

The Rapid Estimation of the Copper Content of Sea-water.

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THE recent recognition of the importance of copper in nutritional anæmia and the work of Prytherch (1931) on its unique action in stimulating oyster larvæ to become attached have rendered the analytical determination of minute amounts of copper a matter of some interest.

In a former paper (1932) it was shown that the copper present in sea-water could be concentrated by electro-deposition and subsequently redissolved and estimated colorimetrically using sodium diethyl-dithiocarbamate according to the method of Callan and Henderson (1929).

In this the deposition required three hours at 90°–100° C., though but little attention was necessary during that period.

Another method of concentration has since been tried, in which, after adding the 0.1 per cent aqueous solution of the reagent and allowing a few minutes for the colour to develop, the coloured compound is extracted with an organic solvent. For this purpose the chemists of the British Drug Houses Ltd., who introduced the method, recommend chloroform, carbon tetrachloride, or other immiscible solvent.

This method is useful in testing coloured solutions containing copper and obviously increases the delicacy; it has been found to do so to an extent which renders the method suitable for direct use with sea or river water, though unlike sea-water, river water can be concentrated greatly by evaporation if necessary. Furthermore, it has been found that only a trace of the yellowish or brownish colouring matter present in the fresh or brackish waters examined is imparted to chloroform when shaken up with the sample. Iron salts give with the reagent a colour rather like that produced by copper. The presence of much iron would accordingly render the results erroneous, though it requires 1.32 mg. per litre of ferric iron to give as much colour as 0.01 mg. per litre of copper. Only in fresh water would there be any risk of error from iron.

The analysis is made by adding 10 ml. of reagent to 100 or 200 ml. of sample and after about ten minutes the process of extraction is carried out by adding 10.0 ml. of chloroform to 100 or preferably 200 ml. of the sample in a separating funnel. The mixture is shaken violently by hand

for exactly two minutes and allowed to stand. The chloroform extract is then drawn off into one of the colorimeter cups and suffices to fill it to a depth of about 52 mm. The other cup receives a similar extract, but made from a standard solution containing 0.01 mg. of copper in 100 ml., or twice that amount in 200 ml. The same volume of sample and standard should always be extracted.

It is advisable also to make a blank extraction of the sample, without reagent, as traces of the brown or yellowish substances which colour fresh waters are taken up, also the pigment may be partly extracted from plankton present. It has been found that a colorimeter setting of 2.0 mm. of the standard can just be distinguished from 50.0 mm. of pure chloroform. The blank given with pure sea-water is the same, 2 mm., whereas the water of the River Yealm, a moorland river of brownish colour, gave as blank 5.2 mm. and the clear yellowish water of the Conway River, at low tide (sp. gr. 1.001), gave only 5.5 mm. On the other hand apparently colourless water from an oyster tank gave 6.5 mm. blank, equivalent to 0.0013 mg. copper in 100 ml., or 13 mg. per cubic metre.

Larger volumes, up to 1 litre, may be extracted if necessary. Even with only 200 ml. and 10 ml. of solvent, the removal of the coloured copper compound is by no means complete; further extraction will remove colour not only from the liquid, but from the droplets of the organic solvent which adhere to the walls, so it is not easy to ascertain the percentage left behind after one extraction. This does not however vitiate the results provided the extractions are carried out in an identical manner for sample and standard.

Determinations carried out as described, using 100 ml. or 200 ml. samples, gave the following results, expressed in milligrams of copper per cubic metre:

Conway River, high water 16, low water 36. River Exe, at Lympstone, Exmouth, high water 8, low water 14. River Yealm, not tidal, 0. A non-moorland tributary stream of River Yealm, 5. The oyster tanks at Lympstone and Conway varied from 0-15. None of the figures here given are of significance in bringing about the attachment of oyster larvæ, which, according to Prytherch, settle down when the copper content of the water lies between 150 and 500 mg. per cubic metre. This range contained 97.5 per cent of the larvæ he observed, with *Ostrea virginica*. The British species is *O. edulis* and may behave differently. I am indebted to Mr. H. P. Sherwood of the Conway Fisheries Experiment Station for kindly providing me with a number of water samples.

As regards the solvent used for extraction, McFarlane (1932) states that chloroform and carbon tetrachloride are unsatisfactory, but he gives no reason. He recommends the purest amyl alcohol as the colour of the extract fades with the "technical" variety. In chloroform solution

fading has never been suspected to occur within the usual duration of a day's work, nor has the solvent proved unsatisfactory, save as regards the incompleteness of a single extraction. Evaporation of the solvent must be guarded against.

In order to compare the solvents chloroform, carbon tetrachloride, and amyl alcohol, 10.0 ml. of each was added to 100 ml. of Plymouth tap water, using ordinary methylated chloroform, carbon tetrachloride "purified," and amyl alcohol "analytical reagent, pyridine and nitrogen free." The soft tap water has a brownish tint of which amyl alcohol extracted between four and five times as much as did either of the others, this of course is a disadvantage. Of the 10 ml., the amounts recovered were, respectively, 8.7, 9.8, and 6.9 ml. after standing overnight.

With 200 ml. of sea-water and 10.0 ml. of solvent the volumes recovered were respectively 7.4, 9.7, and 4.0 ml. The alcohol floats, which is a disadvantage, as the large volume of sea-water has to be drawn off from the separating funnel before it, whereas the other two sink and time is saved in drawing them off. The concentration of copper compound in the alcohol is about 1.5 times, and in the tetrachloride 0.66 times that in chloroform; but taking into account the volumes recovered, that in the tetrachloride is almost exactly the same as in chloroform, whereas only 0.8 times as much is obtained with the alcohol. The comparisons were made after standing overnight. The alcohol does not separate as readily as do the others, but separates from sea-water better than from fresh.

SUMMARY.

1. The copper content of sea and river waters can be rapidly determined using the sodium diethyl-dithiocarbamate method followed by extraction with chloroform.

2. This solvent takes up only traces of the natural colouring matters found in waters, but blank estimations should be made with some river waters and occasionally with sea-waters on account of the presence of plankton.

3. Carbon tetrachloride can be used instead of chloroform as solvent, it extracts less per unit volume, but the volume recovered is larger, the resultant yield being identical. Amyl alcohol extracts more than chloroform, but the volume recovered is smaller, the resultant yield being about 0.8 times that of the others. The alcohol takes up more colouring matter from river water, does not separate as readily as the others, and floats, whereas they, more conveniently, sink. For work in hot climates its higher boiling-point may be an advantage.

4. In fresh water iron salts in large excess might cause error; this can be checked by the electro-deposition method.

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