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The Nitrite Content of Sea-Water.

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With 1 Figure in the Text.

THE estimation of chemically bound nitrogen compounds in sea-water has, in the past, been a matter of considerable difficulty because of the small quantities of these substances involved. They are present in the form of free and saline ammonia, nitrites, nitrates and organically bound nitrogen. The ammonia has usually been estimated by distilling it off in alkaline solution and determining colorimetrically in the slightly acid distillate by means of Nessler's reagent. The nitrites and nitrates are first of all reduced to ammonia, which is then distilled off in alkaline solution and estimated as before. The organic nitrogen compounds are first converted to ammonia by the Kjeldahl method or a modification thereof which is estimated as before.

Even when comparatively large quantities of sea-water are used, the error of these methods is considerable, as is admitted by the investigators themselves. In making a series of analyses of the nitrogen compounds in sea-water by these methods I found the chief difficulty to be in the use of the Nessler reagent. In such dilute solutions of ammonia the addition of even a slight excess of Nessler reagent produced a greenish tint which interfered with the matching of the colours. In addition the process is long and requires considerable quantities of sea-water.

Recently Gad-Andresen (**5**) has described a method for the estimation of nitrogen compounds in sea-water which, as it is volumetric, is a great advance on previous methods. The process consists of a preliminary conversion of nitrites, nitrates, and organic nitrogen to ammonia and subsequent decomposition of this with alkaline hypobromite in Barcroft's differential manometer. The nitrogen gas liberated is measured by the difference of pressure, and, the constants of the apparatus being known, the ammonia, and thus the other compounds, can be estimated. The free and saline ammonia are estimated by distilling off in alkaline solution after concentration of the sample, and measuring the liberated nitrogen as before.

A series of test analyses were made, using this method, and it was found that with solutions of artificial sea-water containing known amounts of nitrates and ammonia, the agreement between theoretical and calculated results was good, the error being about ± 5 per cent, which is the accuracy claimed by Gad-Andresen. The only disadvantage of the method for these compounds seems to be the length of time required to complete a series of analyses. Its advantages lie in its accuracy and the comparatively small quantities of sea-water required.

With regard to nitrites it was found that the method was unsuitable. The samples are preserved by adding 1 c.c. per litre of a saturated solution of sulphur dioxide or concentrated sulphuric acid. Solutions of sulphur dioxide, *i.e.* sulphurous acid, are unstable and decompose on standing to give sulphur and sulphuric acid,

$$3\,H_2SO_3 \longrightarrow S + 2\,H_2SO_4 + H_2O,$$

and sulphuric acid even in the cold in dilute solutions, decomposes nitrites to give nitric acid and nitric oxide.

$$3\,HNO_2 \longrightarrow HNO_3 + 2\,NO + H_2O.$$

To find whether this would take place in sea-water in the great dilution in which nitrite is known to be present, 500 c.c. sea-water were treated with 0·5 c.c. sulphuric acid as described and distilled. A sample of the original sea-water tested with Ilosvay's modification of Griess' reagent gave in thirty minutes a faint pink coloration indicating the presence of a small quantity of nitrite. The first 100 c.c. of the distillate gave a slightly stronger reaction than the original sea-water, showing that all the nitrite had not been decomposed by the sulphuric acid. As distillation was started a few minutes after adding the sulphuric acid, and other samples which had been preserved with sulphuric acid gave negative results, it must be concluded that it is unsafe to use sulphuric acid for the preservation of nitrites in sea-water. No reaction was obtained with subsequent portions of the distillate, nor did the residue in the flask give a positive result as would be necessary for estimation by Gad-Andresen's method. Unfortunately also, the nitrite on decomposing gives rise to nitrates which will then affect the nitrate estimation in the sample.

To estimate the nitrite in sea-water, it seemed worth attempting to use a colorimetric method as there are several of great delicacy. Eventually the Griess method as modified by Ilosvay (**6**) and applied to sea-water by Buch (**3**) was adopted. It has the advantage over other methods in that it is sufficiently sensitive to enable the nitrite in sea-water to be estimated directly without any preliminary concentration of the nitrite as is necessary with the m-phenylenediamine method or the Trommsdorf method. The reaction depends on the formation of a pink azo dye by the action of the nitrite in acid solution on a mixture of α-napthylamine and sulphanilic acid.

The two following solutions are required :—

Sulphanilic acid. One gram of sulphanilic acid is dissolved with the aid of heat in 14·7 grams of glacial acetic acid mixed with an equal bulk of water. More water is gradually added to the warmed liquid with constant stirring till 285 c.c. have been used altogether.

α-Napthylamine. 0·2 gram of α-napthylamine is dissolved with the aid of heat in 14·7 grams of glacial acetic acid mixed with twice its bulk of water. More water is added till 325 c.c. have been used altogether.

These solutions are kept separately and mixed in equal proportions just before they are required. The solution of α-napthylamine tends to become pink on keeping owing to the development of nitrite in the solution from ammonia in the air. Should this occur a fresh solution must be prepared. These solutions are not affected by light.

