THE DETERMINATION OF
TRACE METALS IN SEA WATER

A thesis presented to the University of Cape Town
for the degree of Master of Science

by

Michael John Orren B.Sc. (Hons.)

Department of Chemistry,
University of Cape Town.
October 1965.

CONTENTS.

SUMMARY.

In this thesis, some methods which have been used for the determination of traces of copper, iron and manganese were critically discussed. Special emphasis was laid on spectrophotometric procedures. Techniques were improved or modified where necessary. Sea water samples, collected in the South East Atlantic Ocean up to 600 miles offshore, were analysed for copper, and further samples from a sea area within 100-150 miles of the coast were analysed for copper, iron and manganese. An attempt was also made to improve the sampling technique by the use of cation exchange resin.

Samples of sea water were collected by a special technique which minimized contamination of samples by traces of metals. The samples were then stored frozen in polythene containers until ready for analysis. On storage of sea water in polythene at room temperature, marked losses of trace metals occurred. The processes which might remove metals from solution were discussed. Special cleaning procedures were devised to remove all traces of metals from the apparatus used.

Copper in sea water samples ~~were~~ was determined by complexing the cupric ions present with diethyldithiocarbamate ions. The complex was then extracted into xylene and determined spectrophotometrically.

Iron in sea water samples was first reduced to the divalent state and then complexed with bathophenanthroline. The complex was extracted into isoamyl alcohol and determined spectrophotometrically.

A method was devised for the determination of manganese

in sea water samples. This involved coprecipitation of hydrated manganese dioxide with a carrier of magnesium hydroxide. The precipitate was taken up into sulphuric acid, and, after removal of halide ions, manganese ions were oxidized to permanganate ions, which were determined spectrophotometrically. A special procedure was developed in order to estimate the reagent blank.

The general factors controlling the concentration of trace metals in the ocean were discussed as were the most likely forms of the metals present in sea water. It was necessary to carry out a detailed analysis of the oceanographic conditions in the area studied in order to relate the distribution of copper to water masses and ocean currents. The seasonal variation of copper in coastal waters was discussed and was related to upwelling of deeper water to the surface layers. Samples taken from the surf zone showed low concentrations of copper, iron and manganese. The concentrations of iron and manganese increased after much rain, but copper concentrations remained fairly constant.

The concentration of iron in coastal water was fairly high, but decreased as the distance from the shore increased. The concentration of manganese was fairly constant in the few samples obtained.

Due to the absence of large rivers in the area, metal concentrations in the area studied were almost exclusively controlled by oceanographic factors such as currents and upwelling.

A procedure involving the sorption of traces of copper, iron and manganese on cation exchange resin was developed and investigated. This procedure also determined metals present in a particulate form. Experimental techniques were

iii/...

developed for analysis of particulate metals. Copper in sea water could be successfully determined with the ion exchange technique. Manganese was not sorbed strongly by the resin. Iron, present mainly as colloidal hydrated ferric oxide, was not successfully determined by the method.

1. INTRODUCTION.

1.1 Objective.

In this thesis, a reconnaisance of the concentrations of the trace metals copper, iron and manganese in South African ocean waters, was carried out. Special attention was given to the distribution of copper.

There were three main objects in carrying out the work. Firstly it was considered necessary to examine known methods of analysis critically, improve these when required, and to devise new techniques when necessary. Secondly, since South African waters have not been surveyed previously, the data obtained should be of considerable interest. Thirdly it was hoped to develop a better sampling procedure.

1.2 General background to the problem.

Although 70.8% of the earths surface is covered by sea water,(23) very little is known of the chemistry of the oceans. The sea is an extremely complex chemical system in which all the naturally occurring elements, except indium, tellurium, hafnium, osmium, iridium, platinum, polonium, astatine, francium, actinium and protactinium have been detected. Eleven so-called "major" constituents comprise approximately 99.9% of the total dissolved solids in the sea (Table 1); the remaining elements are present in trace concentrations. A marine trace element has been defined by Goldberg(34a) as an element ".... whose marine abundance is less than 10^3 atoms/10^6 atoms chlorine". Typical values of some selected trace elements are given in Table 2. In addition to the inorganic constituents, much organic matter,(40) including amino-acids (18 so far identified), proteins, fatty acids, complex lipids, carbohydrates, purines, citric and malic acids, and vitamins (Vitamin B_{12} and thiamine), is present in the ocean, and nearly all these compounds are capable of metal-ligand complex formation.

TABLE 1 - Major Constituents of sea water(70)
(Based on sea water of 19 °/oo chlorinity*)

Ion	g/Kg water	Ion	g/Kg water
Na^+	10.5561	Cl^-	18.9799
Mg^{++}	1.2720	$SO_4^=$	2.6486
Ca^{++}	0.4001	HCO_3^-	0.1397
K^+	0.3800	Br^-	0.0646
Sr^{++}	0.0133	F^-	0.0013
		H_3BO_3	0.0260

* The sign "°/oo" means parts per thousand by weight. Chlorinity is defined by Defant.(23)

TABLE 2 - Concentration of some trace elements in sea water.(70)

Ion	µg/l. water	Ion	µg/l. water
Ag	0.1 to 2	Ni	0.07 to 6
Cd	0.1	Pb	8
Co	0.04 to 3	Se	4 to 6
I	40 to 60	Ti	5
Mo	0.3 to 16	Zn	3 to 20

In this thesis attention was focussed on the trace

3/...

metals in sea water, as a more complete understanding of their distribution holds the key to many problems in ocean science. Apart from the interesting geochemical aspects of the equilibrium set up between metal solutions and sediments, trace metals such as copper, iron and manganese are essential for normal functioning of such fundamentally vital processes as photosynthesis in phytoplankton.(45b) The photoplankton form the primary link in the marine food-chain as they utilize inorganic nutrients (phosphate and nitrate mainly) and produce organic material by photosynthesis. The essential nature of trace elements may be highlighted by the example of the role played by iron. In the absence of iron, no photosynthesis can occur even under the most favourable circumstances.(45b) The phytoplankton are adapted to utilize the minute marine concentrations (micrograms per litre level) of trace elements, and in plankton, the concentrations of these elements are much higher than that of the surrounding water. Copper, iron and manganese may be from 7,000 to 20,000 times more concentrated in plankton(92) than in sea water, and, in fact, all forms of marine life contain relatively high concentrations of trace metals when compared to the surrounding water. (For example see Goldberg(34b)). An accurate knowledge of trace element concentrations is again important for possible future exploitation of marine reserves of some of these elements. Although extraction at these extreme dilutions is difficult, ion exchange(22) and ion flotation(79) techniques have been applied successfully, and extraction from the sea will no doubt become economically attractive as terrestial reserves diminish.

The three metals, copper, iron and manganese were chosen for more detailed study as they have known physiological effects and are fairly typical trace elements. Published determinations of these metals have shown scatters of several orders of magnitude (Table 3). They are mostly based on single analyses of surface samples widely separated in space and time. Cooper(20) has expressed the opinion that most of the discrepancies in published analyses were due to metallic contamination introduced during the sampling procedure.

TABLE 3 - Previous determinations of copper, iron and manganese.

Mean Cu µg/l	Reference	Mean Fe µg/l	Reference	Mean Mn µg/l	Reference
0.2 to 90	Richards(70) (1957) review	0 to 60	Richards(70) (1957) review	0.7 to 16	Richards(70) (1957) review
19 to 27	Riley & Sinhaseni(71) (1958)	25 to 50	Armstrong(3) (1957)	1 to 3	Ishibashi(44) *et al.* (1960)
		5 to 32	Laevastu(49) & Thompson (1958)	0.6 to 1.4	Loveridge(51) *et al.* (1960)
0.9 to 1.5	Loveridge(51) *et al.* (1960)	0 to 82	Schaefer(76) Bishop (1958)	0.5 to 4	Skopintsev(82a) and Popova (1960)
4 to 5	Tikhonov(89b) & Zhavoronkina (1960)	13 to 30	Dobrzhanskaya(28a,b) and Pshenina (1958, 1959).	0.3 to 8	Hood(41) *et al* (1961)
30	Skopintsev(82a) and Popova (1960)	4 to 156	Hashitani(38) Yamamoto (1959)	2.7 to 4.5	Fabricand(29) *et al.* (1962)
2.5 to 3.6	Zhavoronkina(95) (1960)	1 to 10	Skopintsev(82a) & Popova (1960)	5 to 15	Parker(67) *et al.* (1963)
0.12 to 1.0	Bowles(11) & Nicks (1961)	1.6 to 3.2	Zhavoronkina(95) (1960)	0 to 200?	Skopintsev(82b) (1963)

Mean Cu µg/1	Reference	Mean ~~Cu~~ Fe µg/1	Reference	Mean ~~Cu~~ Mn µg/1	Ref.
1.3 to 2	Fabricand(29) et al. (1962)	43 to 205	Sananman(74) & Lear (1961)		
0.75	Fonselius(31) & Koroleff (1963)	5 to 7	Fabricand(29) et al. (1962)		
2	Magee(52) & Rahman (1965)	2 to 5	Menzel(55) & Spaeth (1962)		
		5 to 78	Parker(67) et al. (1963)		

The form in which the metals exist in the sea is not well-known, due to the complexity of the ocean system. Most authors(50) divide the metal content into two fractions, viz. "particulate" and "soluble" metal. The former is that fraction held on a 0.45 micron millipore filter; the latter, that which passes through. This rather crude classification is occasioned by analytical difficulties and ignorance of the complex equilibria in very dilute solutions. For instance, "soluble" metals may exist as ions, be complexed with organic or inorganic ligands, or even present in the form of colloidal suspensions stabilized by the organic matter present. "Particulate" matter may include terrestial detritus and fine suspensions of clays etc.

1.3 Sampling.

Trace element sampling is usually performed by lowering a non-metallic sampler to the desired depth, closing it, and then after transferring to another vessel, either

6/...

freezing the sample solid for shore analysis, or, rarely, analysing it aboard ship. Very few analyses are done at sea, mainly because of operating difficulties, a few of which are given below:

(i) Unless specially designed, spectrophotometers and other instruments do not operate successfully at sea on small oceanographic research vessels.

(ii) Handling of samples and reagents is very difficult on a moving ship.

(iii) Contamination may easily arise from brass and steel fittings aboard ship.

(iv) Most analysts are not such efficient workers aboard ship as they are ashore.

The trace metal concentration of samples has been found to decrease with time on storage at room temperature in polythene containers (as discussed later). Storage in glass vessels at room temperature can give rise to misleading results by sorption on the walls of the container,(85) and glass can also release significant quantities of metals into a sample.(28) In order to minimize losses, samples taken at sea are usually frozen in polythene containers and analysed ashore.

1.4 Comparison of instrumental techniques.

Analysis generally involves some preliminary concentration stage, such as solvent-extraction or carrier co-precipitation followed by application of instrumental methods such as:

(i) Emission spectography

(ii) X-ray fluorescence spectroscopy

(iii) Polarography

(iv) Neutron activation analysis

(v) Absorption spectrophotometry
(vi) Atomic absorption spectrometry

A statistical comparison of the results of methods (i), (iii), (iv) and (v) was carried out by Cook *et al.*(19) A common sample, containing copper, manganese, chromium and mercury at low parts per million concentrations, was independently analysed by 9 laboratories. 551 analyses were carried out altogether. The following results were obtained for the precision of a single determination at the 95% confidence level. The figures quoted are derived from the formula $\frac{ts}{x} \times 100$ where s is the standard deviation, x is the overall mean, and t is Students factor for n-1 degrees of freedom at the 0.05 probability level.

Emission spectrography	± 40%
Polarography	± 25%
Neutron activation	± 20%
Absorption spectrophotometry	± 10%

Emission spectrography has been very useful for outlining the general distribution of elements in the ocean(70) as many elements are determined rapidly and simultaneously. Apparatus is, however, expensive, great care is required in analysis, especially in the preparation of standards, and precision is poor at low levels. X-ray fluorescence spectroscopy suffers from the same disadvantages for marine work. Polarography is useful if a preliminary separation and concentration of elements can be carried out, but difficulty arises in the selection of suitable "trace element free" supporting electrolyte and suppressor solutions. Activation analysis requires high neutron fluxes which are available at certain large centres only and often extensive radiochemical separations are needed to remove interfering elements. Atomic absorption techniques have been applied to sea water, but, although showing promise,

8/...

these methods have not as yet been widely used.

In this work, emphasis has been placed on absorption spectrophotometric techniques as these combine the advantages of ease of operation, general availability (most laboratories are equipped with spectrophotometers) and moderate to good precision even at extremely low concentration levels. Spectrophotometric methods have wide applicability and bring trace analysis within the scope of modest laboratories - enabling studies to proceed at many centres on a wide front.

2. EXPERIMENTAL.

2.1 General.

Sea water samples were obtained with a commercially available sampler(39) designed by the National Institution of Oceanography, England. The sampler consisted of a polypropylene tube open at both ends. After attaching to a wire, the sampler was lowered to the required depth and a weight was allowed to slide down the supporting wire. The impact of the weight on a trigger caused two spring-loaded rubber seals to close off the tube ends and thus trap the sample. The sampler was then hauled to the surface and the sea water (1,200 ml) transferred to two 400 ml capacity polythene containers by means of polypropylene taps let into the sampler tube. Prior to filling, the polythene containers were rinsed once with the sample. When large samples were collected for the determination of copper, iron and manganese, the sampler was lowered several times until the required number of samples was obtained. All samples were frozen at -18°C as soon as possible and kept frozen until ready for analysis.

Initially, (April 1964 cruise) the sampler was lowered into the sea near the bow of the slowly moving ship and closed just below the surface. It was thought that sampling as far forward as possible would avoid contamination from the ships hull. The procedure was inconvenient (danger of falling overboard and height of ships bow), however, and subsequent samples were taken by lowering the sampler on a 4 mm stainless steel wire to a depth of 20 m. At this depth, contamination from the hulls of the ship or ship discharges could be assumed to be negligible, whereas the trace metal content would most probably be the same as that at the surface. (The upper 20-50 m of the ocean are thoroughly mixed by wave action and turbulent current

10/...

flow,(23) and stratification of the upper 20 m is only encountered close inshore after a period of calm, sunny weather). A special set of samples for investigation of the variation of copper concentration with depth, was collected at depths of 20,400,720 and 1160 m at a position off Cape Point (Fig. 6, station 28).

Contamination of samples becomes a serious problem when working with metal concentrations in the ppb (parts per 10^9, w/v) range. Precautions taken to avoid contamination included special cleaning procedures (see 6.1) and protection of all samples from exposure to dust (or spray, aboard ship). All reagents were of high quality and were specially purified in some cases to remove all traces of metals present as impurities. (See 6.10). Unless otherwise stated, the term "water" refers to double glass-distilled water (6.10).

A further difficulty encountered in trace analysis arises from the sorption of ions on the surfaces of containers.(85,33) Sorption on glass was essentially negligible during the relatively short period of analysis, as may be inferred from calibration data obtained using glass apparatus. Storage of sea water samples in polythene at room temperature (c. 20°C) for some days resulted in appreciable losses of copper and iron, whereas freezing to -18°C preserved the trace metals far better. (See 3.4).

The determinations of copper and iron involve organic solvent extraction and it was considered advisable to extract samples of sea water with the organic reagents (xylene and isoamyl alcohol) to check that no coloring matter was extracted. Extraction of inshore samples under the same conditions used for analysis, excluding the reagent, gave negligibly low colorations. These inshore samples were known to be far more likely to contain coloring matter than any of the samples analysed.

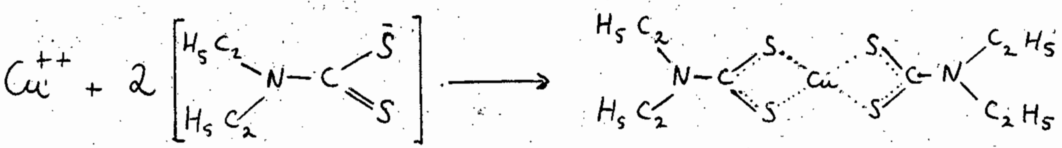
Frozen samples were brought to room temperature by a standard technique (6.5) designed both to minimize losses on polythene and to eliminate contamination during the thawing process carried out in hot, tap water.

2.2 Development of a method for copper determination.

Copper in sea water samples was determined by a sensitive spectrophotometric procedure involving the addition of excess sodium diethyldithiocarbamate reagent followed by solvent extraction of the copper complex. Full details of the procedure appear in section 6.2.

2.2.1 Background to the procedure.

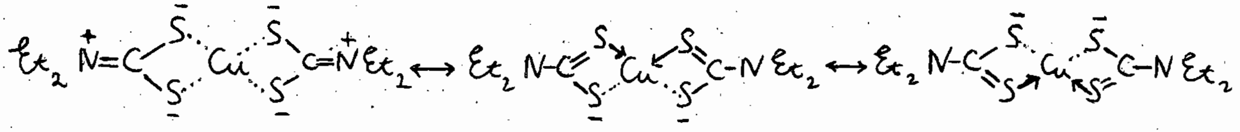
The analytical method was based on the reaction of divalent copper ions, in slightly acid or alkaline aqueous solution, with diethyldithiocarbamate (abbreviated DDC) ligands (I) to form a complex of the form $Cu(DDC)_2$(II).(75) The complex is insoluble in water, but may be extracted with organic solvents such as xylene, carbon tetrachloride and chloroform, among others. The brown complex, in organic solution, shows maximum absorption at a wavelength of about 435 mμ.



I

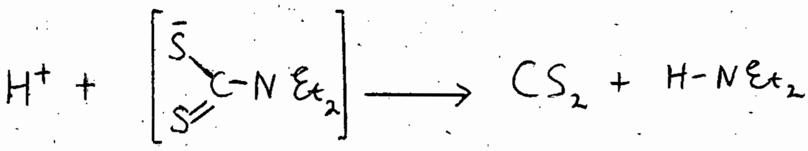
II

The natural logarithm of the equilibrium constant of this reaction(54) (at 20°C, in a 75% ethanol/25% water (w/w) mixture) is 28.8. The structure of II, as shown by infra red spectral investigation,(14) is a resonance hybrid of the canonical forms III, IV and V, each of which probably contributes equally to the structure. This is pertinent to understand why the complex is stable.



III IV V

The decomposition of DDC ions in solution is directly dependent on the pH and proceeds by the following reaction.(75)



The half lives(75) of DDC ions at various pH values are:-

pH 5.0	half life	4.9 minutes
6.0	"	51 "
7.0	"	8.3 hours
8.0	"	3.5 days

Within the range of pH of the sea(23) (7.0 to 8.5), this reaction is sufficiently slow to allow the reaction with copper ions to proceed without interference. It was, however, necessary to devise a modified technique to determine copper in acidic solution (see below).

2.2.2 Procedure.

An approximately 350 fold excess of sodium diethyldithiocarbamate (DDC reagent, see 6.10) was added to the sea water sample, and the resulting copper complex was extracted with 5 ml xylene for one hour. An excess of the DDC reagent was considered necessary to prevent decomposition of the complex.(15a) Excess reagent does not alter the colour intensity.(15a) Although the distribution of the copper complex between the organic and aqueous phases usually attains equilibrium after about 40 minutes,(15a) under the conditions of the procedure (see 6.2) extraction was carried out for one hour to ensure that equilibrium was attained.

The organic layer was separated in a separating funnel, as this procedure was found to be superior to that used by Chow and Thompson,(15a) which involved the use of a medicine dropper to separate the xylene layer.

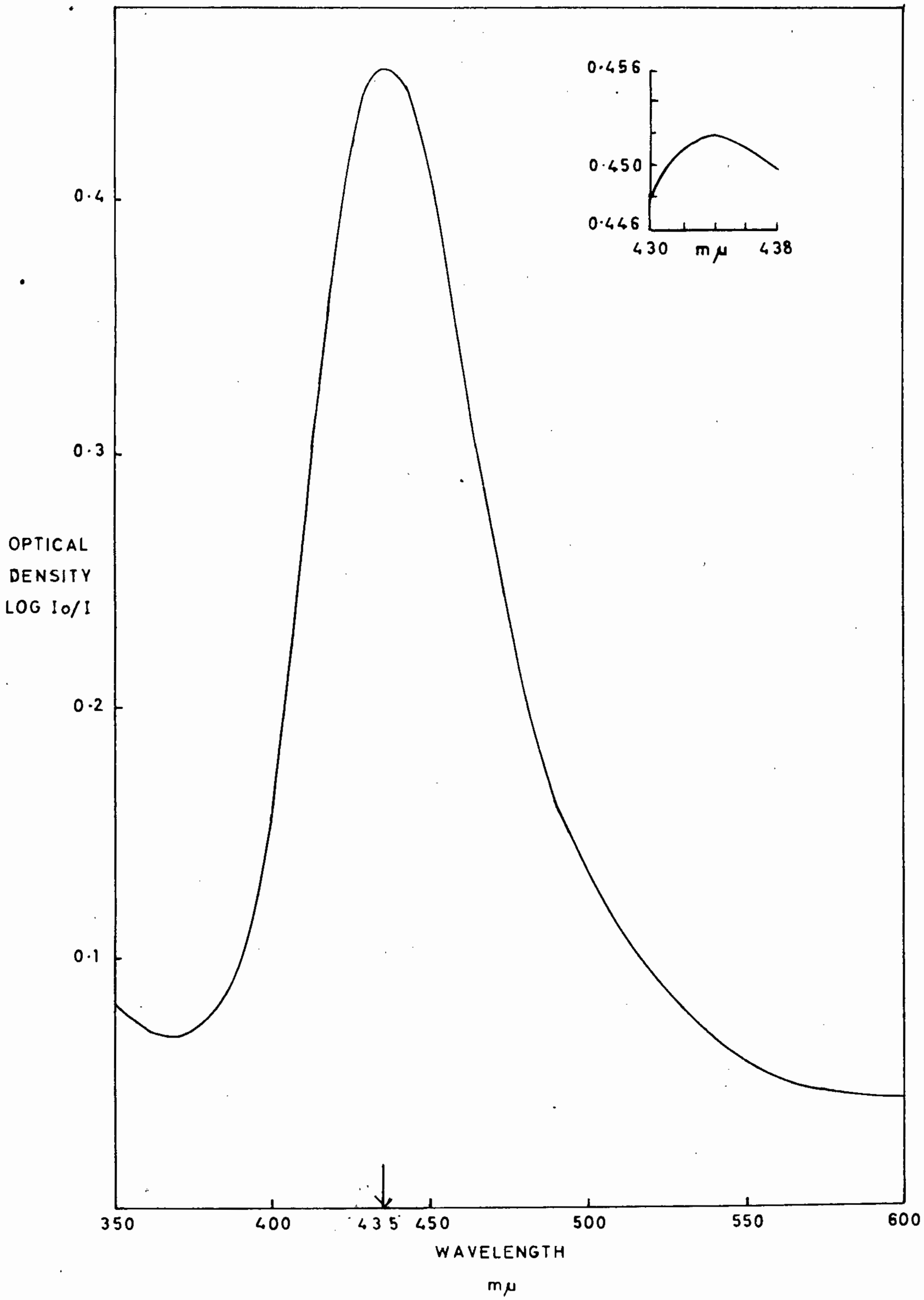
It was found necessary to allow the extracted samples to stand for about an hour before separating off the xylene layer. This removed any interference from manganese (see 3.1.1) and entrained water in the xylene layers separated within this time. If samples were allowed to stand in the separating funnels, high results were invariably obtained, perhaps due to the evaporation of some xylene, and the samples were left in the stoppered polythene containers in which extraction was carried out. Separation was then performed rapidly.

