contain 0·0100 mg. To this was added 0·0150 mg., and analysis showed a loss of 0·0008 mg. The result is not conclusive as the blank on the sea-water was so high.

To test the effect of varying bulk, two lots of natural sea-water were examined, one in its natural state, the other after evaporation to small bulk. The amounts found were 0·0318 mg. and 0·0316 mg. respectively. A third portion, precipitated by strong ammonia only, gave 0·0312 mg.

Filtrates from the iron precipitate were also examined. The amount found by analysis of two lots of a sea-water were 0·0378 mg. and 0·0391 mg. To one filtrate 0·0237 mg. was added and 0·0266 mg. found, a gain of 0·0029 mg. No more phosphate was added to the other and 0·0025 mg. was found, using a very dilute standard. In another experiment the figures for the original analyses were 0·0336 mg. and 0·0348 mg.; 0·0237 mg. was added to each filtrate and gains of 0·0013 mg. and 0·003 mg. were obtained. These gains are all small, the average being 0·0018 mg., half the blank on the reagents. Finally the filtrate from the sample mentioned above as having been concentrated to small bulk was examined after the addition of 0·0237 mg. The gain in this case was higher, 0·0058 mg.

The fact that the errors on the filtrates, though small, were all positive, made it seem possible that there was still phosphorus, though not necessarily phosphoric acid, unprecipitated. To test this three lots of 500 ccm. were taken; two were analysed in their natural condition, giving 0·0336 mg. and 0·0348 mg. The third portion was boiled for three-quarters of an hour with 10 ccm. of decinormal potassium permanganate in Jena glass; it was then acidified with HCl. and boiled for two hours longer. On analysis 0·0558 mg. was found, a gain of 0·0216 mg. Another sea-water gave 0·0415 mg. and 0·0566 mg. for the natural and oxidised portions, a gain of 0·0147 mg. The filtrate from the oxidised portion was analysed without the addition of more phosphoric acid and 0·0018 mg. found. A similar experiment made earlier by the gravimetric method showed an increase from 0·082 mg. to 0·147 mg. on oxidation. In one case an untreated water was found to contain 0·09 mg. by the gravimetric method; the filtrate was oxidised and yielded a further 0·07 mg

There are two possible explanations of this increase of the phosphates by oxidation. One is that there may be in sea-water a small quantity of organic matter which hinders but does not completely prevent the separation of the phosphoric acid by iron; the objection to this is that the action would probably be irregular and the duplicate analyses would differ more widely than they do. The other, to which the writer inclines, is that there is a considerable amount of phosphorus present in forms other than phosphoric acid, perhaps as phosphites or as an organic compound, which is oxidised to phosphoric acid by potassium permanganate.

The ratio of phosphoric acid found in the untreated sample to the total shows a tendency to constancy, but it has not been determined yet whether the permanganate method converts the whole of the phosphorus into phosphoric acid, and experiments on this are in progress.

The samples of sea-water, with two exceptions, were taken at the Knap Buoy, half a mile outside the lighthouse on Plymouth Breakwater. The other two were taken close to the rocks under the Laboratory.

The results are given in the following table, and also the method by which they were obtained.

The salinity was determined against the *International Normal Water*.

SURFACE SAMPLES TAKEN AT THE KNAP BUOY.

Date.	G.M.T.	S. ‰	Method.	P_2O_5, mg. per litre.	
				Found in duplicates.	Mean.
1915					
April 21	10.30 a.m.	—	Iron and gravimetric	—	0·1
,, 23	—	—	,, ,,	—	0·1
June 21	10.30 a.m.	—	,, ,,	—	0·049
July 5	noon	—	,, ,,	—	0·064
,, 12	10.30 a.m.	—	,, ,,	—	0·09
,, 14	11.40 a.m.	34·83	,, ,,	—	0·082
Sept. 13	11.30 a.m.	34·92	,, ,,	—	0·04
,, 21	10.30 a.m.	34·96	Ammonia and colour	—	0·046
Nov. 24	11.35 a.m.	34·78	,, ,,	0·042, 0·041	0·0415
,, 26	11.45 a.m.	34·43	,, ,,	0·040, 0·034	0·037
,, 29	11.10 a.m.	34·14	,, ,,	0·040, 0·037	0·0385
Dec. 2	12.20 p.m.	—	,, ,,	0·0484, 0·0435	0·0460
,, 9	10.30 a.m.	31·46	,, ,,	0·049, 0·047	0·048
,, 13	11.30 a.m.	—	,, ,,	0·044, 0·041	0·0425
,, 16	12.10 p.m.	26·20	,, ,,	—	0·043
,, 20	11.25 a.m.	29·69	,, ,,	0·058, 0·064	0·061
1916					
Jan. 3	11.35 a.m.	25·66	,, ,,	0·057, 0·057	0·057
,, 14	1.55 p.m.	33·87	Iron and colour	0·0318, 0·0316	0·0317
,, 18	2.30 p.m.	33·93	,, ,,	0·0336, 0·0348	0·0342
,, 24	11.20 a.m.	33·42	,, ,,	0·0378, 0·0391	0·0384
Feb. 5	12.30 p.m.	31·58	,, ,,	0·0507, 0·0414	0·0460