The quantitative application of the method to sea-water has been described by Buch (**3**), who showed that the effect of varying salinity was negligible, and that the effect of varying temperature could be avoided by having standard and unknown at the same temperature during colour development. One or two modifications of his method have been adopted for ease of colour reading and for convenience at sea.

Standard solutions are prepared containing 0·01 mg. nitrite nitrogen per litre and 0·005 mg. nitrite nitrogen per litre and 100 c.c. of each placed in separate beakers. 100 c.c. of the sea-water sample to be examined are placed in another beaker and 4 c.c. of the mixed Griess-Ilosvay reagent added to each. After standing for a length of time corresponding to the amount of nitrite present the colours are compared in Hehner tubes in an apparatus such as is described by Atkins (**2**) for the colorimetric estimation of phosphates in sea-water. The tubes are rested on a thin glass plate, through which light is reflected by an opal glass plate, the light being cut off from the remainder of the cylinders by a black case. The height of the liquid in the stronger solution is adjusted till the colours are of equal intensity. From the relative heights the amount of nitrite in the sea-water sample can be estimated. For 0·01 mg. nitrite N. per litre, fifteen minutes at room temperature gives a readable colour. If the sample is more dilute the time should be increased to half an hour or one hour. As the colour development goes on for at least two hours comparisons must only be made on samples to which the reagent has been added simultaneously.

The dilute standard solutions change their nitrite content readily, and, to ensure accuracy, dilution from a strong solution of nitrite must be made ; this strong solution must be replaced frequently. A suitable standard for dilution may be prepared by dissolving 0·6075 gram pure crystalline potassium nitrite in a litre of distilled water free from nitrite.

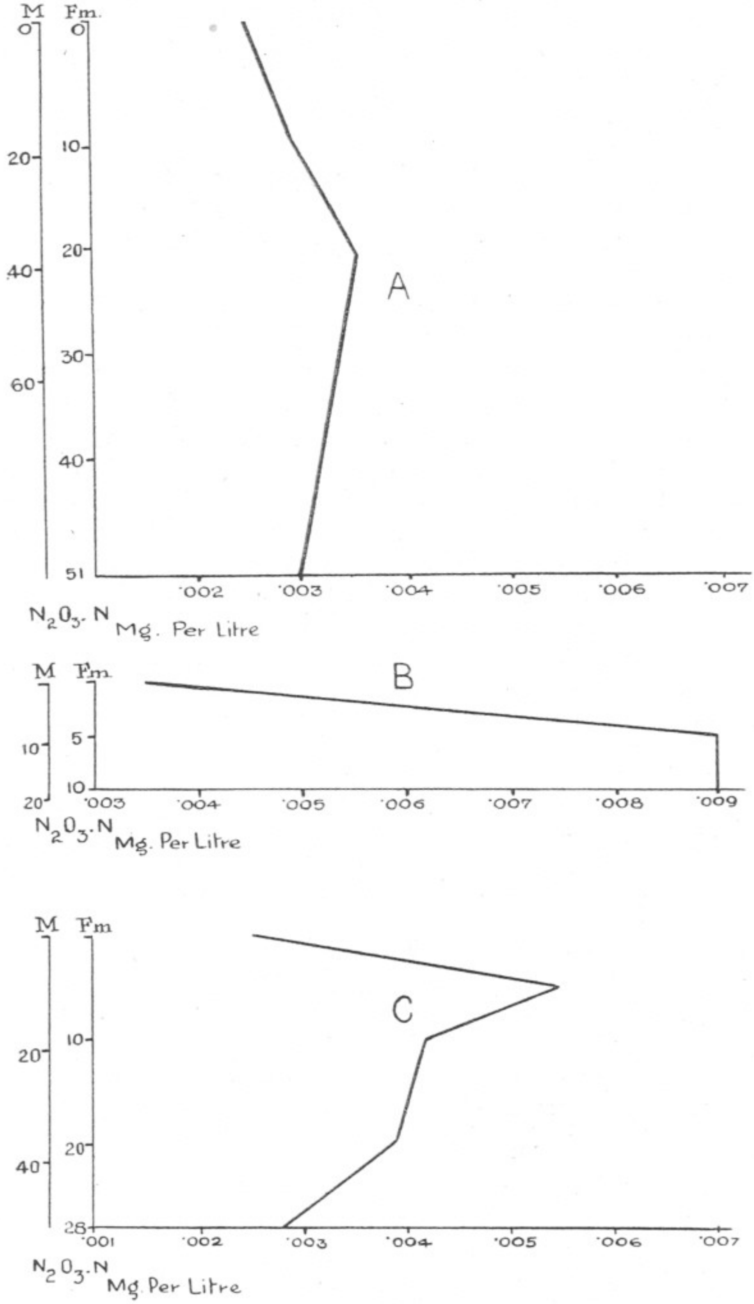
This solution contains 100 mg. nitrite N. per litre. All dilutions must be made with nitrite free distilled water.