When employing new, clean polythene containers for the first time, it was found necessary to "condition" the containers by extracting an artificial sample in them. Very erratic results were obtained when untreated containers were first put in use (see 3.4).

In order to determine the wavelength of maximum absorption of the complex, approximately 10 µg copper was added to 400 ml "aged" sea water (see below), and the copper complex extracted by the standard procedure (6.2). Readings of the optical density of the complex were taken at 10 mµ intervals in the wavelength range 350-600 mµ. Near the peak of maximum absorption, readings were taken at 2 mµ intervals. The absorption spectrum was plotted (Fig. 1) and the wavelength of maximum absorption was found

FIGURE 1

ABSORPTION SPECTRUM OF COPPER DIETHYDITHIOCARBAMATE. INSET SHOWS PEAK AREA.



to be 434 mμ. This was rounded off for convenience to 435 mμ; an insignificant change.

If iron interference (see 3.1.1) was suspected, a rapid test could be made by determining whether the absorption peak of the extract lay at 434 mμ. In the presence of much iron (not from seawater samples but as gross contaminant the colour of the xylene layer was of a significantly darker shade of brown, and the absorption spectrum was found to be distorted. The iron complex spectrum has its maximum absorption at 515 mμ.(75)

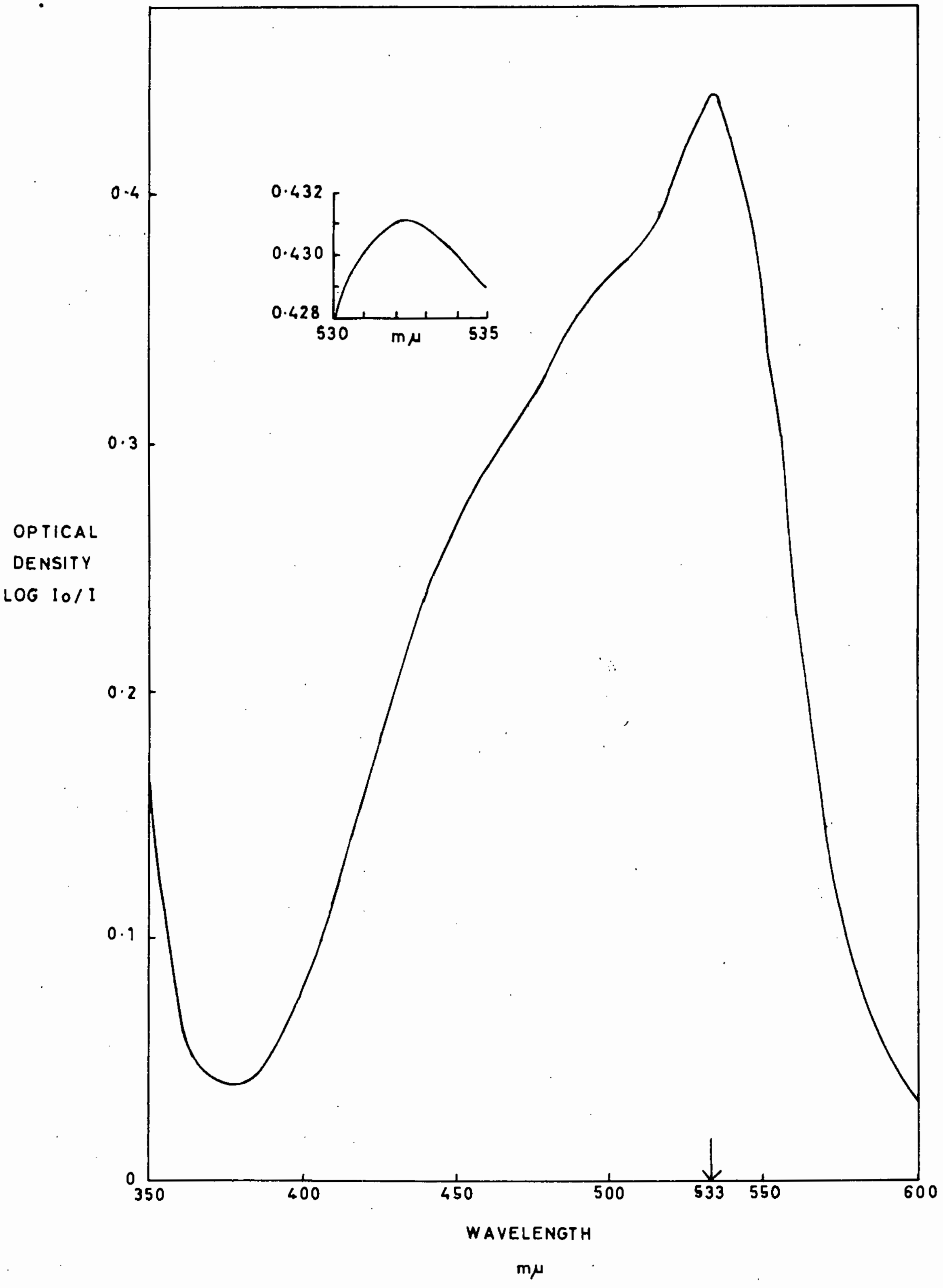
2.2.3 Calibration.

Calibration procedures could not be carried out by using water, as its pH was too low (5.5 to 6.0) and the DDC reagent decomposed. Chow and Thompson(15a) claimed to have calibrated their procedure for determining traces of copper in the sea by adding known amounts of copper to an artificial sea water, made by dissolving reagent grade sodium chloride in water.No purification procedure was used. In this work, it was found that when reagent grade sodium chloride was made up as described by the above authors, the resultant solution contained so much copper as an impurity that the solution became dark brown on adding the DDC reagent. This solution could not therefore be used for calibration purposes, and it was decided rather to use sea water. (In retrospect, it is considered that the copper complex could have been extracted from the artificial sea water with xylene, thus giving a "copper free" sea water for calibration purposes, but this did not occur to the author at the time).

Sea water of low copper content for use in the calibration procedure was prepared by allowing a sample of filtered

FIGURE 2

ABSORPTION SPECTRUM OF IRON-BATHOPHENANTHROLINE COMPLEX. INSET SHOWS PEAK AREA.



sea water (see 6.10) to stand for a week or so in a large polythene container at room temperature. The known loss of trace metals from sea water samples on storage in polythene (see 3.4) reduced the concentration of copper (and iron) in this "aged" sea water to a level satisfactory for use in constructing calibration curves. This was done as surf zone water was expected to have quite a high concentration of copper because of the effects of land drainage, and it was consequently necessary to reduce the copper content to include the range of concentration of copper in offshore water.

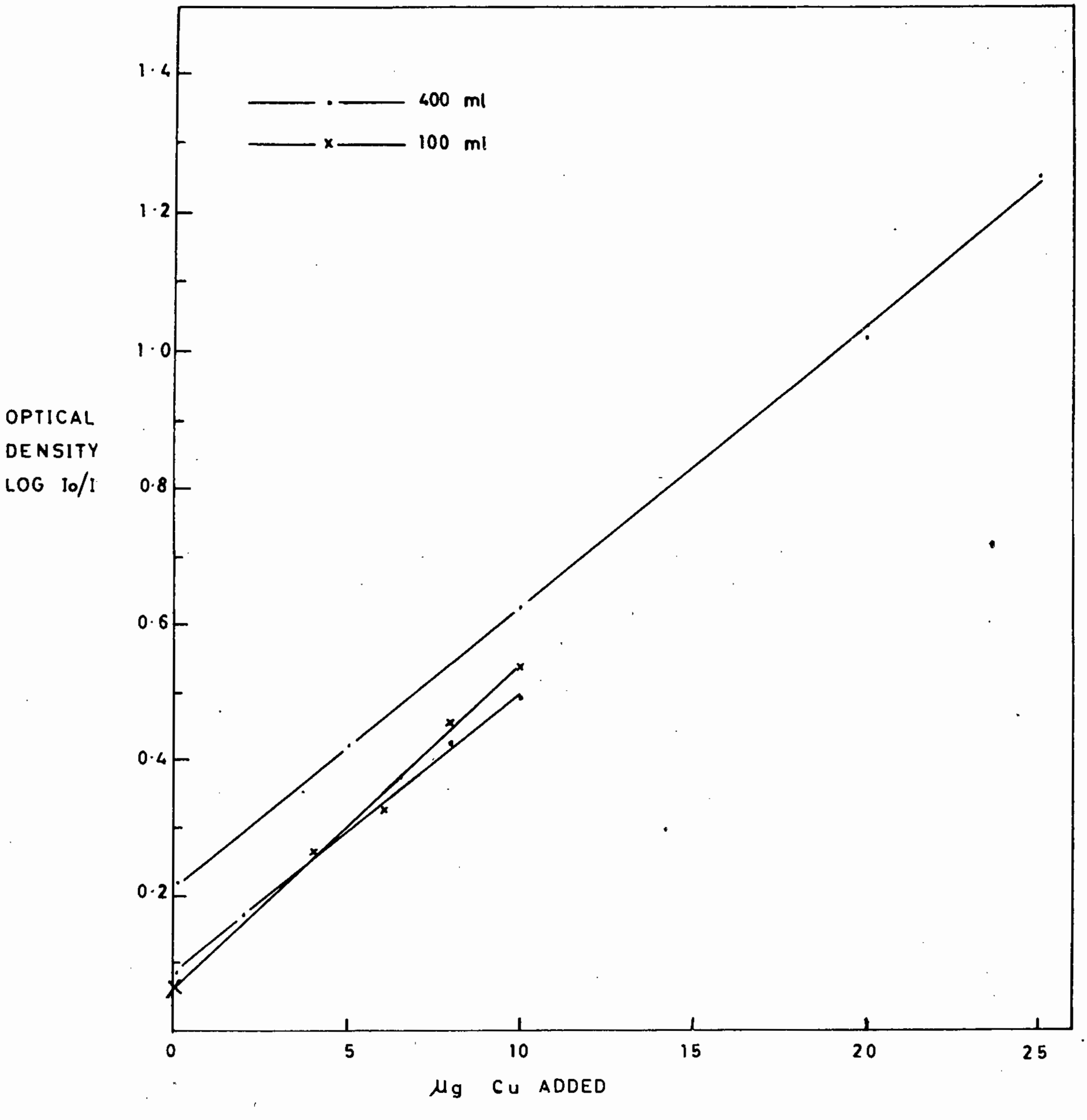
Calibration curves (Fig. 3) were prepared by plotting the results obtained on 400 ml samples of the same batch of "aged" sea water to which known amounts (2, 4, 6, 8 and 10 µg) of copper had been added. An additional point was obtained on a sample to which no copper had been added. A further curve was plotted similarly for the range 0-25 µg from the results obtained by adding 5 µg amounts of copper (up to a maximum of 25 µg) to 400 ml samples of a different batch of "aged" sea water. From these results, Beers Law was observed to hold for the system in the range 0-25 µg copper.

Straight lines were fitted to the experimental points by the method of least squares.(see 6.11) These lines did not pass through the origin due to the small quantity of copper still present in the batches of sea water. A factor corresponding to the increase of optical density per unit (1 µg) copper added was obtained from the regression equations, and the optical density of unknown samples was divided by this factor to transform the results to concentration values.

The regression equations for the ranges 0-25 µg (1) and 0-10 µg (2) were:-

FIGURE 3

COPPER CALIBRATION CURVE



(1) $Y = 0.2158 + 0.0411 X$ where Y = optical density
(2) $Y = 0.0846 + 0.0413 X$ X = µg copper added

The transforming factor used was 0.041 optical density units per µg copper.

In order to establish confidence in the procedure, the results of copper determinations (taken from calibration data in the 0-10 µg range), found by transforming the optical densities using the above factor, were plotted against the copper added. (Fig. 7). The points were fitted to a straight line by the least squares method, and the slope was calculated as 1.00(9), in good agreement with the expected value of 1.00(0).

2.2.4 Determination of the reagent blank.

As mentioned above, the pH of the water was low and reagent blanks could not be determined by applying the general procedure to 400 ml samples of water. An estimate of the reagent blank was obtained by extracting 2 ml DDC reagent with 5 ml xylene for one hour in a polythene container. This blank value was reproducible and remained constant for one batch of reagents. Reagent blanks usually corresponded to about 0.5 µg copper. (Chow and Thompson(15a) apparently did not use a reagent blank, as they made no mention of a blank in their paper).

2.2.5 Sensitivity and precision of the method.

The spectrophotometric sensitivity of the above analytical method, calculated as described by Sandell(75) was found to be 0.005 µg copper/cm^2. For comparison, a value of 0.004 µg copper/cm^2 has been quoted for the sensitivity of the diethyldithiocarbamate method for copper determination.(75) In this case, carbon tetrachloride was used as an extracting agent and the optical density was measured at a wavelength of 436 mμ.

The standard errors of estimate (6.11) were calculated from two individual sets of results, constituting calibration data, and were found to correspond to 0.30 μg ($n = 5$, concentration range 0-25 μg) and 0.11 μg ($n = 5$, concentration range 0-10 μg).

2.2.6 Modified procedure for acid samples.

Samples obtained from the elution of ion exchange columns (see 2.6) and from the procedure for extraction of particulate matter (see 2.5) were strongly acid (c. 2N in hydrochloric acid) and copper was determined by a modified technique (see 6.2.2 for full details). The excess acid was neutralized by an aqueous solution of ammonia and the solution buffered by specially purified triammonium citrate to a pH of 8.2 to 8.5. The pH was adjusted to within the range of pH of the "aged" sea water samples used for calibration (7.9 to 8.5) in order to avoid effects of change of pH on the procedure. (See 3.1.1).

The procedure was not satisfactory as the ammonia solution was not sufficiently free from copper. Purification of the ammonia solution was undertaken by isothermally distilling(43) the ammonia into water (see 6.10). The purified solution gave completely satisfactory results.

A separate calibration was carried out for these samples as the ionic concentration, ionic composition and volume of these samples differed considerably from the previous samples. Known amounts of copper solution (corresponding to 2, 4, 6, 8 and 10 μg) were pipetted into beakers containing a constant amount (2 ml) of redistilled hydrochloric acid. The solutions were diluted with water

18/...

and the copper determined as detailed in 6.2.2.

A blank determination was carried out on a little water containing 2 ml hydrochloric acid. This blank value was found to be greater than the estimated reagent blank found above, and corresponded to a constant value of c.1.5 μg copper for a given batch of reagents. As the quantity of hydrochloric acid present in later samples was not known accurately, additional reagent blanks were carried out in the ion exchange procedure and particulate matter extraction procedures (See 2.6 and 2.$\overset{5}{7}$).

The results (Fig. 3, "100 ml" line) of the calibration procedure were treated as in section 2.2.3, and a factor calculated. The regression equation for this procedure was

$$Y = 0.0667 + 0.0472\,X \qquad Y = \text{optical density}$$
$$X = \mu\text{g copper added}$$

The factor used was 0.047 optical density units per μg copper. As before, the procedure was checked by plotting the copper found, using the factor, against the copper added (Fig. 7 "100 ml" line). A slope of 1.00(0) was found (using a regression equation fitted to the experimenta points), and this was again in good agreement with the expected value.

The sensitivity and the standard error of estimate were calculated as above (2.2.5) and corresponded to 0.004 μg copper/cm^2 and 0.24 μg respectively.

2.2.7 Results.

Samples of sea water (see 2.1) were collected on three deep-sea cruises during April, July and December 1964. The results of their analyses are presented in Table 4. The positions at sea (stations) where the samples were taken (Fig. 6) where chosen in anticipation of the possible comparison with known oceanographical results. Miscellaneous samples of filtered (6.10) sea water, mostly collected at the same time as sea water for the calibration procedures, were taken from the beach at Sea Point. The results of copper determinations on these samples are included for comparison.

TABLE 4 - Results of copper determinations

Station	Position	Depth m	Copper µg/l	Mean µg/l
APRIL 1964 1	35°55'S 14°51'E	0	5.(0) 8.(7)	7
2	37°17'S 13°16'E	0	9.(5) 12.(6)	11
3	38°32'S 10°52'E	0	16.(7) 11.(7)	14
4	39°52'S 8°54'E	0	4.(0) 12.(5)	8
5	41°05'S 11°41'E	0	16.(0)	16
6	42°28'E 14°15'E	0	8.(9) 17.(2)	13

Station	Position	Depth m	Copper µg/l	Mean µg/l
7	43°35'S 17°23'E	0	14.(2) 8.(9)	11
8	44°24'S 20°16'E	0	12.(4) 10.(4)	11
9	42°31'S 20°09'E	0	13.(3) 8.(5)	11
10	41°40'S 17°17'E	0	6.(3) 8.(8)	7
11	40°57'S 15°25'E	0	7.(2) 2.(0)	5
12	40°05'S 15°07'E	0	10.(4) 8.(7)	9
13	40°12'S 14°41'E	0	4.(1) (35)	4
14	39°06'S 13°34'E	0	12.(2) 6.(0)	9
15	38°00'S 15°00'E	0	5.(0) 4.(3)	5
16	39°06'S 17°22'E	0	4.(2) 5.(9)	5
17	40°17'S 19°54'E	0	8.(7) 9.(7)	9
18	38°09'S 19°50'E	0	3.(2) 11.(1)	7

Station	Position	Depth m	Copper µg/1	Mean µg/1
19	37°45'S 18°00'E	0	9.(4) 20.(1)	15
20	36°38'S 16°28'E	0	10.(8) 1.(6)	6
JULY 1964 21	35°14'S 21°14'E	20	13.(4) 12.(2)	13
22	37°30'S 21°44'E	20	2.(6) 1.(8)	2
23	35°18'S 20°36'E	20	3.(6) 2.(9)	3
24	36°03'S 20°10'E	20	2.(7) 6.(6)	5
25	35°29'S 19°47'E	20	4.(7) 10.(8)	8
26	34°13'S 18°43'E	20	16.(2) 6.(6)	11
27	34°13'S 18°17'E	20	1.(4) 10.(9)	6.1
28	34°42'S 17°43'E	20	16.(9) 14.(6)	16
		403	14.(8) 16.(8)	16

Station	Position	Depth m	Copper µg/l	Mean µg/l
		718	9.(9) 10.(6)	10
		1160	6.(7) 9.(2)	8
DECEMBER 1964				
29	35°25'S 17°52'E	20	14.(9)	15
30	34°44'S 18°14'E	20	15.(6) 16.(4)	16
26	34°13'S 18°43'E	20	15.(3) 19.(2)	17
27	34°13'S 18°17'E	20	17.(0) 13.(3)	15
28	34°42'S 17°43'E	20	11.(2) 9.(1)	10
31	35°11'S 17°10'E	20	10.(0)	10

SURF SAMPLES

Station	Position	Depth m	Copper μg/l	Mean μg/l
-	Sea Point,	0	4	
	Beach		4	
	(Rocklands)		0	
	Sea Point Beach	0	5	
	(Aquarium)		4	4

2.3 <u>Development of a method for iron determination.</u>

The iron in sea water samples was reduced to the ferrous state with hydroxylamine and complexed with bathophenanthroline (4, 7-diphenyl-1,10-phenanthroline). The complex was then extracted into isoamyl alcohol and determined spectrophotometrically. Full experimental details are given in section 6.3.

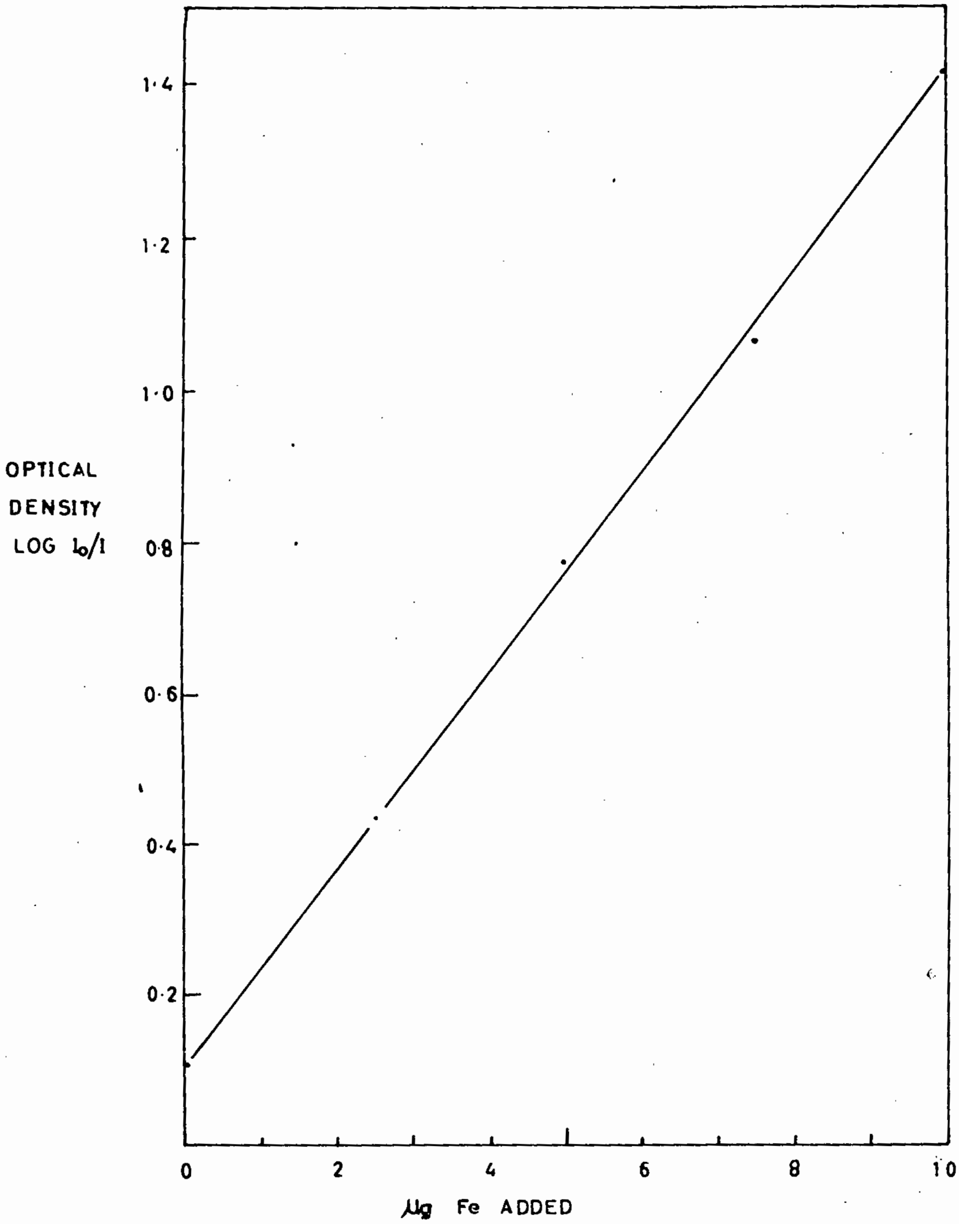
2.3.1 Background to the procedure.