SURFACE SAMPLES TAKEN UNDER LABORATORY.

1916					
Jan. 17	—	—	Iron and colour	0·0602, 0·0572	0·0587
Feb. 11	—	—	,, ,,	0·0408, 0·0421	0·0414

A few points come out clearly from the results.

In the first place, the results are much lower than those obtained by Raben for the Baltic and North Sea, his lowest being 0·14 mg. per litre against 0·1 mg., the highest found at Plymouth. The salinity at the Knap Buoy is nominally 33·5 per thousand up to nearly 35, comparable with a large part of the North Sea. But the North Sea receives enough fresh water from the great rivers of Russia and Germany to keep the salinity of nearly the whole of it below 35 per thousand, while the effect of the land drainage in the western part of the English Channel is confined to a comparatively narrow band along the coast. The effect of an increased supply of land water in increasing the phosphoric acid is seen in the results for December 20 and January 3, when the salinity was very low; a sudden rise occurred then after the figures had been fairly constant for two months or more. This rise did not show itself till a few days after the salinity had fallen, which suggests that much of the phosphorus from the land enters the sea in an incompletely oxidised form and is then converted, by bacterial action, into phosphoric acid.

If the earlier gravimetric results are taken as correct there is a decided seasonal change, the higher values being found in spring and summer, but the writer is not inclined to place much confidence in them. The experiments are being continued and it is hoped that the next few months will settle the question.

It is unfortunate that so far it has not been possible to obtain samples at a greater distance from shore, as it may be that the increased phosphoric acid found after oxidation is a purely littoral or estuarine phenomenon resulting from the form in which part of the phosphoric acid is carried down by land-water. It does not seem likely that it arises from diatoms or bacteria which pass through a paper filter, as the same increase was noticed on oxidising a filtrate from a solution in which iron had been precipitated by ammonia, a very efficient method of removing the finest suspended particles. The approximately constant ratio of the two forms of phosphorus is also an objection to this explanation.

SUMMARY.

1. Phosphoric acid in sea-water may be determined with an accuracy of about 0·003 mg. per litre by concentration with iron and colorimetric examination.

2. If the sea-water be previously oxidised by potassium permanganate the amount found is considerably increased.

3. From September, 1915, to February, 1916, the average amount of phosphoric acid in water collected half a mile outside Plymouth Break-

water was 0·044 mg. per litre; this showed signs of an increase when the supply of land-water rose after rain. The figures are much lower than those found by Raben for the Baltic and North Sea.

4. The amounts found by another method during the previous spring and summer are higher, but the figures cannot be considered quite trustworthy.

Note.—Since the above was written, it has been found that boiling with potassium permanganate does not oxidise the whole of the phosphorus with certainty. Duplicate analyses of a sample taken on Jan. 17th, 1916, both gave 0·0190 mg. per litre. Two other portions were oxidised and found to contain 0·0449 mg. and 0·0569 mg. per litre.

An attempt has also been made to determine whether any of the phosphoric acid is reduced to other forms in the short interval between the collection of the water and the beginning of the analysis. A sample of water was sterilised with toluol immediately after taking. A single determination showed 0·0350 mg. per litre in the original sample, and 0·0375 mg. after oxidation. But the water for over a week had been extraordinarily clear and free from suspended matter, far more so than any of the samples given in the table, so that the experiment cannot be considered conclusive.

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