A series of samples containing quantities of nitrite nitrogen, varying from 0·01 mg. to 0·002 mg. per litre treated as above, could be easily distinguished, and a closer approximation to their real value obtained by comparing in Hehner tubes. For example, a sample containing 0·01 mg. per litre compared with a sample containing 0·005 mg. per litre gave a reading of 96 against 42, *i.e.* 0·0046 mg. per litre; a sample containing 0·002 mg. per litre compared with the sample containing 0·005 mg. per litre gave a reading of 95 against 35, *i.e.* 0·0018 mg. per litre.

An examination of the nitrite content of sea-water at different places and at different depths in the Clyde Sea Area was made. The samples were collected in Winchester or half Winchester quart bottles, and the analysis of the samples carried out within a few hours of their collection and generally at sea. The reason for this is that the nitrite values change readily on keeping, as will be shown later. A few of the results are shown in text Fig. 1: (*a*) is a station in the estuary, (*b*) is a station at the head of Loch Long, and (*c*) is a station half-way down the same loch. The figures for temperature, salinity, and nitrite content are shown below.

	Depth in fm.	Temp. °C.	Salinity.	Nitrite N. mg. per litre.
(*a*) Gantock Station.	0	13·73	29·86	0·0025
23.6.25.	5	8·56	31·78	0·0027
	10	8·25	32·01	0·0029
	20	8·29	32·18	0·0035
	35	8·36	32·37	0·0033
	51	8·37	32·26	0·0030
(*b*) Arrochar Station.	0	14·43	26·31	0·0035
17.6.25.	5	9·41	32·26	0·0090
	10	7·53	33·54	0·0090
(*c*) Thornbank Station.	0	11·29	31·14	0·0025
26.6.25.	5	7·73	31·91	0·0056
	10	7·98	32·04	0·0042
	20	8·07	32·18	0·0039
	28	8·10	33·52	0·0028

Over practically the whole of the Clyde Sea Area during June and August the nitrite decreased from bottom to top, the values obtained during that time rarely exceeding 0·01 mg. per litre. Occasionally values were lower than 0·001 mg. per litre.



TEXT FIG. 1. Graphs showing the nitrite content of sea-water at various depths, at A. Gantock Station. B. Arrochar Station. C. Thornbank Station. The ordinates are depths in fathoms and metres, the abscissæ denote milligrams of nitrite nitrogen per litre.

Recently Domogalla, Juday and Peterson (**4**) have made a study of the different forms of nitrogen in Lake Mendota, a fresh-water lake. The nitrite nitrogen, which approaches its minimum in June, is of the same order of size as the amounts found in the sea. Their results show in general during the summer, a greater percentage of nitrite in the lower layers than in the surface layers in agreement with the results found so far in the sea.

To find the effect of algal plankton on the nitrite content, diatom cultures, a series which Miss Marshall kindly supplied, prepared as described by Allen and Nelson (**1**), were tested for nitrite. The original enriched sea-water contained from 0·001 mg. to 0·003 mg. per litre, and as the cultures developed the nitrite content increased rapidly. The nitrite content continued to increase long after the diatoms had ceased increasing. The relation of the nitrite increase to the diatom growth is complicated by many factors, notably the strength of the light, the quantity of nitrate present, and the nature of the flask. It is proposed to investigate this further.

The cause of the change of nitrite in the sea is probably complex. According to Buch samples kept in the dark may increase their nitrite content rapidly, *e.g.* from 0·004 mg. to 0·028 mg. per litre in two days. Kept in a clear glass bottle the nitrite content did not change. There seem to be at least five different ways in which the nitrite content of the sea may be changed.

1. By bacterial action.

This has been a much disputed source. The presence of the necessary bacteria has been demonstrated, but there is still doubt as regards their place in the nitrogen metabolism of the sea.

2. By ultra-violet light.

It was first observed by Thiele (**8**) that solutions of nitrates on exposure to ultra-violet light give an increasingly strong reaction for nitrites. Moore and Webster (**7**) later showed that the same happened in sunlight, and that more effect was produced in solutions in quartz vessels than in solutions in glass vessels, thus demonstrating that the shorter wavelength rays were responsible.

3. By the algal plankton.

Moore and Webster showed that plants absorb the nitrite produced by the action of the ultra-violet light acting on the nitrate.

4. By influx from rivers and from the atmosphere.

Moore and Webster showed that night air contains less nitrite than day air, and that this is precipitated from the atmosphere. Uncontaminated rivers have only a small percentage of nitrite.

5. Finally the nitrite may be decreased by oxidation to nitrate.

There is no increase of nitrite directly due to animal life as it is not a normal product of animal metabolism. Both nitrates and ammonia are produced which may indirectly give rise to nitrites.

SUMMARY.

1. The method of water sample preservation used by Gad-Andresen is not suitable for nitrite or nitrate estimation.

2. Using Buch's method it has been shown that the variation in nitrite during the summer is from less than 0·001 mg. to about 0·01 mg. per litre and that the deeper water layers have more than the surface layers.

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