Bathophenanthroline (B) forms a strongly coloured, stable, hexacovalent ion of the form $(FeB_3)^{++}$ with divalent iron in weakly acid, neutral or alkaline solution. The red complex ion is readily extracted into isoamyl alcohol and the solution shows maximum absorption(75) at a wavelength of about 533 mμ. The reaction(75) is as follows, where BH^+ is the bathophenanthrolinium ion. $B + H^+ \longrightarrow BH^+$

24/...

FIGURE 4

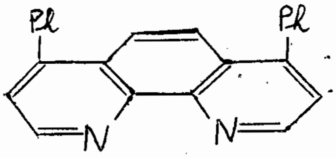
IRON CALIBRATION CURVE



$3BH^+ + Fe^{++} \longrightarrow [FeB_3]^{++} + 3H^+$

Overall reaction

$3B + Fe^{++} \longrightarrow [FeB_3]^{++}$



(B)

The natural logarithm of the equilibrium constant of the overall reaction(54) is 26.6 (measured at 18°C, using a 10% ethanol/90% water (w/w) solution). In the complex ion, the iron atom is coordinated with the six nitrogen atoms.(75)

The bathophenanthroline reagent forms stable complexes over a wide range of pH, but in this work the complex was formed in a solution buffered to pH 4.0 to 4.5 to avoid interfering ions (see 3.1.2).

2.3.2 Procedure.

A sample of sea water was acidified with dilute hydrochloric acid to bring all forms of colloidal hydrated ferric oxide into solution.(84) The iron was then reduced to the divalent state by the action of hydroxylamine hydrochloride. The solution was buffered to pH 4.0 to 4.5 by the addition of sodium acetate, and an approximately 300 fold excess of bathophenanthroline reagent introduced.(50) The complex was then extracted into 15ml isoamyl alcohol.

Both the hydroxylamine hydrochloride and sodium acetate reagents contained relatively large amounts of iron as an impurity, and were specially purified (6.10).

The wavelength of maximum absorption of the complex was determined by treating a solution containing about 3 µg iron in 100 ml water by the standard procedure (6.3) and determining the optical density values of the extract at

25/...

wavelength intervals of 10 mμ over the range 350 to 600 mμ. Near the maximum absorption peak, readings were taken at 1 mμ intervals. The absorption spectrum was plotted (Fig. 2) and indicated that maximum absorption occurred at 533 mμ. This value was used throughout.

2.3.3 Calibration.

Calibration was carried out by a procedure closely resembling that used for copper. Known amounts of iron (2.5,5,7.5, and 10 μg) were added to 100 ml samples of the same batch of "aged" sea water and an additional point was obtained on a sample to which no iron was added. Beers Law was valid for the data in the range 0 to 10 μg (Fig.4).

The regression equation fitting the experimental data for the range 0 to 10 μg iron was

$Y = 0.1079 + 0.1320X$ Y = optical density

X = μg iron added

The factor for transforming optical density values to concentration was accordingly 0.132 optical density units per μg iron.

The procedure was checked by plotting the results of iron determination, obtained from transformation of the calibration data, against the iron added (Fig. 8). The slope of the best straight line representing the data (obtained by the least squares method) was 1.00(2). This agreed well with the expected value of 1.00(0).

2.3.4 Determination of the reagent blank.

A sample of 100 ml water was treated by the standard procedure (6.3) and the optical density of the extract was transformed to concentration by the factor above.

26/...

This reagent blank was constant for one batch of reagents and corresponded to about 0.4 µg iron.

2.3.5 Sensitivity and precision of the method.

The spectrophotometric sensitivity of the method was 0.0025 µg iron/cm^2. This value is in exact numerical agreement with the sensitivity calculated from an ethanol/water solution of the complex(75) at a wavelength of 533 mµ. The standard error of estimate, calculated from the calibration data, was found to be 0.28 µg (n = 5, concentration range 0 to 10 µg).

2.3.6 Modified procedure for acid samples.

As mentioned above (2.2.6) some samples were strongly acid. In order to determine iron in these samples, a modified procedure was used. (See 6.3.2). As the samples were already sufficiently acid to ensure that all iron was in solution, hydroxylamine was added directly, and the solution was adjusted to a pH of 4 to 4.5, after the addition of sodium acetate, by adding approximately 4N sodium hydroxide. It was found necessary to purify the sodium hydroxide solution as described in 6.10. The bathophenanthroline reagent was then added and the procedure continued in the usual manner.

As the presence of salts does not interfere with the complex formation,(84) it was not considered necessary to construct a separate calibration curve. A special blank determination was, however, carried out on samples to account for the impurity introduced by the varying amounts of hydrochloric acid and sodium hydroxide present. (See 2.6 and 2.$\overset{5}{7}$).

27/...

2.3.7 Results.

Samples of sea water for the determination of iron could only be collected on a single cruise during December 1964. The results of the determinations are shown in Table 5. The positions at sea (stations) where the samples were collected are shown in Fig. 6. Some determinations of iron in filtered samples of sea water from the Sea Point beach were included for comparison.

TABLE 5 - Results of iron determinations.
(December 1964)

Station	Position	Depth m	Iron µg/l	Mean µg/l
26	34°13'S 18°43'E	20	9.(7) 13.(4)	12
27	34°13'S 18°17'E	20	11.(2)	11
29	35°25'S 17°52'E	20	9.(3) 5.(6)	7
31	35°11'S 17°10'E	20	0.(4) (16.1) 0.(8) 0.(2) 0.(0) 0.(6)	0.4
-	Sea Point Beach (Aquarium)	0	5 4 14	8

28/...

2.4 Development of a method for manganese determination.

Manganese was concentrated from sea water samples by adding potassium hydroxide, which, in the presence of oxygen, precipitates manganese as the insoluble hydrated dioxide.(75) Magnesium ion is present in moderate amounts in sea water (Table 1) and no additional carrier need be added to the sea water sample as precipitation of magnesium hydroxide carries down (as shown by the results of this work, see 3.1.3) the fine precipitate of manganese dioxide. The precipitate was dissolved in concentrated sulphuric acid, and, after removal of halide ions, the manganese was oxidised to permanganate ion by potassium periodate. The permanganate was determined spectrophotometrically. Full details of the procedure are given in section 6.4.

2.4.1 Background to the procedure.

Oxidation of manganous ion to permanganate may be carried by using either periodate or persulphate ions.(75) For the determination of manganese in sea water, the periodate oxidation has been found more satisfactory.(7) The reaction is as follows:

$$2\,Mn^{++} + 5IO_4^- + 3H_2O \longrightarrow 2\,Mn\,O_4^- + 5IO_3^- + 6H^+$$

According to Sandell,(75) the reaction proceeds fairly rapidly in a hot solution containing nitric acid or sulphuric acid; the presence of small amounts of silver ion is advantageous as this catalyses the oxidation process; in the presence of excess periodate, the oxidised solution is stable for long periods, and the excess reagent does not affect the colour intensity.

2.4.2 Procedure.

It was originally intended to use the procedure of Barnes(7) to determine manganese in sea water. Preliminary work showed that this procedure was unsuitable for the following reasons:

(i) A large (c. 80 ml) final volume of solution was obtained in which the faint permanganate colour could not be detected.

(ii) Oxidation was incomplete. Only 30 minutes were allowed for oxidation;(7) after 1 hour, the reaction is only about 86% complete.(75)

The precipitation step was adapted from the procedure given by Barnes,(7) with the following modifications:

(i) The sea water sample was stirred while adding the potassium hydroxide reagent, and, after allowing the precipitate to settle for an hour, the solution was stirred again. The single stirring recommended(7) gave low recoveries of manganese.

(ii) Twelve hours(7) was found to be too short a time for the precipitate to settle. After 15-17 hours the precipitate was more compact and removal of the supernatant solution was easier. A siphon was used to remove most of the supernatant liquid without disturbing the precipitate.

In Barnes' procedure,(7) 1.5 ml of concentrated sulphuric acid is added to the mixture of precipitate with about 100 ml of supernatant solution. Hence the final solution would be very dilute. In order to avoid this difficulty, the precipitate was separated by centrifuging and decanting the supernatant liquid. This reduced the total volume to

30/...

about 10 ml, and had the further advantage of lowering the amount of halide ion in the solution of the dissolved precipitate.

In order to obtain a solution having the optimum acid concentration for the oxidation procedure (i.e. about 2N in sulphuric acid), only 1 ml (pipette) of concentrated sulphuric acid was added to dissolve the precipitate instead of the 1.5 ml recommended.(7)

The volume of the solution containing manganese was kept to a minimum throughout. The water used in washing the centrifuge tubes was removed by cautious evaporation to a volume of about 12 ml. It was found convenient to graduate the beakers used in order to ascertain when evaporation had proceeded to this stage.

Halide ions were precipitated by the addition of 2 ml 2N silver nitrate reagent followed by dropwise addition of the reagent until precipitation was complete. A slight excess was then added. Barnes(7) suggested that an (unspecified) excess of silver nitrate should be added. An excess of the order of 20 mg silver nitrate has been recommended as a catalyst by Sandell,(75) and in this work the excess reagent added was about 30 mg (1 to 2 drops of the 2N silver nitrate reagent solution).

The silver halides were removed by filtration through a sintered glass plate. The precipitate was washed with two portions of dilute (1%, v/v) nitric acid, special care being taken to ensure adequate washing with the minimum amount of dilute acid, in order to keep the solution volume as low as possible.

The oxidation was performed by adding potassium periodat to the solution in the proportions recommended by Sandell.(75) The solution was then rapidly heated to near boiling and kept on a boiling water bath for 1½ hours. Under these conditions, oxidation to permanganate should be about 89% complete, using information quoted by Sandell.(75)

The small final volume of the solution (c. 14 ml) caused some difficulty, as a precipitate, mainly composed of silver sulphate, separated slowly on cooling, yielding a turbid solution unsuitable for spectrophotometry. Interference from this source was avoided by cooling the solution in ice, encouraging precipitation and finally centrifuging to give a clear solution. Almost all the silver sulphate was removed by this procedure. If the solution was not cooled, the precipitation continued for fairly long periods (a few hours).

The optical densities obtained from samples of various final volumes were standardized by reducing all values to that which a 10 ml volume of solution would have.

The absorption spectrum of permanganate ion is well known,(75) and the optical densities were measured at a wavelength of 525 mμ.

2.4.3 Calibration.

Calibration data were obtained by adding known amounts (about 2,4,6,8 and 10 μg) of manganese to 1000 ml samples of the same batch of "aged" sea water and determining the optical density after applying the procedure. An additional point was obtained on a sample to which no manganese was added. Beers law was valid over the range tested (0-11 μg). The data were plotted, (Fig. 5) as was done in the calibration procedures for copper and iron, and the regression equation calculated. This was

32/...

$Y = 0.0257 + 0.0164X$ Y = optical density
X = µg manganese added

The factor used was 0.016(4) optical density units per µg manganese.

A check of the procedure was carried out by plotting the manganese found using the factor, against the µg manganese added (Fig. 9). The slope of the best straight line fitting the points was 1.00(6), showing satisfactory agreement with the expected value of 1.00(0).

2.4.4 Determination of the reagent blank. (See section 3.1.3)

A stock solution containing magnesium and calcium ions in approximately the same ratio as present in sea water (Table 1) was prepared by dissolving 132.2 g hydrated magnesium chloride and 19.2 g dried calcium chloride (70-75% $CaCl_2$) in water and making up to 250 ml. 20 ml of this solution contains 1.27 g magnesium and about 0.4 g calcium.

10, 20 and 30 ml aliquots of this stock solution were pipetted into beakers containing 990, 980 and 970 ml of water respectively to give a final volume of 1000 ml each. The manganese in the samples was determined by the usual procedure and the optical densities found were plotted against the volume of the stock solution added. (Fig. 10).

A straight line was fitted to the experimental points by the least squares method. The regression equation was

$Y = 0.0250 + 0.0058X$ Y = optical density
X = ml stock solution added.

The extrapolated optical density for X = 0 is 0.025 units, which corresponds to about 1.5 µg manganese. As a single batch of reagents was used throughout, a blank of 1.5 µg was subtracted from all samples.

2.4.5 Sensitivity and precision of the method.

The spectrophotometric sensitivity of the procedure was 0.030 µg manganese/cm^2. The quoted value$^{(75)}$ for permanganate ion is 0.027 µg manganese/cm^2. The standard error of estimate, calculated from calibration data, was 0.57 µg (n = 5, concentration range 0 to 11 µg).

2.4.6 Treatment of acid samples.

It was not possible to modify the above technique to determine manganese in samples containing large amounts of hydrochloric acid, as the large quantity of silver halides precipitated on addition of silver nitrate interfered with the procedure and results were erratic. Interference was caused by the large volume of silver nitrate added and the increased volume of dilute nitric acid required to wash the large quantity of precipitate. Both these factors increased the final volume of the solution and so reduced the colour intensity.

Laboratory facilities were such that removal of excess hydrochloric acid, by evaporation to fumes of sulphur trioxide with sulphuric acid, was not possible.

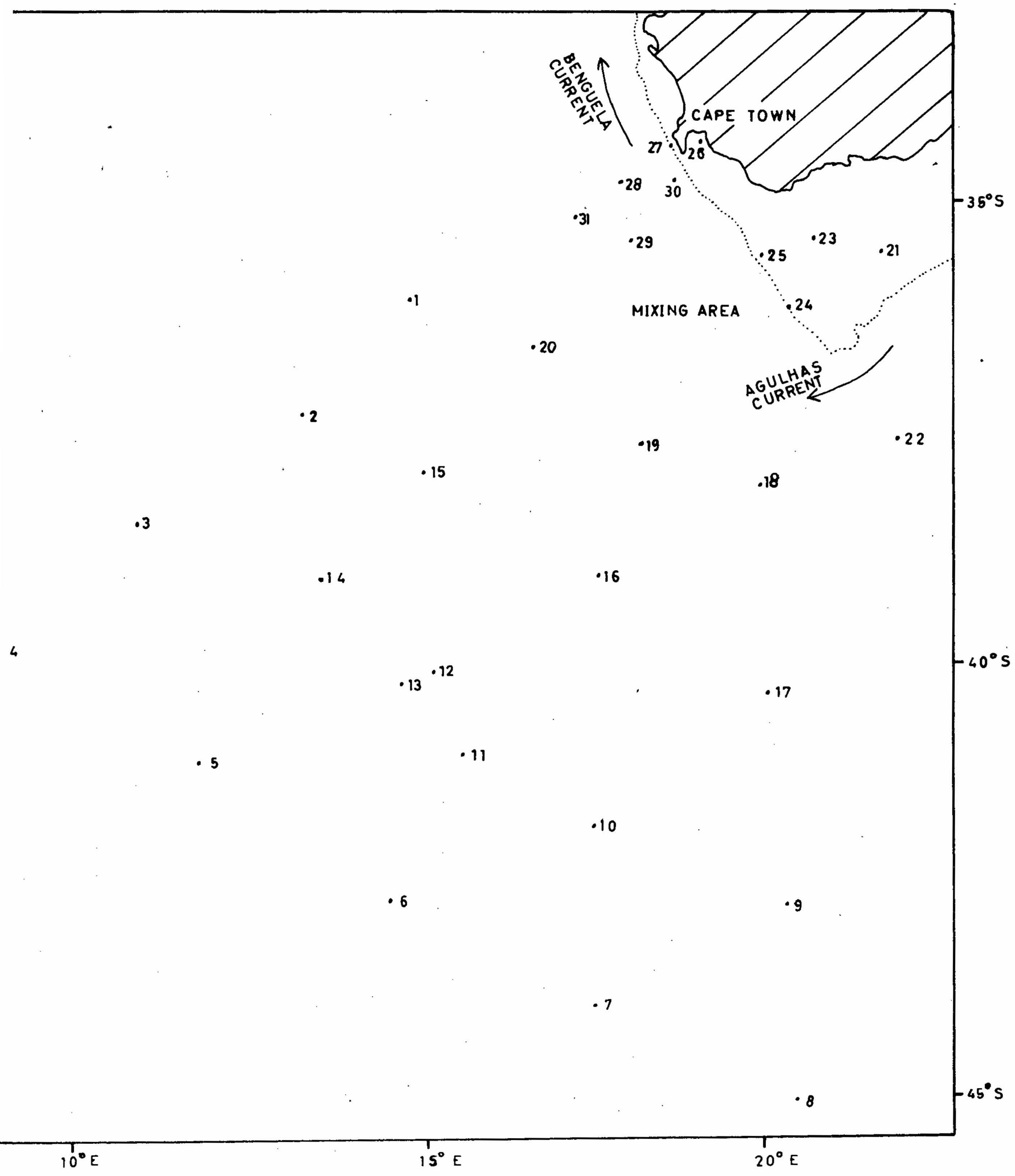
2.4.7 Results.

Samples of sea water for manganese determination were collected during the deep-sea cruise in December 1964. Table 6 lists the results obtained and the sampling positions are shown in Fig. 6. Filtered samples of sea water from the beach at Sea Point were analysed for manganese

34/...

FIGURE 6

MAP OF STATIONS SAMPLED



and the results are also shown in Table 6.

TABLE 6 - Results of manganese determinations.
(December 1964).

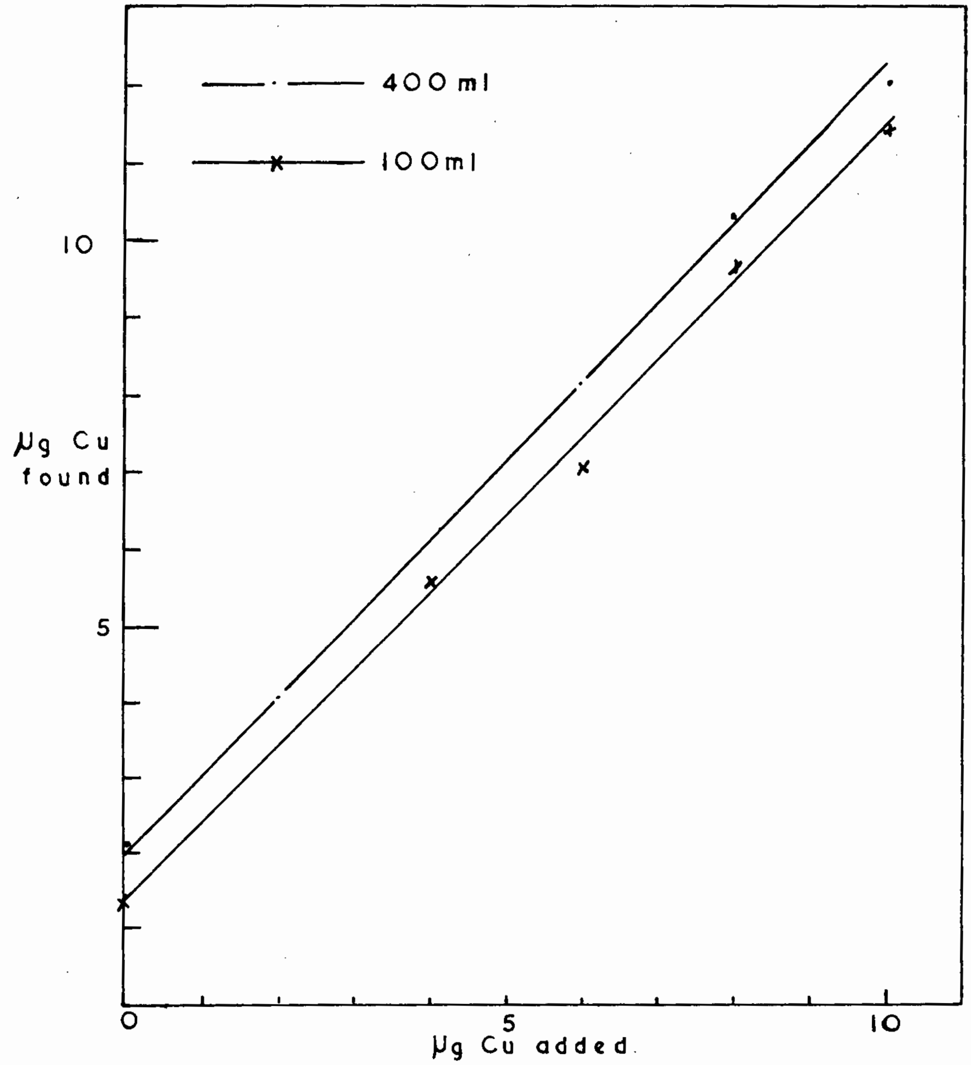
Station	Positions	Depth m	Manganese µg/l
26	34°13'S 18°43'E	20	0.6
27	34°13'S	20	3.(3)
	18°17'E	20	4.(3)
29	35°25'S 17°52'E	20	2.(1)
-	Sea Point Beach (Aquarium)	0	1.(1)
			3.(0)
			6

2.5 Development of a method for determination of particulate copper and iron.

A sample of sea water was filtered through a millipore HA filter (0.45 µ pores) supported in an all-perspex apparatus (see 3.3.2). The filter was removed and the metals brought into solution with hot, dilute hydrochloric acid. Aliquots of the acid solution were taken for determination of copper and iron. Full details of the procedures are given in section 6.8.

FIGURE 7

TEST OF PROCEDURE FOR COPPER DETERMINATION



2.5.1 Background to the procedure.

The procedure for the determination of particulate iron was adapted from an existing method,(84) which was intended to give an estimate of the suspended iron readily available for plankton utilization. Extraction of the particulate material with dilute (c. 0.55N) hydrochloric acid at 100°C for 10 minutes liberates iron from most substances present in a suspended form in the sea.(84) Mineral combinations of iron and that present in stable organic complexes will not be readily extracted. Evaporation with perchloric acid mixtures is required to liberate strongly bound iron,(50) but laboratory facilities were such that this was not possible.

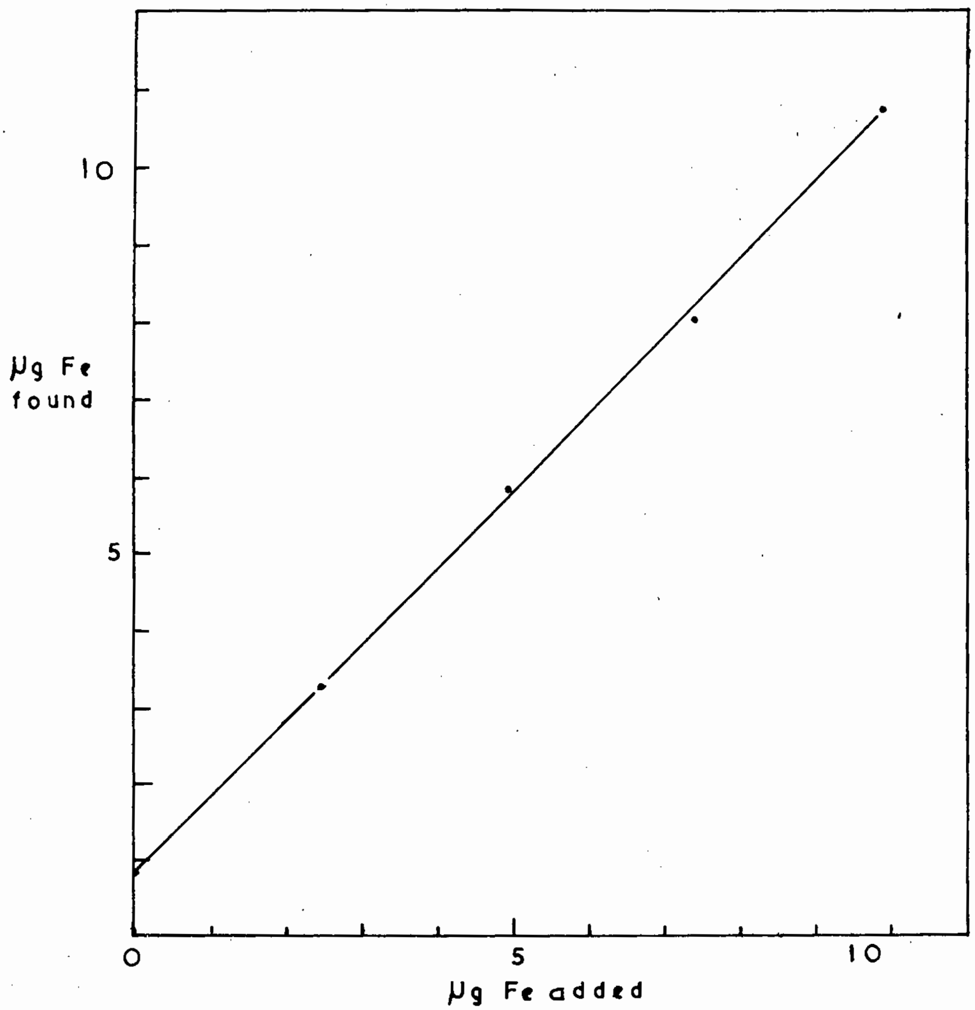
Very few studies have been made of the distribution of particulate copper.(40) In this work, a method was developed which was basically similar to that used for iron. Copper was extracted from the filter under the conditions used for iron above. As was the case with iron, copper held in stable organic forms or in mineral combination will probably not be readily extracted.

2.5.2 Procedure.

The acid extraction was carried out by using 11 ml dilute hydrochloric acid instead of the 10 ml recommended by Strickland and Parsons.(84) These workers used the entire 10 ml acid extract to determine iron by adding the reagent (2,2'- dipyridyl) directly to the extract. The use of the more sensitive bathophenanthroline in the present work enabled iron to be determined using a 5 ml aliquot of the extract, and a further 5 ml was then available for copper determination. The use of 11 ml of the acid made it easy to pipette out two 5 ml aliquots.

FIGURE 8

TEST OF PROCEDURE FOR IRON DETERMINATION



Copper and iron in the aliquots were determined as described in sections 6.2.2 and 6.3.2 respectively. A blank determination was done as described in 6.8.

2.6 Ion exchange procedures.

Sea water samples were passed through small columns of sodium form IRC 50 cation exchange resin at a flow rate of 2-3 ml/min. The sorbed ions were eluted with an excess of hydrochloric acid and aliquots of the acid solution were taken for copper and iron determination.

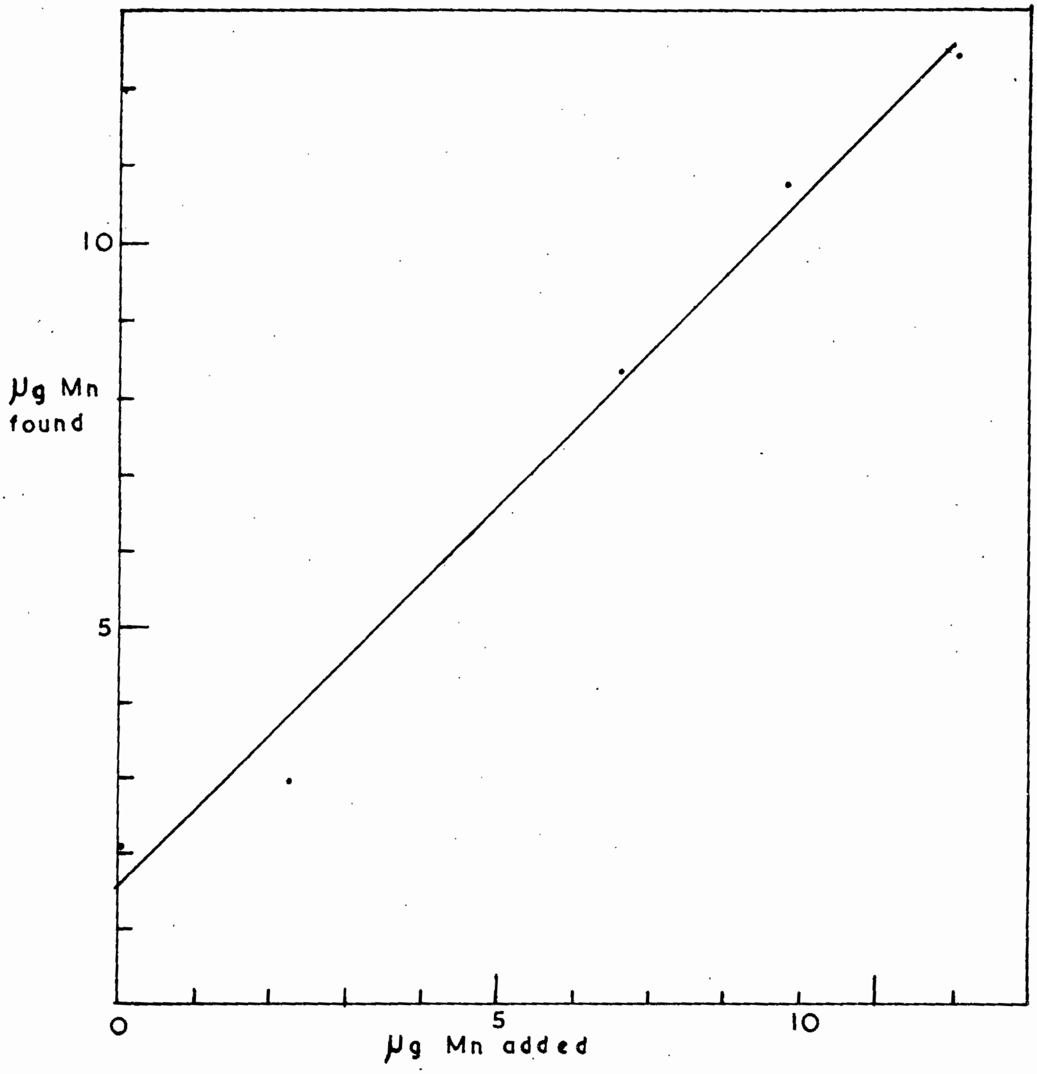
2.6.1 Background to the procedure.

The resin used (Amberlite IRC 50 carboxylic acid ion exchanger) is of the cross-linked methacrylic acid type and has carboxyl (-COOH) active groups. The resin is highly insoluble and is stable in acid and alkaline solutions over a wide temperature range.(48) The resin was supplied as spherical white beads with an effective particle size of 0.3 to 0.5 mm and was used in this form. This size range was considered satisfactory for analytical use of this resin.(48)

A marked change in volume of the resin occurs on exchanging sodium and hydrogen ions. On adding sodium ions to hydrogen form resin, the volume of the resin bed increased by about 50%. The effect was reversible. The swelling and contraction apparently did not damage the resin structure,(48) but some difficulty was introduced when eluting with small volumes of acid, as the contraction of the resin bed led to volume changes. For example, after the sample had run through the column, and the level of liquid in the column had been adjusted so that the resin was only just covered, addition of 10 ml acid caused a contraction of the resin bed

FIGURE 9

TEST FOR PROCEDURE FOR MANGANESE DETERMINATION



and after adding 10 ml water to wash the column, about 25 ml eluted liquid was collected.

2.6.2. Procedure (See 6.7 for full details).

An excess of resin was used throughout as the procedure used to convert the resin to the sodium form did not proceed to completion (see 3.3.2). All work was carried out at room temperature (c. 20°C).

Samples of sea water of known metal content were filtered through the columns and the effluent was sampled for determination of copper, iron and manganese. Metal ions were eluted with hydrochloric acid at a flow rate of 0.2 to 0.3 ml/min.,(27) and the columns were then washed with water. Metal ions were then determined in the eluted solution.

In the experimental test of the ion exchange method discussed in 3.3, a single large batch of filtered sea water was used. 1000 ml quantities of this sea water were "spiked", by adding approximately known amounts of standard copper and iron solutions, to give levels of concentration varying from near-zero to 30 µg metal/l. 400 and 100 ml samples of the spiked solution were taken for copper and iron determination respectively, and the remaining 500 ml sample was placed in a clean sea water sampler and passed through the special filter assembly and ion-exchange column. (Fig. 18). After filtration was complete, the filter was removed and the metal present was determined as described in 2.5 and 6.8. The column was then eluted with hydrochloric acid and the effluent analysed for copper and iron.

2.6.3 Results.

The results of the ion exchange experiments are

38/...

presented in Tables 7 to 10. The results of the ion exchange method for determination of metals in the sea appear in Table 14.

TABLE 7 - Sorption of copper on IRC 50 cation exchange resin

Initial Concentration µg	Effluent concentration µg	Flow rate ml/min	% retained on resin	% recovery	Recovered µg
14.(5)	2.(1)	4.0	86	78	11.(3)
	0.(8)	4.5	95	65	9.(4)
	0.0	2.9	100	92	13.(3)
	0.(6)	2.7	96	88	12.(8)

TABLE 8 - Sorption of iron ~~i~~on IRC 50 cation exchange resin

Initial concentration µg	Effluent concentration µg	Flow rate ml/min	% retained on resin	% recovery	Recovered µg
4.(2)	* 2.(8)	2.0	-	17	0.7
	0.7	1.9	83	79	3.(3)
	1.(3)	1.3	69	17	0.7
	1.(6)	2.0	50	52	2.(2)

* Sample contaminated.

39/...

TABLE 9 - Sorption of manganese on IRC 50 cation exchange resin

Initial concentration µg	Effluent concentration µg	Flow rate ml/min	% retained on resin
24.(5)	14.(5)	2.1	41
	13.(9)	2.1	43
20.(0)	11.(0)	1.3	45
	4.(1)	1.3	79

TABLE 10 - Sorption of copper and iron combined on IRC 50 cation exchange resin at 15 µg/l concentration level

Initial concentration µg	Effluent concentration µg	Flow rate ml/min	Recovered µg	% recovery
Iron	2.(4)	1.3	0.(9)	15
6.(1)	2.(8)	1.4	1.(4)	23
	* 3.(7)	1.2	0.(5)	8
	1.(1)	1.0	0.(3)	5
Copper	2.(4)	1.3	1.(7)	28
6.(0)	2.(3)	1.4	1.(2)	20
	* 4.(5)	1.2	1.(5)	25
	2.(4)	1.0	1.(4)	23

* Sample contaminated

2.7 Investigation of losses of metals on storage in polythene

A stock solution of filtered sea water was treated with known amounts of copper, iron and manganese standard solutions to give concentration levels of about 20 µg metal/l for copper and iron and about 10 µg/l for manganese. These levels were chosen in order that experimental concentrations used might resemble those found in the ocean as closely as possible. The stock solution was well mixed, and samples taken from it were analysed for copper, iron and manganese. Twelve 400 ml samples of the stock solution were taken in polythene containers and placed in a refrigerator at -18°C. A further twenty four 400 ml samples in polythene containers were allowed to stand undisturbed at room temperature. (Samples were protected from the radiant heating effect of sunlight).

The room temperature was measured twice daily (about 8 a.m. and 5 p.m.) and did not vary appreciably (4°C range of temperature) during the experiment.

Sets of the samples kept at room temperature were analysed for copper, iron and manganese after one, two, three and seven days storage. Sets of frozen samples were analysed similarly after eight and forty days storage respectively.

Full experimental details of the above procedure are given in 6.6, and the results appear in Table 11.

41/...

TABLE 11 - Results of prolonged storage of frozen and unfrozen samples in polythene containers

Time (days)	Storage temperature (°C)	Concentration (µg/1000 ml)				
		Copper	Mean	Iron	Mean	Manganese
0	20 ± 2	26.(9) 23.(8) -	25.(3	24.(1) 24.(9) 26.(0)	25.(0)	7.(5)
1	20 ± 2	14.(3) 24.(4) 18.(1)	18.(9	20.(5) 22.(0) 23.(1)	21.(9)	10.(9)
2	20 ± 2	16.(7) 12.(9)	14.(8)	19.(5) -	19.(5)	3.(1)
3	20 ± 2	23.(1) 15.(8) 11.(8)	16.(9	19.(7) 19.(6) 16.(9)	18.(7)	7.(3)
7	20 ± 2	15.(6) 20.(2) 16.(0)	17.(3	12.(0) 12.(9) 19.(5)	14.(8)	6.(7)
8	-18	18.(2) 23.(6) 23.(0)	21.(6	14.(8) 21.(9) 18.(9)	18.(5)	9.(6)
40	-18	25.(0) 20.(7)	22.(8	20.(0) 16.(4)	18.(2)	-

3. DISCUSSION.

3.1 Analytical methods.

3.1.1 Copper.

In the following section, a brief survey was made of some of the more common techniques which have been used to determine copper in sea water, and this was followed by a discussion of some spectrophotometric reagents used for the determination of traces of copper.

Spectrochemical analysis has been applied to the determination of copper in sea water.(95) This paper was unfortunately only available in abstract form and no details of the procedure were given.

The residue obtained from evaporation of 5 ml sea water was analysed using x-ray fluorescence techniques by Natelson *et al.*(61) The results obtained were very high and this was probably due to some contamination introduced during the analytical procedure.

Atomic absorption spectroscopy was used to determine copper in many ocean samples,(29) but the precision obtained was relatively poor (coefficient of variation *c.* 25%). Preliminary concentration of the copper in a sea water sample has been obtained by adding ammonium pyrrolidine dithiocarbamate, to complex the copper, followed by solvent extraction of the copper complex with ethyl acetate. The ethyl acetate extract was then analysed for copper by the direct application of atomic absorption spectroscopy.(52) This technique gave results of much improved precision when compared with the above technique (29) in which sea water was aspirated directly into the flame.

Coprecipitation of copper sulphide, with mercury (82a) or cadmium (24) sulphides as carriers, has been used to concentrate copper from sea water. The copper in the precipitate was then determined spectrophotometrically as the diethyldithio-carbamate complex. Coprecipitation with calcium carbonate, using ferric ion as a carrier, has been applied (56) to the extraction of copper (and manganese) from natural water. The precipitation process is incomplete. A column of oxycellulose has been used to collect traces (1-20 μg) of copper (and iron) from 100 ml samples. (77) This technique has not been applied to sea water.

Direct analysis of a sea water sample by polarographic procedures, in order to determine copper, was compared to the spectrophotometric procedure, involving solvent extraction of the diethydithio-carbamate, by Tikhonov and Zhavoronkina. (89a) These authors found the latter procedure to be more accurate. Polarographic techniques may be satisfactorily applied to sea water, however, if a preliminary extraction of the copper is carried out by using dithizone as a complexing agent and extracting the copper dithizonate into carbon tetrachloride. (89b)

Many spectrophotometric reagents for the determination of traces of copper in water have been introduced since the review of Sandell (75) in 1959. A few reagents which have been investigated recently are nickel diethyldithio phosphate, (62) 2,2'-bicinchonic acid, (59) 4,4'-diphenyl-6,6'-dimethyl-2,2'-biquinoline, (60) n-pentyl-2-pyridylketoxime, (90) and dyes such as fast Sulphon Black F, (8) acid alizarin black (42) SN, and the o,o'-dihydroxyazo dyes of 8-~~amino~~hydroxyquinoline. (6)

Many of these reagents may be made highly specific for copper by the use of suitable masking techniques, but their lack of adequate sensitivity precludes their use in the direct determination of copper in sea water.

Copper has been determined in sea water samples by spectrophotometric determination of the copper-dithizone complex using the mixed colour(31) method. This procedure suffers from the usual disadvantages of the use of dithizone, viz. poor reagent stability and lack of specificity. The results obtained showed a wide scatter. The authors considered the higher values to be due to sample contamination but offered no explanation for the extremely low results obtained in some cases. Some errors inherent in the technique might contribute to the scatter. Samples were stored unfrozen, in polythene containers, for a period of some days before analysis. The results obtained in this work(3.4) indicate that a considerable decrease in concentration of copper may occur on storing in polythene if samples are not frozen. Losses on storage may well account for the low results obtained by these authors.

Oxalyl dihydrazide and sodium ethyl-bis (tetrazolylazo) acetate were used to determine copper in sea water samples,(11) and while the former reagent was found to be more specific for copper, the latter was more convenient to use. Tuck and Osborn(91) compared five spectrophotometric reagents for the determination of copper in water, viz. sodium diethyldithiocarbamate, biscyclohexanoneoxalyldihydrazone, oxalyl dihydrazide, 2,9-dimethyl-1,10-phenanthroline (neocuproine) and 2,2'-biquinoline. These authors considered 2,2'-biquinoline to be most suitable as it was not affected by common interfering ions (iron, nickel and zinc).

A comparison of the use of the reagents sodium diethyldithiocarbamate and 2,2'-biquinoline for determining copper in sea water(10) showed that there were no essential differences in precision. The former reagent was found to be more suitable for all normal work, and the use of the more specific 2,2'-biquinoline was only necessary in sea water polluted by other metal ions, such as is present in estuaries.

Sodium diethyldithiocarbamate was chosen as the spectrophotometric reagent for copper, as it is both sensitive and precise (see above). Further, this reagent is readily available and convenient to use.

The method used was adapted with some modifications from that proposed by Chow and Thompson.(15a) Many metal ions (zinc, indium, cadmium, mercury, silver, lead, tin and antimony) react with diethyldithiocarbamate ion,(75) but the interference from these is prevented by using solvent extraction. Major interfering ions are those of iron, nickel, cobalt, bismuth, manganese and uranium.(75)

Iron interferes at the pH of sea water only if present in large excess.(78) The colour intensities of nickel, cobalt and bismuth diethyldithiocarbamates in xylene are much weaker (less than ⅛ as intense) than that of the copper complex,(75) and these ions do not interfere at the low concentrations present in sea water.(15a) Manganese imparts a transient pink coloration to the organic extract, and this may be avoided by allowing the sample to stand for 30 minutes or so.(75) The uranium (uranyl) complex is insoluble in xylene.(75)

Oxidising and reducing agents present in sea water do not interfere. Variations in salinity have very little influence on the formation of the complex. A change of one salinity unit alters the slope of the calibration curve by about 0.1%. A change of unit pH alters the calibration by about 2%.(15a)

The pH and salinity of samples taken in this work varied very little (pH 7.9 to 8.4; salinity 34.5°/oo to 35.5°/oo).

Carbon tetrachloride and chloroform are normally preferred for extraction of the copper diethyldithiocarbamate.(75 Other solvents may be used, however, and xylene was chosen for the following reasons.

(i) Solubility considerations.

As large samples (c.400 ml) were extracted with small volumes (c. 5 ml) of solvent to improve the sensitivity of the method, the solubility of the solvent in water became important. The low solubility (0.2 g/l) of xylene was advantageous when compared to that of carbon tetrachloride (0.8 g/l) and chloroform (10 g/l).

(ii) Extraction efficiency.

97% of the complex may be extracted by a single extraction with xylene,(15a) whereas carbon tetrachloride and chloroform only extract about 60% of the complex in one extraction.(75) Xylene has the disadvantage that the single extraction only reaches equilibrium after about 40 minutes;(15a) compared to extraction times of a few minutes for the other solvents.(75) Repeated extractions followed by volume adjustment and drying to remove occluded solution are, however, required when chloroform and carbon tetrachloride are used.

(iii) Emulsion formation.

Carbon tetrachloride and chloroform have a tendency to form emulsions when shaken with sea water, due to the presence of organic material. Xylene seldom formed emulsions, with the exception of those samples taken close inshore, which contained large quantities of suspended matter.

(iv) Photochemical stability.

Solutions of the copper complex in xylene were stable, and the color showed no fading after periods of up to six hours, provided the samples were not exposed to strong light. Normal artificial room lighting had no effect. Solutions in the other solvents are only stable if kept in the dark.(75)

The estimated reagent blank is subject to error, as it assumes that the extraction of 2 ml reagent solution with 5 ml xylene may be compared with extractions carried out on much greater volumes of aqueous solution. The distribution coefficient of copper diethyldithiocarbamate between xylene and water could not be used to calculate the extraction efficiency as the salt concentration and pH of the sodium diethyldithiocarbamate reagent were appreciably different from the sea water in which the distribution coefficient was known.

In the early stages of the work on the determination of copper, difficulty was experienced with fading of the color intensity of the complex. This was traced to the use of a (presumably impure) certain batch of xylene. On rare occasions, in later work, rapid fading of samples occurred and this could not be explained. A possible reason could be the effect of traces of mercury, which decolorize the copper complex,(86) as the laboratory in which the work was done was previously used to store deep-sea thermometers. Mercury from broken thermometers was originally also stored in the room, and the possibility exists that traces of mercury vapour were responsible for the fading.

3.1.2 Iron.

A brief survey of some recent methods of determination of iron in water follows.

Iron has been determined by spectrochemical analysis(95) of sea water, but no details were available (cf. 3.1.1).

Natelson <u>et al</u>(61) applied an x-ray fluorescence technique to the determination of iron in sea water, but their results were, as in the case of copper, very high.

Atomic absorption spectroscopy determinations(29) of iron in sea water showed a poor precision (cf. similar determinations of copper in 3.1.1).

An extremely sensitive, combined isotope dilution and solvent extraction technique has been developed recently(83) for the determination of very low concentrations of iron in water. At concentration levels of 1 and 0.1 µg iron/l coefficients of variation of 4% and 8% respectively, were obtained. This method has not as yet been applied to sea water.

In the presence of traces of iron, the fluorescence of the aluminium Pontachrome Blue Black R complex is decreased, and the reaction forms the basis of a fluorimetric method of determining iron.(9) This method is claimed to have three times the sensitivity of bathophenanthroline, but has not been widely used.

Few of the many spectrophotometric reagents for determination of traces of iron in water have the required sensitivity for use in sea water. More recent determinations of trace iron, such as by complexing with formaldoxime(53) or anthranilic acid(25) are also not sufficiently sensitive. Thioglycollic acid(88) is a sensitive reagent for determining trace iron, but the interference of copper creates difficulties.

The most sensitive spectrophotometric reagents for iron are those based on six fold coordination of divalent iron with the nitrogen atoms of organic ligands. 1,10-phenanthroline and 2,2'-dipyridyl reagents, have been widely used(3,2~~3~~6a,2~~3~~6b,46,81) for determining traces of iron in sea water. The related compound, bathophenanthroline (4, 7-diphenyl-1,10-phenanthroline) has been most favoured(33,49,67,74) in recent years mainly because of its high sensitivity (0.1 to 0.3 µg iron/1 may be detected) and its convenience in use. The increased sensitivity is enhanced by the fact that the ferrous bathophenanthroline ion may readily be extracted into organic solvents, in contrast to the ferrous complexes of the above reagents.(75) A new reagent, tripyridyl triaz~~o~~ine, is equivalent in sensitivity(17) to bathophenanthroline, but is less convenient to use,(94) as the complex is difficult to extract.

Bathophenanthroline was considered to be the most suitable spectrophotometric reagent for this work, and the method of Strickland and Parsons(84) was adapted for use.

Many metals (silver, bismuth, cadmium, mercury, zinc, beryllium, molybdenum, tungsten, copper, nickel, cobalt and tin) interfere by either forming precipitates (silver and bismuth) or complexes with the reagent.(75)

Chloride and sulphate do not interfere in moderate amounts,(75) such as are present in the ocean. Other interfering anions(75) (oxalate, tartrate, phosphate and fluoride) are not present in sufficient quantity in sea water to interfere.

None of the above metal ions interferes with the determination of iron in sea water, if the pH of the sample was adjusted to 4.0 to 4.5 before forming the complex.(50)

Isoamyl alcohol is the generally recommended solvent(75) for extracting the ferrous bathophenanthroline complex and was used in this work. Nitrobenzene(75) has been proposed for certain purposes.

Extraction of the complex from 100 ml samples by 25 to 30 ml isoamyl alcohol, followed by determination in 10 cm path length spectrophotometer cuvettes, has been recommended.(84) 10 cm cuvettes were not available and the method was modified by extracting 100 ml of sample with 15 ml solvent followed by determination in 5 cm path length spectrophotometer cuvettes. The isoamyl alcohol is slightly soluble in water (c. 2.3 g/100 ml) but this did not create any problems, nor was the sensitivity significantly altered (see 2.3.5) by the modified extraction. The phases separated rapidly with no emulsion formation. (The separation process was probably facilitated by the presence of ethanol from the bathophenanthroline reagent).

After separation, the extract was made up to 15 ml with acetone. This ensured that a constant volume was used, and the acetone removed cloudiness due to entrained water droplets.(84) The extract was stable for at least four hours in artificial room lighting.

The molar extinction coefficient of the iron-bathophenanthroline complex extracted as above was 22,100. Comparison with the literature value(75) (22,400) indicated that the extraction efficiency was 98 to 99%.

Very few experimental difficulties were experienced with this procedure and it was fairly rapid compared to the methods for copper and manganese determination.

3.1.3 Manganese

Comparatively little work has been published on analyses of traces of manganese in water.

Atomic absorption spectroscopy determinations(29) of manganese in sea water were of poor precision, as was the case with copper and iron determinations (see above).

Manganese may be concentrated from sea water samples by coprecipitation of manganese sulphide with mercury sulphide,(82a) or more usually, by coprecipitation of the hydrated oxide with iron,(72) lanthanum(67) or aluminium(44) as carriers. Rona <u>et al</u>(72) extracted manganese from the mixed oxide precipitate (iron as carrier) by forming manganese diethyldithiocarbamate and solvent extracting the complex. Manganese was then determined by neutron activation techniques. Other workers dissolved the precipitate and oxidised the manganese to permanganate ion, which was then determined spectrophotometrically.

The catalytic action of manganous ions forms the basis of several very sensitive spectrophotometric procedures. For example, the rate of oxidation of triethylamine by periodate ion to form a yellow dye,

52/...

is a function of the manganese concentration.(64) The detection limit is *c*.0.5 μg manganese/l. Methods based on the catalytic action of trace manganese on the oxidation of leucomalachite(30,84) green dye have detection limits of *c*.0.1 μg manganese/l as several hundred dye molecules are produced by the action of each manganese atom. However, these procedures suffer from the disadvantage that, even with careful control of temperature, total salt content and pH, standard samples must be run at frequent intervals. It seems that the methods are very sensitive to small random changes in procedure, and so are unsuitable for routine use.

For this work, a method was devised in which the manganese was coprecipitated with magnesium hydroxide. No carrier was necessary as the sample contained magnesium. The manganese was oxidised by potassium periodate to permanganate and determined spectrophotometrically.

Coloured substances interfere with the method,(75) but are present in negligible quantities in sea water. Bismuth and tin cause turbidity(75) but both are present in such small quantities in sea water that their influence is negligible. Chromium interference caused by oxidation to the hexavalent state,(75) is negligible again on account of the small quantities present.(70) Interference from iron was minimized by adding phosphoric acid, which also assists in preventing precipitation of iodates of manganese.(75) Nitrite and sulphite, if present, are destroyed during the acidification stage. Chloride, bromide and iodide ions interfere, as they are readily oxidised by permanganate ion,(75) and must be removed before the oxidation step is performed.

Various steps in the procedure were discussed in section 2.4.2, and further discussion of some points follows.

There was some difficulty in establishing a reagent blank for the determination of manganese. "Manganese free" sea water could not be used as there was no way of ensuring that manganese was in fact absent. Artificial sea water contains manganese as an impurity in unknown concentrations. Distilled water could not be used as no magnesium carrier was present. An estimate of the reagent blank was found by the following procedure.

As the manganese method involves only the magnesium ion in sea water, it was decided to attempt to make up an artificial sea water containing only magnesium ion. Manganese would presumably be present as an impurity in the magnesium salt used. By varying the magnesium content of the water, the manganese content would vary proportionally, and an estimate of the manganese contributed by the reagents could be made as follows.

let a = amount of manganese contributed by the reagents
b = " " " " " 10 ml (say stock solution (see below)
c_1, c_2, c_3 = total manganese found, expressed as an optical density, on adding 10, 20 and 30 ml stock solution to 990, 980 and 970 ml water respectively.

Then

$$c_1 = a + b$$
$$c_2 = a + 2b$$
$$c_3 = a + 3b$$

A plot of "c" against "b" will, when extrapolated to zero "b", give the value of "a" in optical density units.

A stock solution of magnesium and calcium chlorides was made up such that 20 ml contained the same amount of magnesium and calcium ions as are present in 1000 ml sea water. (Calcium was added as a certain amount of calcium is present in the precipitate from normal sea water). The addition of 10,20 and 30 ml stock solution to water which was then made up to 1000 ml, gave 1 sample (20 ml added) of about the same concentration of magnesium and calcium as is present in sea water, and the remaining two samples contained ½ and 1½ times the concentration in sea water respectively. Each sample was then analysed for manganese according to the procedure.

A plot of the results showed (Fig. 10) that the points lay almost along a straight line, and a straight line was fitted to the data by the least squares method. The regression equation was

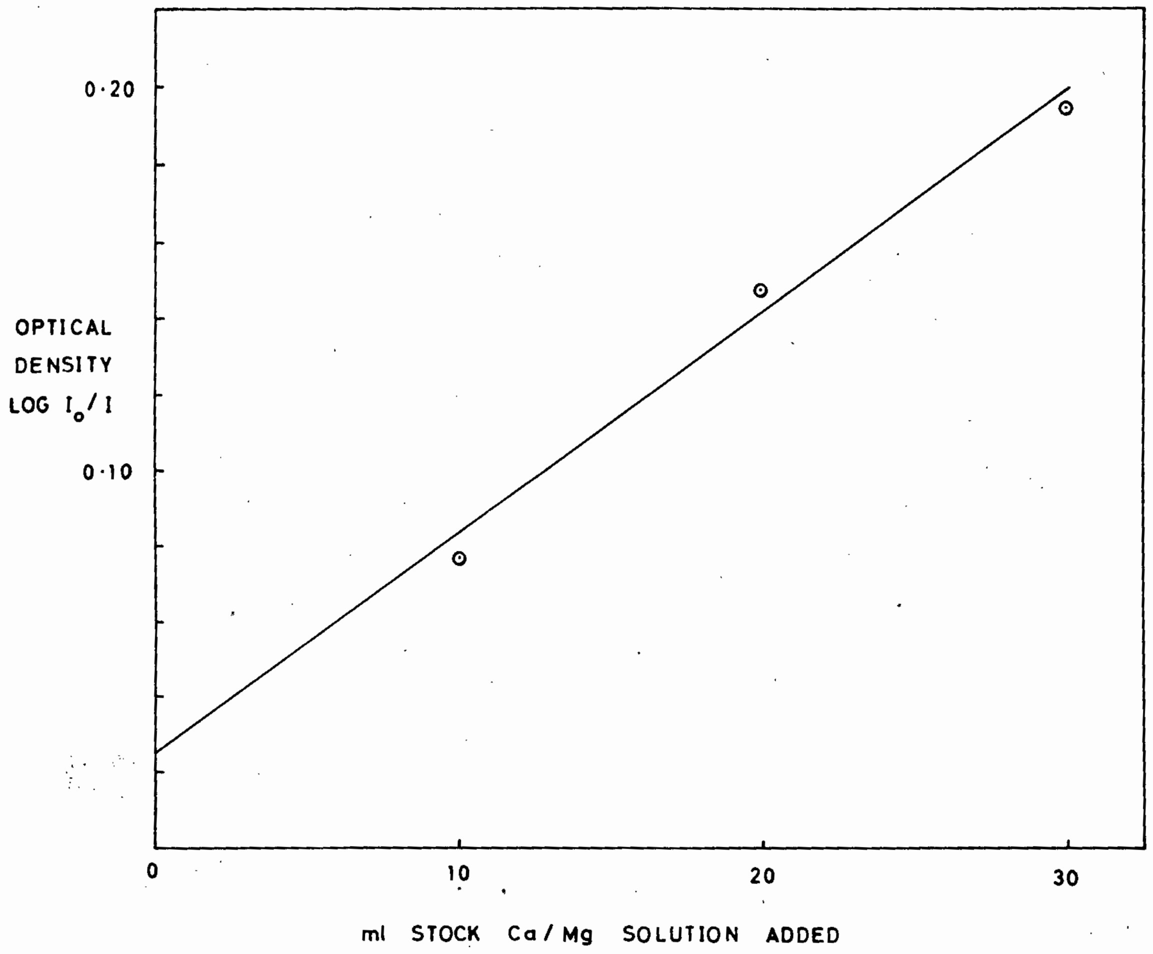
$$Y = 0.0250 + 0.0058\,X \qquad \begin{array}{l} Y = \text{optical density} \\ X = \text{ml stock solution added} \end{array}$$

The value of 0.025 was transformed to concentration units and taken as being an estimate of the reagent blank. It is of interest to note that the reagent grade salt solution contained <u>c</u>.0.4 µg manganese/ml.

The above treatment assumed that a linear relationship was involved. This again implied that the precipitation of magnesium and manganese was complete under widely varying conditions of salt concentration and pH, and that

FIGURE 10

MANGANESE BLANK DETERMINATION



the other major ions present in sea water did not influence precipitation.

The value obtained seems reasonable, however, as the lowest manganese concentration recorded at any time during the experimental work was 1.5 µg, i.e. equal to the reagent blank determined above. It is likely therefore that the above assumptions are valid and that the "reagent blank" is a good estimate of the manganese introduced by the reagents.

On first appearances, it could be agreed that the reagent blank might be attributed to species other than permanganate. Such deductions are considered most doubtful as the final solutions were in all cases clear and had the characteristic permanganate color.

The lower sensitivity (see 2.4.5) of the method (0.027 µg manganese/cm^2 compared to the generally accepted value(15) of 0.030 µg manganese cm^2) was probably due to

(i) Incomplete precipitation of manganese

(ii) Incomplete oxidation to permanganate

A rough estimate of the relative influence of factors (i) and (ii) may be made. In this work, the factor transforming optical density to manganese concentration was 0.016 (2.4.3). For pure permanganate ion at normal concentrations, the factor, calculated on a similar basis, is 0.018 optical density units per µg manganese (as permanganate).

The efficiency of the procedure may be expressed as a percentage:-

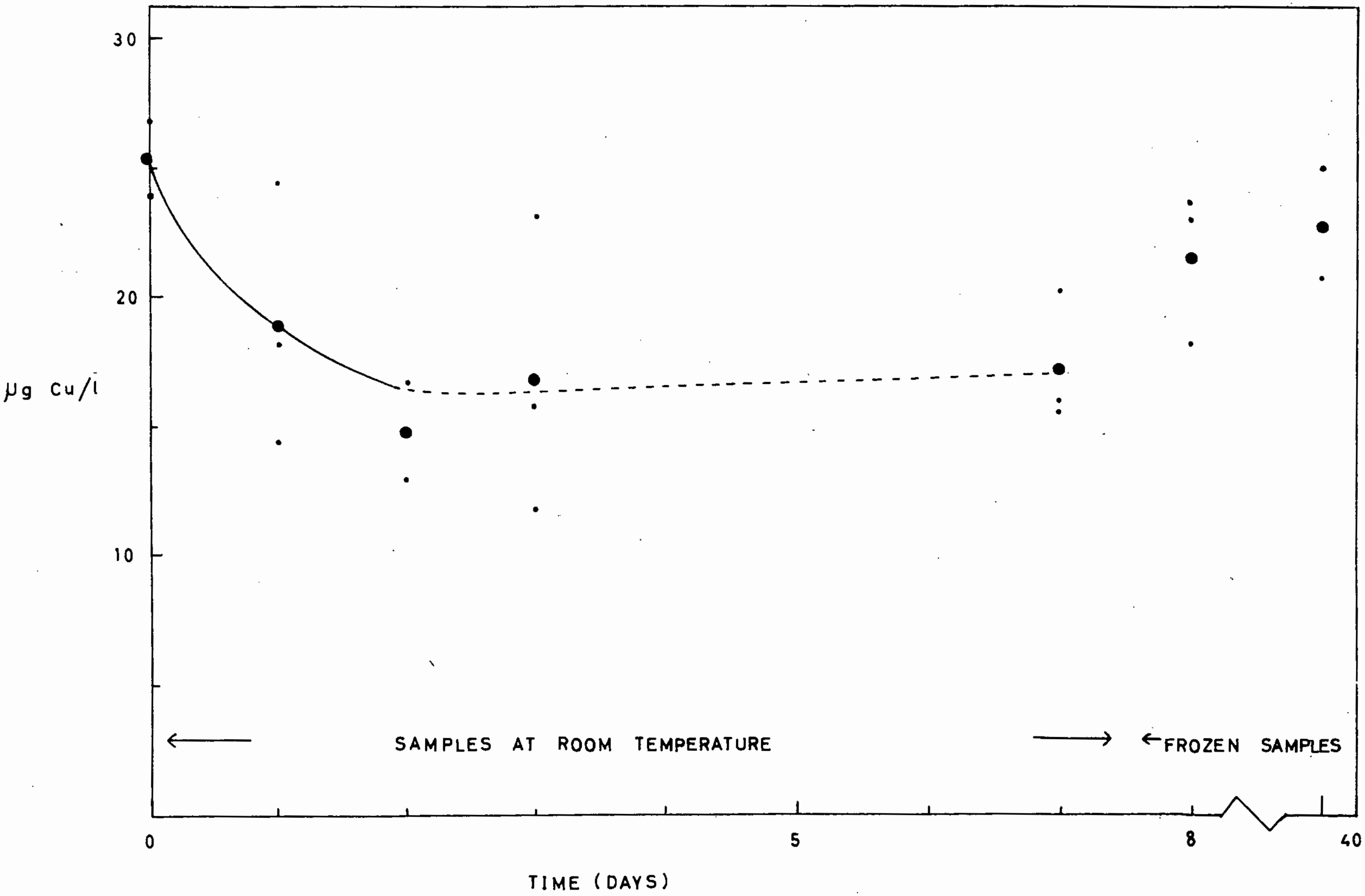
$$\frac{0.016}{0.018} \times 100$$

$$= 88.9\%$$

56/...

FIGURE 11

VARIATION OF CONCENTRATION OF COPPER SAMPLES STORED IN POLYTHENE



86% of the manganese, present in solutions of approximately the same concentrations as used here, is oxidised to permanganate in 1 hour, and 91% is oxidized in 2 hours.(75) Interpolation of the percentage oxidised in 1½ hours gave a value of 89%.

This value is identical to the efficiency of the procedure as expressed above, and it appears that incomplete oxidation is responsible for the decrease of sensitivity, and that the coprecipitation of manganese is essentially quantitative.

The manganese method developed suffers from the following disadvantages,

(i) It was tedious and slow. (A maximum of six samples could be determined in a two day period).

(ii) Large samples (*c.* 1000 ml) were necessary, and determination of replicate samples was limited by the large quantities of sample needed.

3.2 Distribution of copper, iron and manganese

3.2.1 General.

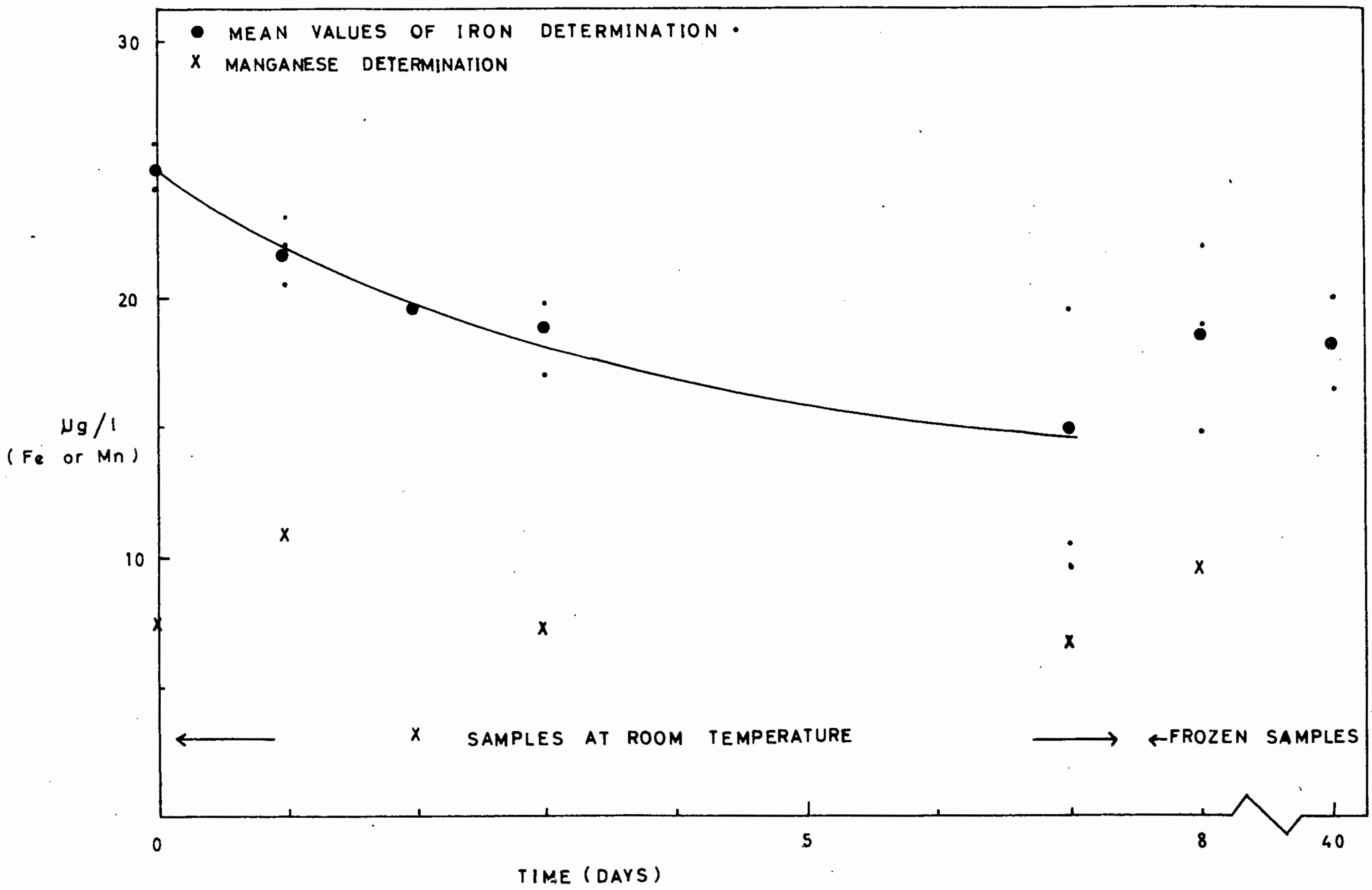
A scheme of processes which may control the concentration of trace metals in the sea has recently been published.(78) A brief description of these processes follows.

Elements are supplied to the sea by -

(i) Stream supply - Rivers etc. add metals in particulate, colloidal and soluble forms to the sea. This material then mixes and disperses.

FIGURE 12

VARIATION IN CONCENTRATION OF IRON AND MANGANESE SAMPLES STORED IN POLYTHENE



$$T = A/\frac{dA}{dt}$$

T = residence time (years)
A = Total amount of an element in suspension or solution in the ocean
dA/dt = Amount of an element introduced into the ocean per year.

The development of such an equation involves the following assumptions,

(a) The chemical composition of the ocean is in a steady state of equilibrium.
(b) The amount of material introduced per unit time is exactly balanced by an equal deposition of sediment.
(c) Complete mixing occurs in the ocean in times which are short compared to residence times.

Despite these assumptions, the residence time is a valuable aid to the understanding of the general distribution of the elements. Some values(35) of T in years are -

Sodium	2.6×10^8
Copper	6.5×10^4
Silicon	1.0×10^4
Manganese	7×10^3
Iron	1.4×10^2

Sodium has an extremely long residence time and is well-mixed throughout the oceans. Copper has a relatively long residence time for a trace metal, and should be reasonably evenly distributed throughout the sea. Silicon enters the sea mainly as suspended clay minerals and these rapidly settle out. Managanese and iron, however, have residence times which are short compared to the time of mixing, and an irregular distribution of these metals is to be expected. These metals are rapidly deposited from sea water and probably prevail as solid phases throughout their passage through the hydrosphere.(35)

The assumptions involved in calculating "T" are only valid when applied to the oceans as a whole and do not apply in near-shore waters where internal modification occurs.

Water masses in the ocean are identified by their characteristic temperature and salinity values,(23) which remain fairly constant for a given water mass. Within such a mass, the salinity is an excellent indicator of mixing processes(23) and for a trace metal of moderately long residence time, the concentration of metal within a water mass should vary proportionally with salinity. Salinity variations in water masses are small, indicating a well-mixed state, and so the trace metal content of a water mass may be expected to be relatively constant. Mixing in the ocean occurs in both vertical and horizontal directions and is most marked in the (always) turbulent flow of ocean currents.(23)

In near-shore environments, moderate to strong ocean currents are usually present, and under these conditions, an element of limited stability (i.e. short residence time) may be rapidly mixed within a current and so appear to be characteristic of that current.

The area studied (Fig. 6) contained areas of upwelling (stations 26,27) and of strong ocean currents (station 22). Continental shelf and slope areas (stations 21, 23, 24, 25, 30) and deep sea environments (stations 1 to ~~1~~20) were also sampled.

As mentioned earlier (1.2) the forms of metal present in the sea are arbitrarily divided into "particulate" (those held by a millipore filter of 0.45 µ pores) and "soluble" metal (soluble metal includes all ionic, colloidal and complexed forms). "Particulate metals" consist of all forms of suspended material such as organic matter, fine clays etc. which contain traces of metals.

Copper is added to the ocean largely as cupric ions.(69) These are removed by sorption on hydrolysate sediments and on oxidates such as manganese nodules.(69)

In sea water, any cuprous ions are rapidly oxidised to the cupric form(69). Sillen(80) has used equilibrium constants in various combinations in an attempt to describe the ionic and solid phase forms of copper in the sea. He concludes that the most likely forms of ionic copper are Cu^{++} and $CuOH^+$ in approximately equal quantities. The main solid phase will probably be $Cu_2(OH)_3Cl$ with appreciable amounts of $Cu_4(OH)SO_4$. These calculations assume equilibrium conditions and "infinite dilution". Activity coefficients of the ions in the sea are not well known. Most of the data used were for reactions at 25°C, whereas the mean temperature of the oceans(23) is 3.8°C. The calculations can thus at best only estimate the forms present in the ocean.

Goldberg and Arrhenius(35) state that about 10% of the copper in the sea is present in an ion-pair complex with sulphate ion (Cu SO4). Of the remaining non-sulphate associated forms, over 90% would be present as hydrated cupric ions or $CuCl^+$, together with minor amounts of $CuCl_2$, $CuCl_3^-$ and $CuCl_4^=$.

Normal sea water is under-saturated with respect to copper, on the basis of calculations(47) using the solubility product of $Cu_2(OH)_3Cl$, so little copper may be expected as this solid phase. Cationic forms of copper thus probably prevail in the ocean.

Copper ions are, however, strongly ~~a~~bsorbed by manganese and iron oxides, suspended minerals and especially by organic matter.(35) Most of the particulate copper will probably be associated with these materials.

Iron is added to the ocean mainly as colloidal forms of hydrated ferric oxide together with a little ferr~~i~~ous ion, which at the pH of sea water immediately oxidises and initially forms a colloidal suspension of ferric oxide.(69)

At the pH of sea water, the concentrations of ionic iron (Fe^{++}, $FeOH^{+}$, Fe^{+++}) in true solution at equilibrium are extremely low (less than 10^{-7} µg/l) and even under conditions far from equilibrium it is unlikely that detectable amounts of ionic iron are present.(80) The soluble iron found after microfiltration probably consists of stable organic complexes or uncharged species such as $Fe(OH)_3$ and polymers of this type.(80) Fluoride, chloride and sulphate complexing of ferric iron cannot compete with hydroxide complexing at the concentrations found in the sea.(80)

Most of the iron in the sea is accordingly in a colloidal or particulate form. The colloidal forms are probably stabilized by the presence of organic compounds. The "particulate" iron is probably present in suspensions of minerals (clays) or may be organically bound in plankton.

62/...

Manganese reaches the ocean as colloidal quadrivalent oxides and in the bivalent form, which like iron, is converted to the colloidal oxide at the pH of the ocean.(69) An important difference between iron and manganese is that manganous ions are apparently more stable in the sea than ferrous ions.(35)

Sillen(80) has calculated that the most likely ionic species of manganese in the sea are manganous ion and permanganate ion, but both would only be present at concentrations of c. 10^{-9} µg/l. Sillen considers it unlikely that these ions could exist for long in the sea and proposed that uncharged hydroxy complexes ($Mn(OH)_3$, $Mn(OH)_4$) and organic complexes were responsible for the concentrations of "soluble" manganese in the sea.

However, Goldberg and Arrhenius(35) consider that the manganese is mainly present as manganous ion in aquato- or chloro-complexes and that 85% of the manganese was present in true solution. Their view and that of Sillen may be reconciled as the former authors suggest that thermodynamically unstable species, such as manganous ion, can exist, as there are few active surfaces in the ocean on which the reaction may proceed to equilibrium.

It thus appears that manganese is present in both soluble and particulate forms with the soluble species being more abundant.

Conclusions as to the nature of the organic complexes of copper, iron and manganese cannot be made at this stage as too little is known about the organic compounds in the sea.(80)

3.2.2 General oceanographic background.

The area investigated (Fig. 6) is, oceanographically speaking, very interesting and was described some years ago by Clowes.[16] Oceanographic data were collected at the same time as samples for trace metals were taken. These more recent data were now used to describe the general oceanography of the area in brief. (Use was made of the general principles of physical oceanography as described in standard text books, such as that by Defant.[23])

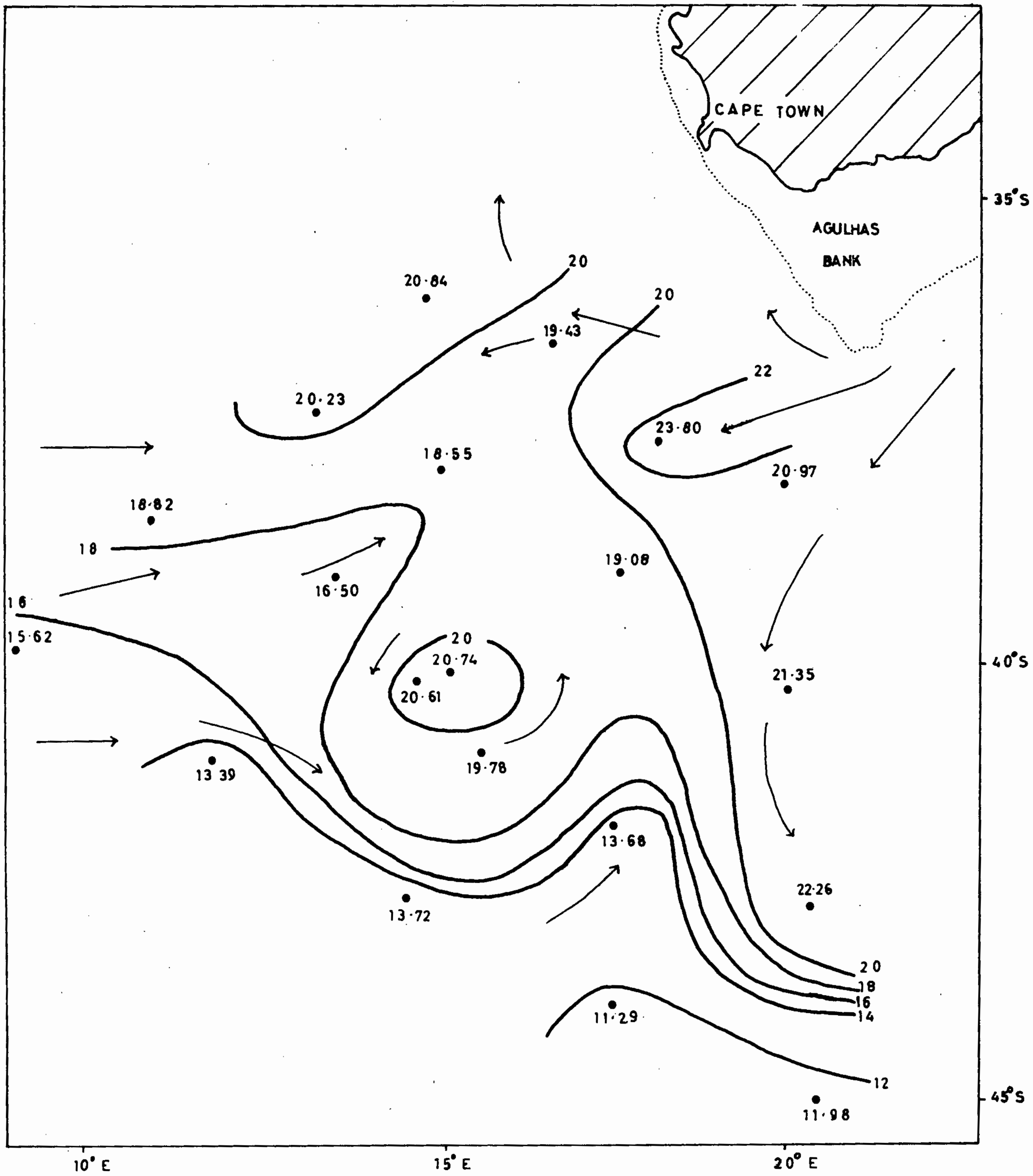
The warm (above 19°C), strong Agulhas current, orginating in Indian Ocean Tropical Water,[65a] flows along the edge of the Agulhas Bank to about 20°E. The current then divides into three streams. Most of the water turns back and returns to the east, but a large part continues on into the S. Atlantic until it meets the West Wind Drift. The remainder of the current turns to the north-west and moves generally northwards past Cape Point. The area of mixing between the West Wind Drift and the Agulhas current is marked by vast oceanic eddies and variable currents.

To the south, near 44°S, a sudden decrease of temperature occurs at the Subtropical Convergence, where cool (c. 15°C) Subtropical Water meets cold (less than 12°C) Subantarctic Water.

Along the west coast of S. Africa, from Cape Point northwards, the phenomenon of upwelling occurs. During summer the prevailing southerly to south-easterly winds produce a current with an offshore-moving component of motion. As water is transported away from the coast, cold

FIGURE 1 3

SURFACE TEMPERATURE(°C) AND CURRENTS IN THE S.E.ATLANTIC DURING APRIL 1964.



deeper water upwells to take its place. This water contains plankton nutrients (phosphate and nitrate) and supports large quantities of plankton when it rises into the photosynthetic zone. (The above description of upwelling is extremely simplified - a full treatment is given by Defant.(23))

Stations 1 to 20 (Fig. 6) were visited during a deep-sea research cruise in April 1964. This cruise was intended to investigate the complex area of interaction of the Agulhas current and the West Wind Drift, and advantage was taken of the opportunity to collect deep-sea samples for copper determination. (Procedures for iron and manganese determination had not been sufficiently developed at that stage). The water masses present at each of station 1 to 20 (determined by T/S analysis)(23) were tabulated in Table 12, and the surface currents and temperatures were shown in Fig. 13.

Table 12: Water masses present at deep-sea stations 1 to 20

Station	Water mass
1	Agulhas Water mixed with Subtropical Water
2	Subtropical Water mixed with decreasing amounts of Agulhas Water
3	Subtropical Water mixed with decreasing amounts of Agulhas Water
4	Subtropical Water present in the West Wind Drift
5	Transition zone between Subtropical and Subantarctic Water
6	Transition zone between Subtropical and Subantarctic Water
7	Subantarctic Water, south of the Subtropical Convergence

Station	Water mass
8	Subantarctic Water, south of the Subtropical Convergence.
9	Mixed Subantarctic and Agulhas Water
10	" " " " "
11	Mainly Agulhas Water mixed with some Subtropical Water
12	Agulhas Water trapped in an anticyclonic eddy
13	Agulhas Water trapped in an anticyclonic eddy
14	Subtropical Water present in the West Wind Drift
15	Uncertain
16	Agulhas Water trapped in an anticyclonic eddy (as stations 12 and 13)
17	Agulhas Water
18	Agulhas Water
19	Strong flow of Agulhas Water
20	Subtropical Water mixed with some Agulhas Water.

Copper samples were collected at stations 21 to 28 during a cruise in July 1964.

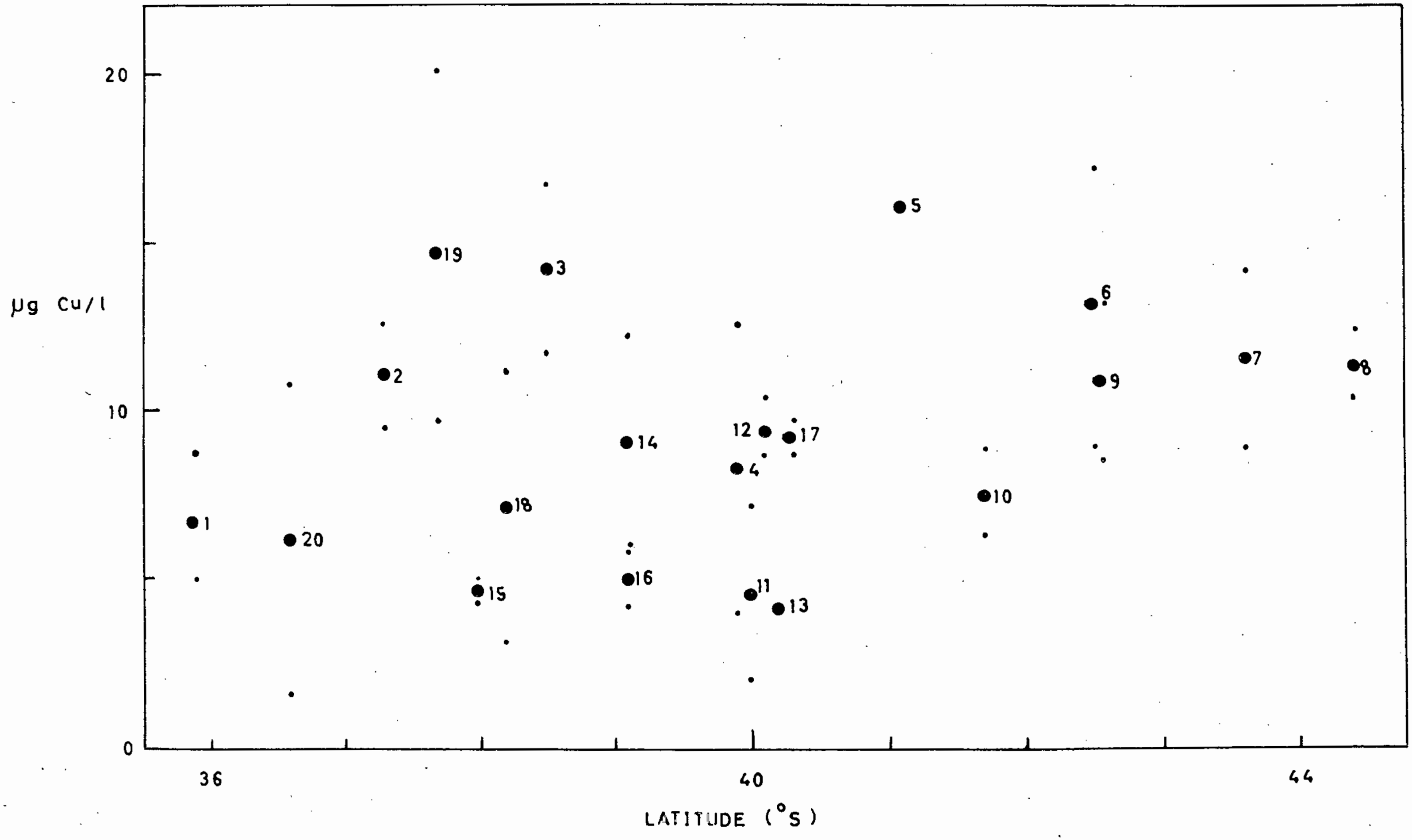
Oceanographic conditions were as follows. Station 22 lay in the centre of a strong flow of Agulhas current water (see Fig. 16). Part of this current moved to the north-west past stations 24 and 25 and then moved close inshore past station 27 off Cape Point. Little upwelling was observed (Fig. 15).

During December 1964, stations 26 to 31 were visited and samples collected for determination of copper, iron and manganese. Oceanographic conditions were no~~t~~w quite different from those in July. Strong upwelling (Fig. 15) took place near Cape Point and in False Bay. The currents

66/....

FIGURE 14

CONCENTRATION OF COPPER AT EACH OF STATIONS 1 TO 20 PLOTTED AGAINST LATITUDE (LARGE POINTS, WITH STATION NUMBERS NEXT TO THEM, ARE MEAN VALUES)



are shown in Fig. 16. Water from the offshore S. Atlantic flows in a north-easterly direction between stations 29 and 31 and then moves round to the north. Stations 28, 29 and 31 lay in this water. A cyclonic eddy(23) causes upwelling just to the south of station 30.

3.2.3 Copper.

As many more samples were taken for copper determination than were obtained for iron and manganese, an attempt was made to link copper concentration with oceanographic conditions.

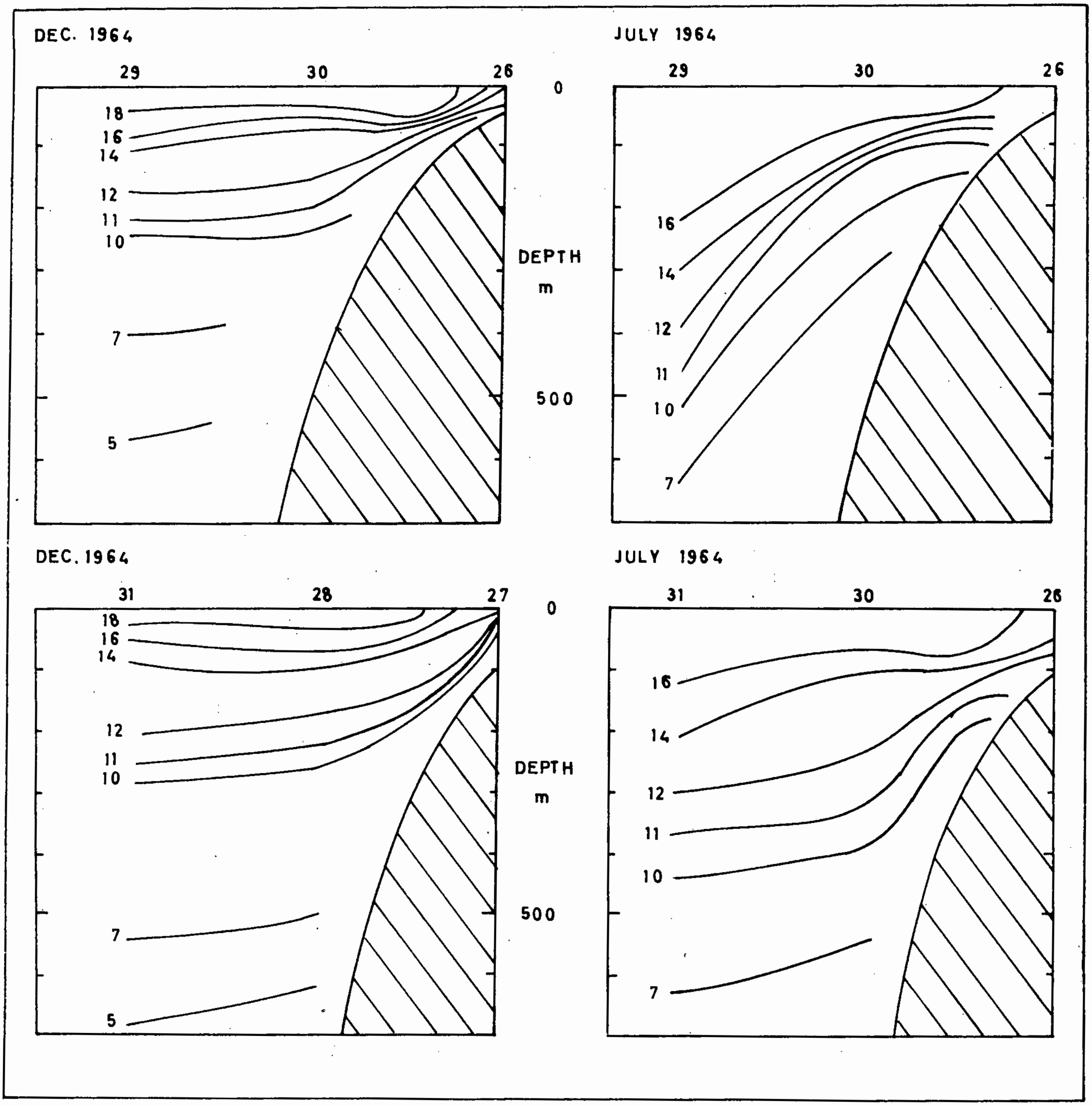
The results for the April 1964 cruise (stations 1 to 20) (Table 4) were plotted against latitude (Fig. 14). Both individual results and mean values (large dots) were plotted. The results showed a large scatter between replicate samples taken from the same 1200 ml sample of sea water. This was considered to be due to the presence of dead or living plankton in the sample, thus rendering it inhomogeneous. The plankton would later release bound copper to the water during storage or determination of the sample, and different quantities of plankton might be present in two 400 ml samples taken from the same water sample. Figure 14 (above) showed little relationship between copper concentration and latitude.

Many variables (ocean currents, upwelling processes and organic activity, all of which are difficult to determine with accuracy) may affect the concentration of copper (and the other metals) in sea water. As only 38 samples were available from the deep-sea area (in which copper concentration was not expected to be homogeneous) normal statistical procedures were not applicable.

67/...

FIGURE 15

TEMPERATURE PROFILES THROUGH STATIONS 26, 27, 28, 29, 30 and 31



A further difficulty in interpretation arose from the inhomogeneity among replicates mentioned above. A laboratory measurement of the precision of the technique apparently had little meaning when ocean samples were determined.

The mean of all 38 determinations was 9.3 µg copper/l. Of these determinations only 5 had concentrations greater than 14 µg copper/l. The frequency distribution of all 38 determinations was drawn (Fig. 20). This appears to be bimodal, with the modes lying at about 4 and 10 µg copper/l.

Comparison with water masses can only be made on a very general basis, as the spread of the data was large.

Samples from Subantarctic Water (stations 7,8) indicated that this water mass had a mean copper content of 11.5 µg/l. All samples from Agulhas water (stations 1, 11, 12, 13, 16, 17, 18, 19) with the exception of that from station 19, had mean values of less than 9.5 µg copper/l. High mean values of copper concentration were found at stations 3, 5, 6 and 19. (The single high value of 20.1 µg/l found at station 19 may perhaps be due to contamination of a sample). The West Wind Drift Water (stations 4, 14) seemed to have mean values ranging from 8 to 9 µg/copper/l.

It appeared that Agulhas Water was associated with a low copper concentration, the West Wind Drift with moderate concentrations while the Subantarctic Water had a moderately high copper content. The high concentrations at stations 5 and 6 were no doubt associated with the upwelling of deep water in cyclonic eddies, which were usually present just to the north of the Subtropical Convergence.(65b) The mean value at station 3 appeared exceptionally high, and the oceanographic conditions gave no explanation of this phenomenon.

The Tropical Water origin of the Agulhas Water(65a) gives an explanation for the low copper content. In the equatorial regions, nutrients and trace metals are taken up by the plankton, which then die and sink to deeper levels. The strong thermocline (temperature gradient) in these regions prevents vertical mixing and upwelling seldom occurs.(23) A net loss of trace metals thus takes place, and the surface water becomes depleted.

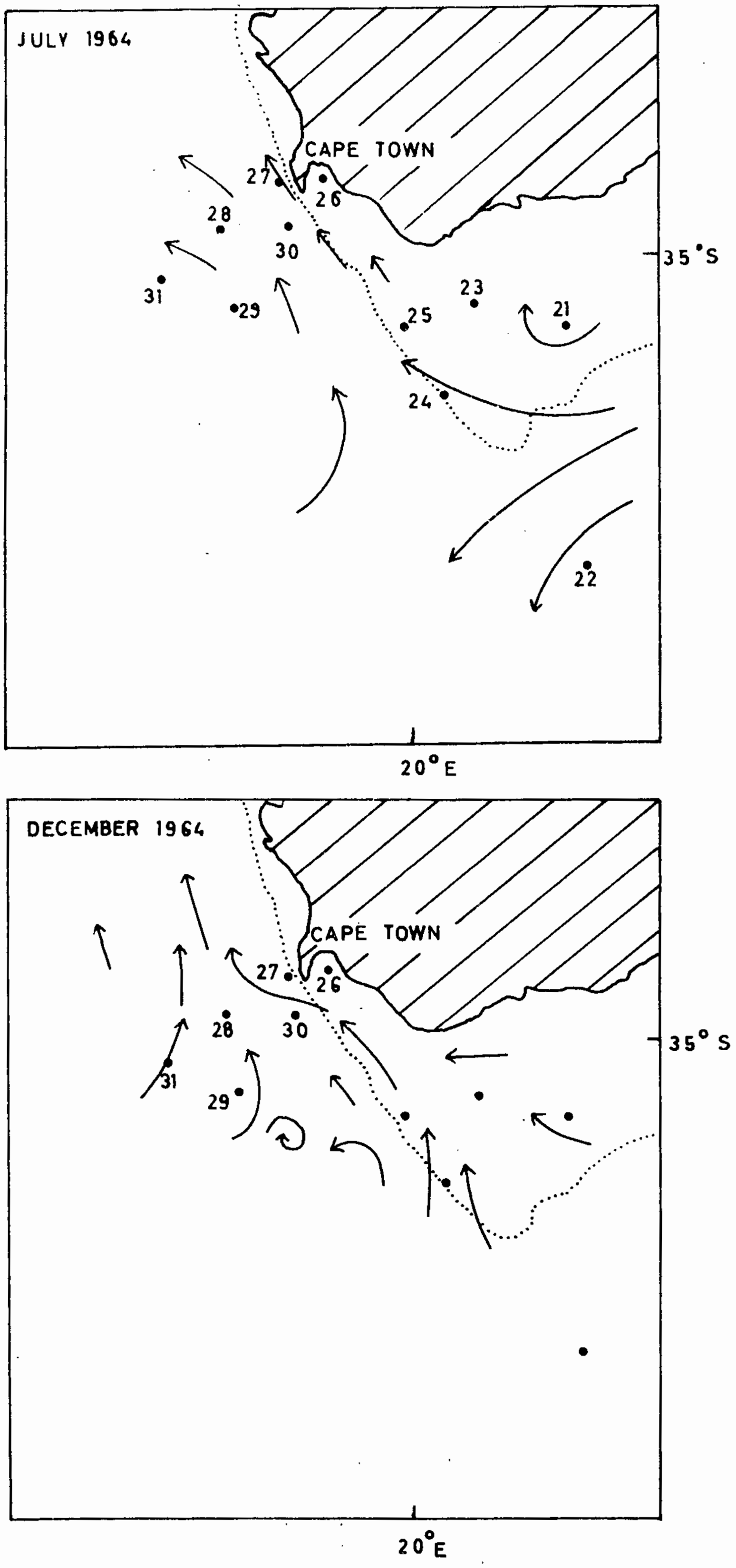
It is of interest to note that the warm Kuroshio current, in the North Pacific Ocean, has a very low concentration(58) of copper (0.5-0.7 µg/l) in its surface layers. This current, like the Agulhas current, has its source in tropical waters. Between the Kuroshio current and the coast of Japan, concentrations of copper increase rapidly, and maximum ~~volume~~ values of 300 µg/l were found close inshore, with very high concentrations (1500 µg/l) in sheltered(58) bays. Land drainage was most probably responsible for the high inshore values.

The Subantarctic Water may derive its higher copper content from water of Antarctic origin, which is rich in trace metals due to glaciation (see above) processes.(78) Further determinations of the copper content of water farther south would be required to substantiate this hypothesis.

Little can be said about the West Wind Drift and Subtropical Water copper content.

During the July 1964 cruise, samples of Agulhas Water were again collected at stations 22, 24 and 25. The mean copper concentration at these stations was 2-5 µg/l the lowest value (2.2 µg/l) being found in the Agulhas current itself. (Fig. 16, station 22).

FIGURE 16
INSHORE CURRENTS DURING JULY AND
DECEMBER 1964



Samples of sea water from stations 21 and 23 differed appreciably in copper content although both stations apparently lay in the same water mass. The lower value found at station 23 indicated the presence of some Agulhas Water, but this was not evident from current charts (Fig. 16). Samples from station 26 in False Bay ~~and~~ had a moderate concentration of copper (11 µg/l). Temperature profiles showed little evidence of upwelling (Fig. 15). Station 27 lay in water of rather low copper content (6 µg/l) for inshore water, and this was probably due to the presence of a current of Agulhas Water, and to the fact that little upwelling had taken place (Fig. 15).

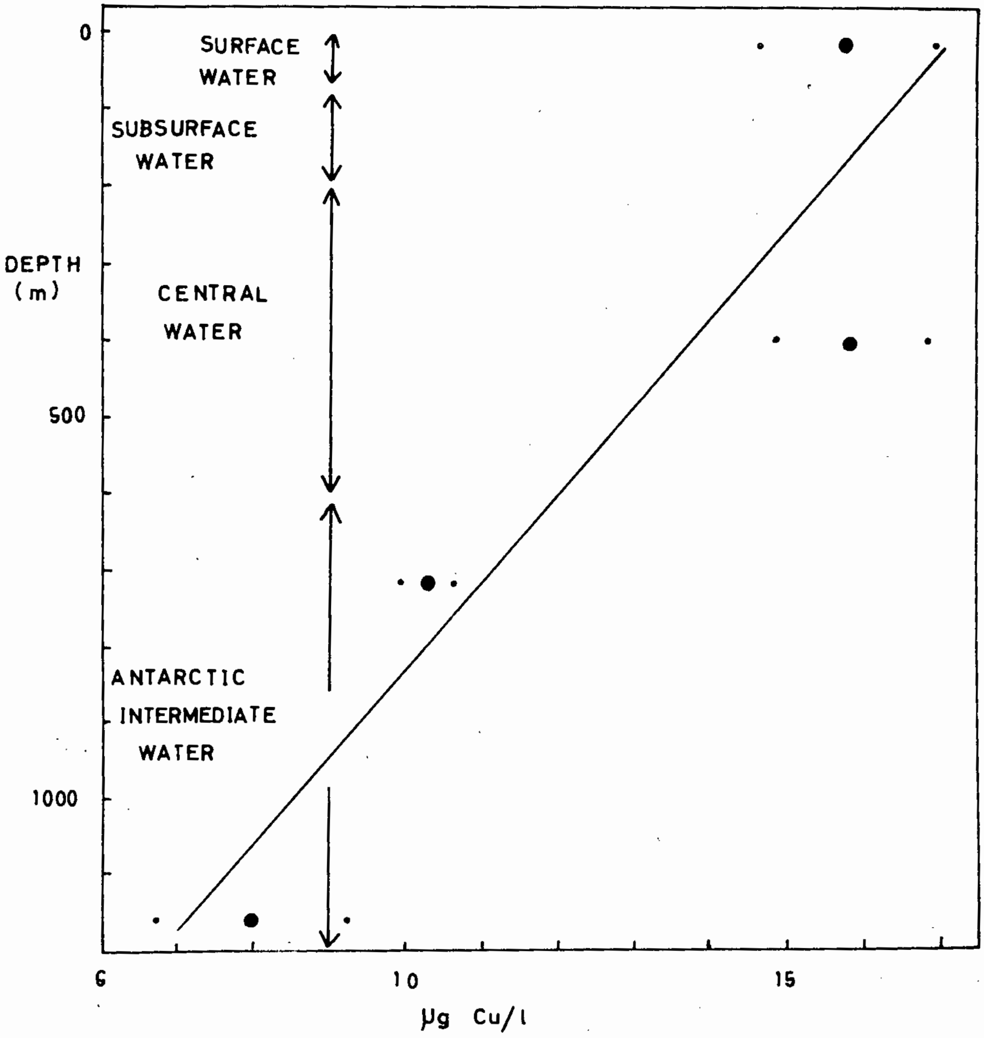
At station 28, a series of samples was obtained from four depths (20, 400, 720 and 1160m). Depths were measured by the use of oceanographic reversing thermometers.(23) The variation of copper content with depth was plotted (Fig. 17). The graph showed that the copper content was fairly high (15-16 µg/l) in the upper 400 m, then decreased with depth to about 8 µg/l at 1200 m.

The water masses present were identified and the approximate boundaries marked on the graph (as oceanographic conditions change gradually on passing from one water mass to another, the boundaries are not clearly defined and extend over some tens of meters).(23) The Antarctic Intermediate Water appeared to have a lower copper content than the other water masses.

The Central Water originates from sinking of surface water in the area just north of the Subtropical Convergence and this water mass then moves northwards at about 500 m to upwell on the west coast of S. Africa.(65b) The concentration of copper in Central Water off Cape Point (station 28) was 15-17 µg/l. The copper content at the source of this

FIGURE 17

DISTRIBUTION OF COPPER CONCENTRATION WITH DEPTH
AT STATION 28



water mass, (stations 5 and 6) is in the range 13 to 16 µg/l and this agrees well with the values measured at station 28.

Samples were taken at stations 26 to 31 during December 1964. Strong upwelling of deeper water was now evident (Fig. 15) at stations 26 and 27, and the copper content was high (15-17 µg/l). The seasonal variation of copper at these two stations is shown in Table 13, and the marked increase in copper content in summer, brought about by upwelling, was very noticeable.

In the English Channel, the concentration(4) of copper is at a maximum in winter (25 µg/l) and a minimum in autumn (1-2 µg/l). The maximum concentration is most probably brought about by land drainage. Land drainage causes a maximum concentration of copper of c. 2 µg/l in San Juan Channel (near Seattle, U.S.A.) during summer.(15b) In the latter area, (a shallow (100 m) near-shore channel) the distribution is complicated by the presence of sulphide zones,(15b) which may precipitate much of the copper.

TABLE 13 Seasonal variations of copper concentration

Station	Season	Winter (July) µg copper/l	Summer (December) µg copper/l
26		11	17
27		6	15

The current diagram (Fig. 16) showed a flow of water from the offshore S. Atlantic moving to the north-east past stations 28, 29 and 31. The copper content of this water mass was about 9-15 µg/l. The high value of 16 µg/1 found at station 30 was most probably caused by upwelling of deep water in the cyclonic eddy south of station 30.

Very low (0-5 µg copper/1) and quite constant concentrations of copper were found in Sea Point surf samples. This was unexpected as land drainage was expected to increase metal concentrations inshore.(78) ~~Abs~~sorption of ions on the large quantities of organic matter and suspended minerals (clay and sand) present may perhaps remove these ions from solution.

3.2.4 Iron.

Sea water samples for iron determination were collected at stations 26, 27, 29 and 31 during December 1964. The inshore upwelled water contained 10 to 13 µg iron/1 and was higher in iron content than water further offshore (0.4 to 9 µg/1). The lowest concentration was found in the current of offshore water (Fig. 16) at station 31, indicating that deep-sea water has a very low iron content.

Samples taken from the surf zone at Sea Point were low in iron concentration (4-5 µg/1), again, like copper, probably due to absorption of iron on suspended matter. A single sample collected after a period of heavy rain had a concentration of 14 µg iron/1. It appears that the iron content in the surf zone can fluctuate appreciably, depending on the local drainage into the sea. Manganese likewise increased in the surf zone after rain, but copper was not affected.

3.2.5 Manganese.

Samples of sea water were collected at stations 27, 26 and 30 for determination of manganese in December 1964. For comparison, two consecutive samples were taken from 20 m depth at station 27. The concentration of manganese showed little variation, but was higher off Cape Point (station 27) than in False Bay (station 26). The concentration of manganese in the water at station 30 may be influenced by the upwelling in the nearby eddy, and was not considered representative of offshore water.

Surf zone samples were moderately low in concentration and increased after a period of heavy rain by a factor of about two.

As there are no large river mouths in the area studied, addition of traces of metals by land drainage appears to be negligibly small, and the concentrations of trace copper were actually higher in summer than in winter, when greater drainage occurs. The trace metal concentration in this area studied thus appears to be controlled almost exclusively by oceanographic processes of upwelling and surface currents, but may be modified by organic processes inshore.

3.3 Development of an ion exchange procedure.

3.3.1 Background.

The number of sea water samples which may be collected and stored in a frozen state, is severely limited by the refrigeration space required aboard ship. The samples cannot be stored in the ordinary "cold rooms" as these do not have a low enough temperature to freeze the samples solid, and special arrangements were necessary aboard ship to keep samples frozen.

Another technique for preserving ions for subsequent

73/...

determination is that of sorbing the ions on to ion exchange resin. This technique has been applied to hydrological field sampling(2) using cation exchangers. Anion exchange resins have been used to collect chloro-complexes of metals such as uranium, gold, zinc, cadmium, iron, copper and manganese after addition of hydrochloric acid to sea water samples.(13, 22) Large quantities of acid are required and the procedure does not lend itself readily to routine use at sea.

Cation exchangers have apparently not been applied to the extraction of trace metals from sea water. Sea water contains an average of about 72 meq ions/1, (Table 1) excluding sodium ions. Use of a resin in the sodium form excludes sodium. It was decided to attempt to extract trace metals from sea water on a sodium form cation exchange resin.

3.3.2 Development of the method.

The choice of a suitable resin was governed by the requirements of

(i) High affinity for copper, iron and manganese
(ii) High exchange capacity, to ensure a compact column size
(iii) Ease of regeneration.

One the basis of these requirements, a carboxylic acid active group type of cation exchanger was chosen. This resin (Amberlite IRC 50) has a marked affinity for copper,(27) and the unusual ability, for a cation exchanger, to select copper in preference to calcium.(48) This effect is probably caused by the nature of the active group which readily forms covalent bonds such as occur in the hydrogen form resin.(48) Copper has a greater ability to form covalent bonds than calcium, and thus is held more strongly by the active group.

Other divalent ions are also readily taken up by the resin(48) and it was expected that iron and manganese ions, if present as divalent ionic species, would be taken up by the resin. A further advantage of this resin for use in sea water is that the exchangeable sodium ions are more readily replaced than those bound to the sulphonic acid type of cation exchanger (sodium form).(48)

The carboxylic acid resins have neutralization curves which closely resemble those of low molecular weight carboxylic acids, and, by analogy, this is taken to indicate that the active group is incompletely dissociated.(48) Below pH 3.5, the resins exist almost entirely in the hydrogen form and do not undergo exchange reactions.(5) The exchange capacity increases markedly with pH as the active group becomes more highly dissociated. At pH 7 the exchange capacity is about 10 meq/g dry resin, i.e. more that double that of a sulphonic acid type of exchanger such as Amberlite IRI20 (4 meq/g capacity).(48) Sea water behaves as a well-buffered, slightly alkaline solution of pH 7 to 8.5.(23) (Values below pH 7.8 are seldom found in the upper layers of sea water). Within this pH range, the carboxylic acid ion exchangers exert nearly all their high exchange capacity.

The very high affinity of the resin for hydrogen ions, as compared to the sulphonic acid resins, ensures rapid and complete regeneration. On addition of the equivalent quantity of mineral acid, all sorbed metal ions are released.(48) As the ions collected from a large quantity of sea water are eluted by a small volume of acid, favourable concentration factors may be achieved.

The order of affinity of the resin for metals such as copper, iron and manganese may be anticipated on theoretical grounds.

A study (1) of the second ionization potentials of divalent ions of approximately equal size, has shown that the effective binding force of a cation increases as the second ionization potential increases. Since the carboxylic acid group is capable of entering into strong covalent bonds, (48) it may be expected that the order of affinity would be related to the effective binding force of the exchanging cations, and this again is related to the second ionization potential.

Ion	Size	Second Ionization Potential
Cu^{2+}	0.70A	20.3 volts
Fe^{2+}	0.74A	16.2 "
Mn^{2+}	0.80A	15.6 "

It is apparent that of the three metals, copper should have the greatest effective binding force, followed by iron and manganese, and accordingly, the theoretical order of affinity should be $Cu^{2+} > Fe^{2+} > Mn^{2+}$

Preliminary experiments were next carried out to determine whether copper, iron and manganese were held by the resin at the levels of concentration found in sea water.

The flow rates of 2 to 4 ml/min used for the preliminary investigation of copper, were chosen as rates of 1-2 ml/min were found satisfactory for sorption of trace copper on phenolic type resins. (48) The recovery of copper was good (Table 7) and appeared to increase with decrease of flow rate. The optimum flow rate was about 2 ml/min and this was used in all subsequent experiments.

77/...

Iron and manganese (Table 8,9) were retained less efficiently by the resin. The results for iron were erratic and this was ascribed to the irreversible formation of hydrated ferric oxide.(73) Manganese was not held strongly, and the experiment was repeated, using a flow rate of *c*.1 ml/min to investigate whether this would improve retention. Inconclusive results were obtained. The figure of 79% retention appears anomalous, and may perhaps be caused by an error in technique. No further work was done on the ion exchange sorption of manganese as the metal could not be determined on elution (see 2.4). The combined sorption of copper and iron was then investigated. Although recoveries were low (Table 10), the experiment demonstrated that simultaneous sampling of the effluent for determination of copper and iron was feasible.

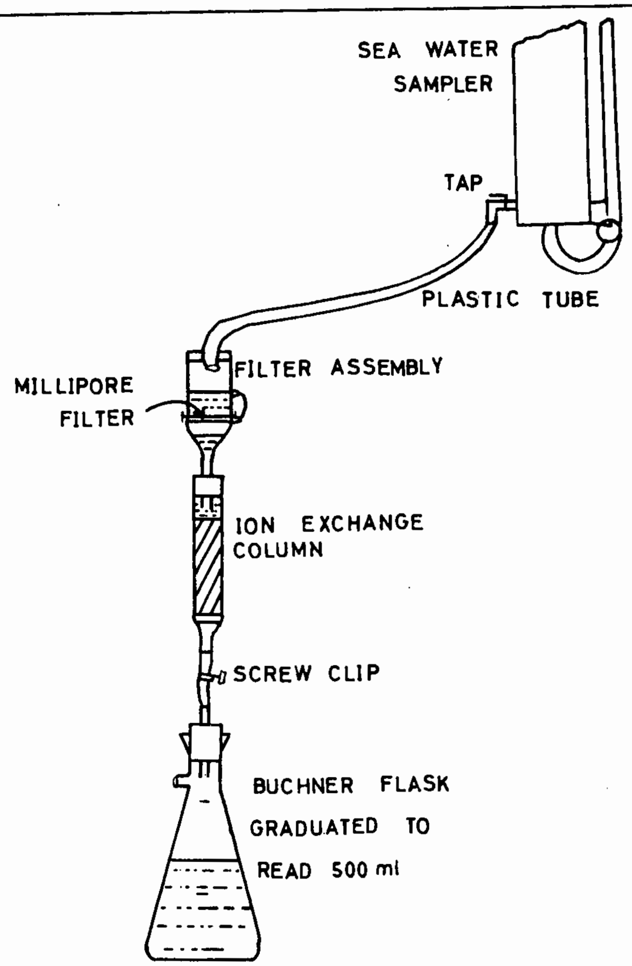
The experimental results for sorption of the three metals copper, iron and manganese were in agreement with the theoretical discussion above.

Initially, the resin was regenerated in bulk, and then transferred back to the columns. This procedure gave high elution blanks (*c*. 4.5 µg iron) in the iron procedure, and in later experiments, regeneration was carried out in the columns. This reduced the blank to *c*. 2.5 µg iron.

The reaction between sodium ions and the hydrogen form resin attains equilibrium very slowly,(48) and complete regeneration was a lengthy process. To avoid delay partial regeneration was carried out by passing excess sodium hydroxide through the column at a fairly high flow rate. An excess of resin (about 50% greater than the theoretical amount) was used to counteract the incomplete conversion. This procedure was rapid and convenient, and was satisfactory

FIGURE 18

APPARATUS FOR FILTRATION AND ION EXCHANGE TREATMENT OF SEA WATER SAMPLES



in practice.

The effects of contamination may be seen in Table 10. The sample marked with an asterisk was contaminated by a few drops of sea water which had briefly contacted a retort stand clamp. The increase in copper and iron was marked.

The analytical determinations of copper, iron and manganese used previously did not distinguish between "soluble" and "particulate" metal forms. An attempt was now made to devise a technique suitable for determining both forms of trace metal in one sampling operation.

A filter assembly to determine particulate matter was designed and made. (Fig. 25). The apparatus was of perspex construction with a lid to prevent the entry of dust. The millipore filter was used without a supporting filter as the support might remove ions by sorption. The perforated perspex disc supported the filter adequately under miled suction. The transparent perspex allowed the operator to adjust the inflow of sample so that the filter did not run dry or overflow. Any failure of the filter could also readily be observed.

The filter was fed with sea water through a flexible plastic tube fitted into the hole in the filter assembly lid. A small hole was pierced in the plastic tube to avoid pressure effects which hindered filtration.

Below the filter was fitted a sodium form ion exchange column to collect the ionic species present. It was found convenient to graduate the reciever to indicate the progress of the procedure. The apparatus is sketched in Fig. 18.

In order to test the procedure under conditions closely

FIGURE 19
KINETIC PLOTS FOR LOSS OF COPPER ON STORAGE

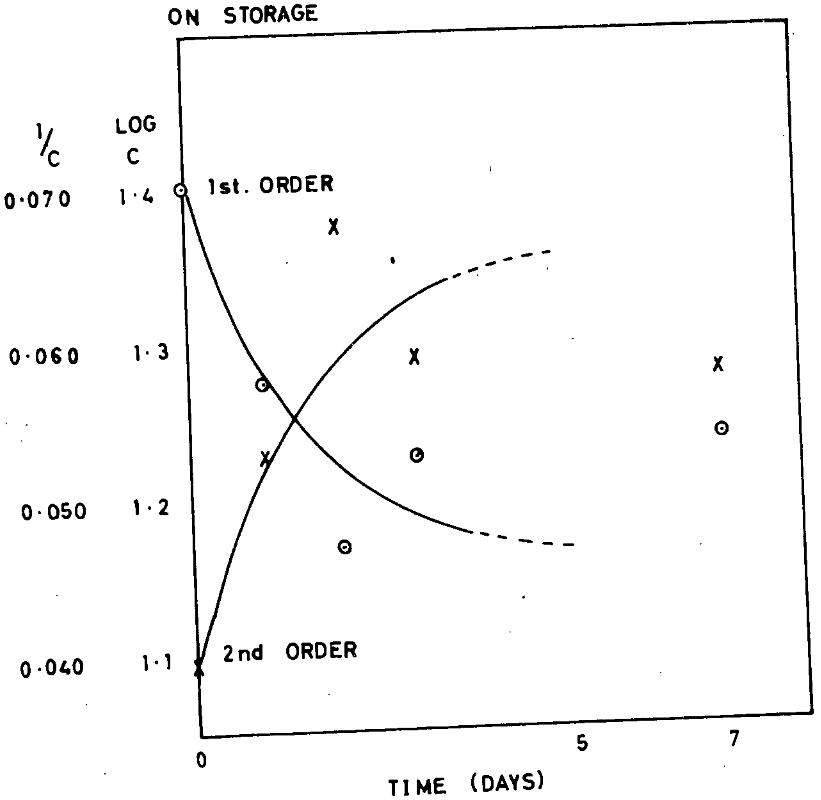
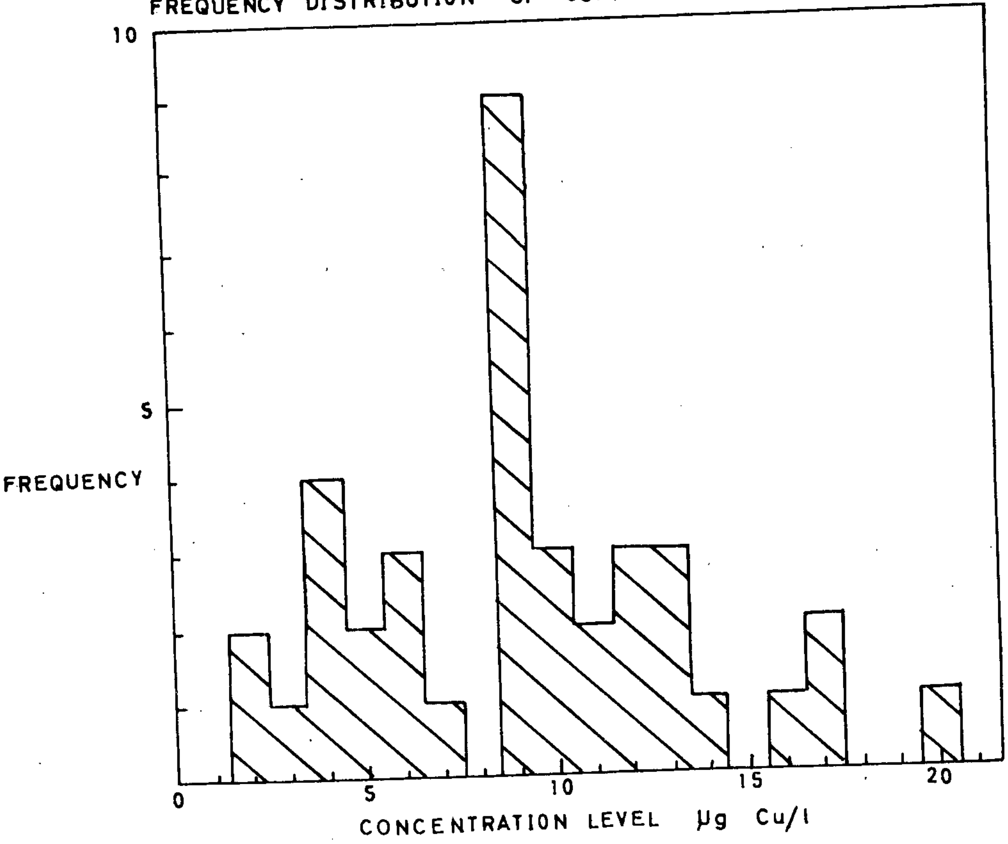


FIGURE 20
FREQUENCY DISTRIBUTION OF COPPER DETERMINATIONS



resembling those at sea, a sea water sampler was used to feed the sea water to the filter. As the processes of ion exchange and filtration should not be affected by ship motion etc., a special ocean cruise was not considered necessary to test the procedure.

Filtered sea water (6.10) was used in the experiments as an unfiltered sample of surf zone sea water contained so much suspended matter (sand, seaweed etc.) that the millipore filter choked up after a short time. In off-shore areas, the sea water contains much less suspended material and prefiltration of samples would not be necessary.

The procedure was as described in 6.7.2. Results were tabulated (Table 14) and the μg copper found by ion exchange plotted against the copper added (determined by the usual procedure).

TABLE 14 - Test of the ion-exchange method for determining copper and iron.

Metal added μg/500 ml	Ion exchange analysis μg/500 ml	Particulate metal μg/500 ml	Total metal μg/500 ml
Copper			
* "0"	0.(9)	-	-
0.8	4.(3)	3.(3)	7.(6)
5.(2)	3.(7)	0	3.(7)
7.(8)	7.(6)	3.(8)	11.(4)
10.(8)	(19)	3.(1)	-
14.(6)	13.1	-	-

80/...

FIGURE 21
KINETIC PLOTS FOR LOSS OF IRON ON STORAGE

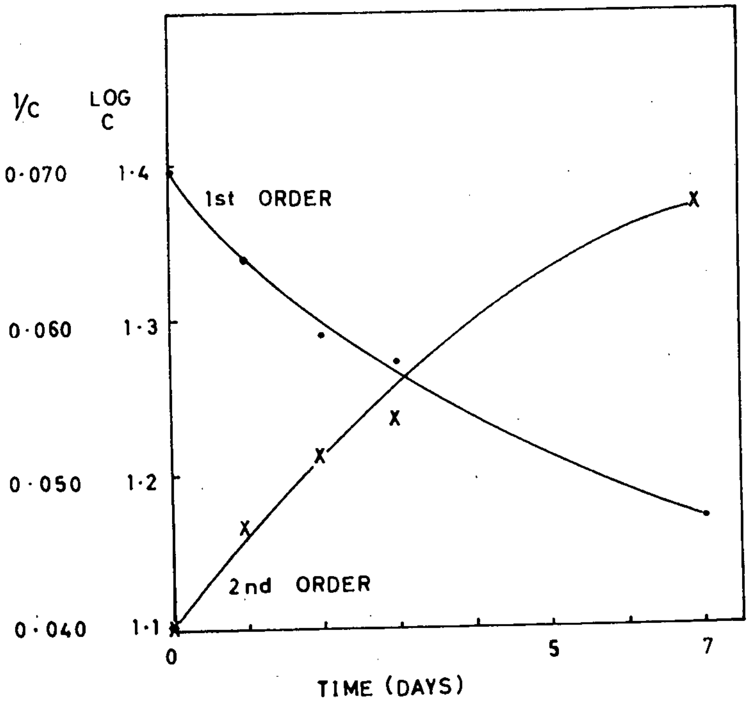
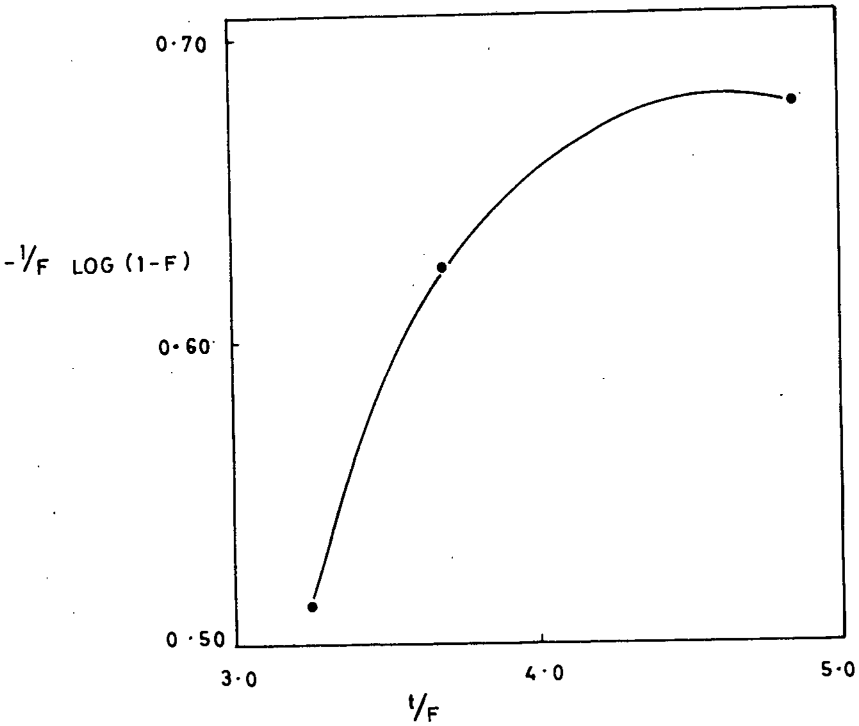


FIGURE 22
TEST FOR TYPE OF DIFFUSION PROCESS



Metal added µg/500 ml	Ion exchange analysis µg/500 ml	Particulate metal µg/500 ml	Total metal µg/500 ml
Iron			
* "0"	1.(4)	0.(6)	2.(0)
0.(5)	0.(4)	1.(9)	2.(3)
2.(7)	0.(7)	3.(9)	4.(6)
4.(4)	2.(3)	5.(1)	7.(4)
8.(9)	2.(5)	8.(6)	11.(1)
11.(9)	2.(9)	9.(7)	12.(6)

* Blank on double-distilled water

Accurately known amounts of copper and iron were not added to the sea water in the "spiking" process. It was found during preliminary experiments on calibration procedures that accurate "spiking" of a large sample (c. 1000 ml) was not always possible. This was probably caused by the fact that the samples were mixed in large (3 1) beakers and sorption of the metal on the glass became significant leading to erratic results. In the calibration procedures, standard solutions were added to samples in the containers in which determination was carried out and the reagents were added immediately afterwards. It was considered that determination of the metal present by the usual procedure would give the best estimate of the concentration of metal present.

The results for copper indicated a linear relationship (Fig. 24) and a straight line was fitted to the data by the method of least squares. The regression equation was

FIGURE 23
IRON DETERMINATION BY THE
ION EXCHANGE METHOD

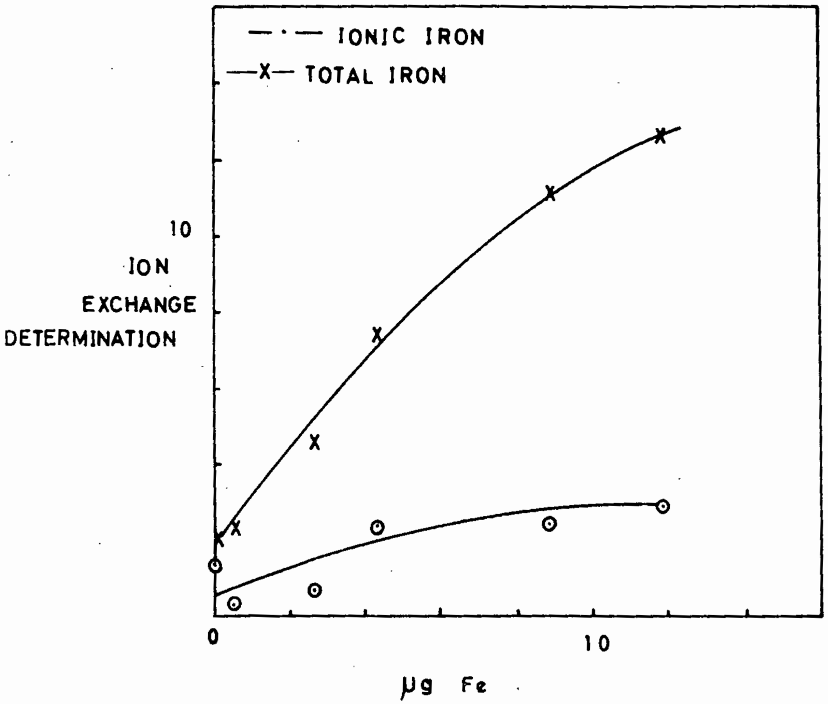
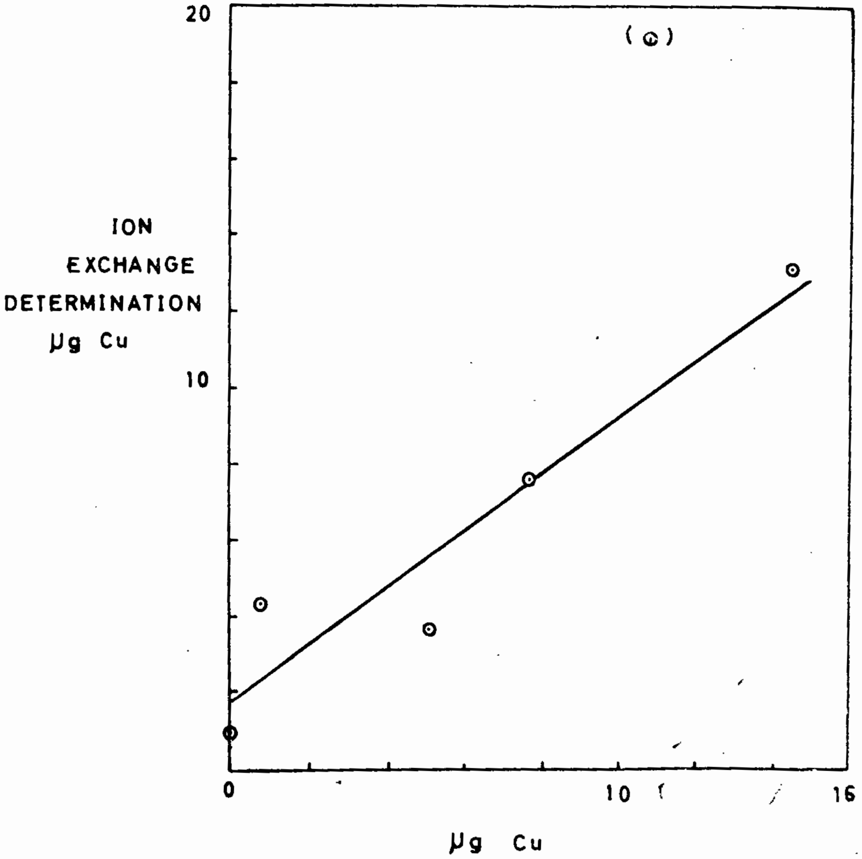


FIGURE 24
COPPER DETERMINATION BY THE
ION EXCHANGE METHOD



$$Y = 1.667 + 0.749\ X$$

Y = µg copper by ion exchange technique

X = µg copper present in the sea water.

The standard error of estimate was 1.42 µg ($n = 5$, concentration range 0-29 µg/l). The very high value of ~~19~~ 38 µg copper/l (bracketed in Table 14 and Fig. 24) was caused by contamination of the sample with a scrap of ash from a visitors cigarette. (Smoking was not normally allowed in the laboratory).

Particulate copper varied from zero to 7.~~0~~6 µg/l and was apparently independent of the ionic copper (cupric ion) added. Three of the four determinations were in the range 6.2-7.6 µg/l and the sample of zero concentration (i.e. equivalent to the blank) may be regarded with some suspicion.

The slope of the graph (Fig. 24) indicated that the ion exchange procedure determined about 75% of the ionic copper added. In this case, both the ion exchange and normal procedures determined ionic copper, so comparison was possible.

The results for the iron determination were quite different (Fig. 23). As the concentration of iron added increased, the amount determined by the ion exchange proceudre increased to a level of about 4 µg/l and then remained constant, while the particulate iron results increased steadily. A plot of the total iron (ionic plus particulate) against the iron present was almost linear. (Fig. 23).

These results were interpreted as indicating that nearly all the ferrous ion added precipitated immediately as hydrated ferric oxide in a non-colloidal form.

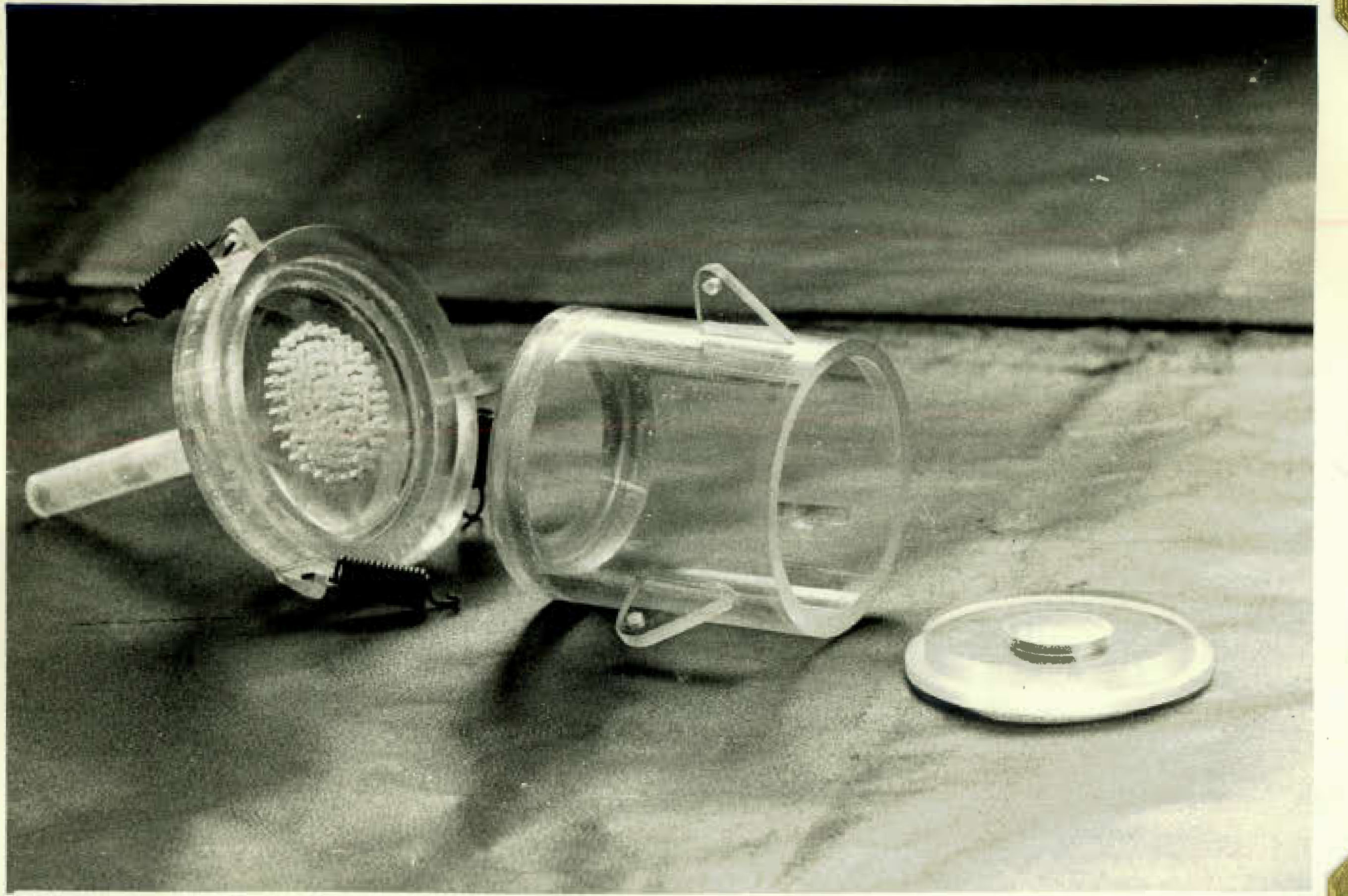


Figure 25. Perspex filter apparatus (9/10 actual size).

At higher concentrations, the formation of colloidal iron oxides which pass through the filter, but are not sorbed on the resin, caused the total iron concentration apparently to level off.

The fact that "soluble iron" to the extent of 4 μg/l was held by the resin indicates that this iron must exist in a charged form. This is perhaps some stable positively charged organic complex. Uncharged compexes would not be held on the column.

Copper was not appreciably sorbed on the precipitated ferric oxide as the particulate copper results do not show any increase with increase of particulate iron.

The ion exchange procedure enables the ionic species present in sea water to be studied and enables a subdivision of the "soluble" fraction of metal to be made. A slight variation of the procedure would enable a detailed study of the trace metal fractions in the sea as follows:

(i) Filter the sea water through a coarse filter, then through the apparatus (Fig. 18). Determine the total particulate metal in both known particle size ranges.

(ii) Treat the effluent from the column with acid to release colloidal and complexed metals and determine them.

This procedure would enable detailed study of ionic species, colloidal and complexed species, and various fractions of suspended material, to be made.

Sorption on ion exchangers may thus successfully be used to determine copper in sea water, but this technique was not suitable for determination of iron and manganese.

Colloidal iron was not collected either by the filter or by the resin and would pass undetected. Manganese was not strongly held by the resin, and like iron, any colloidal manganese would not be detected.

3.4 <u>Losses on storage in polythene</u>.

Although it is well-known that some changes[(3,20)] in trace metal concentration occur on storing samples of sea water in polythene, nothing definite has been published about the extent of loss or the processes involved.

An attempt was made here to study the losses that occur on storing sea water samples for some days in identical polythene containers and to investigate whether any pattern existed.

The results of the experiment were tabulated in Table 11. The concentration of each metal ion was then plotted against time (Figs. 11, 12). The small dots represent individual determinations and the large dots represent mean values.

Study of the plotted points indicated that replicates showed a large scatter, which was most marked in the case of copper. The concentration of copper in samples kept at room temperature decreased by about 30% in the first two days and then remained fairly constant. The frozen samples showed little variation from the original sample concentrations. On a previous occasion, it was also found that the concentration of copper decreased to about 60-70% of its original value on storage in polythene for seven days.

The concentration of iron in samples kept at room temperature showed a steady decrease over the entire period. A puzzling feature was that the frozen samples were noticeably lower in concentration that the original samples.

84/...

The manganese samples stored at room temperature showed little variation with time, but the frozen sample appeared to have a slightly higher concentration. (The sea water sample kept frozen for forty days for manganese determination was unfortunately spoilt).

The scatter observed between identical samples was disappointing. All the polythene bottles were carefully filled to avoid contamination. All bottles had been in use for some months and were thoroughly cleaned before the experiment.

The observed losses of metal may be brought about by

(i) Bacterial utilization
(ii) Precipitation processes
(iii) Sorption on the wall of the vessel

A large increase in bacterial population occurs on confining sea water in a container, as the bacteria multiply on the inside surfaces. Relatively little is known about the marine bacteria,(68) but certain fresh water bacteria are known to utilize ionic manganese and iron in their metabolic processes,(37) and the final product is the metal oxide. Certain fungi also utilize traces of metals(63) and it is possible that fungal forms in sea water may have similar requirements. Organically bound metals in the bacteria would most probably be released during the process of determination. In the case of iron, the sample container was well-shaken before removing the 100 ml sample in order to collect any particulate material which might have settled. Bacterial colonies might well be loosened in this procedure, and on treatment with acid, release their iron. The manganese procedure should likewise release these bound metals in the process of dissolving the precipitate. In the case of copper, where reagents were added directly to the sample container which was then shaken for an hour, it is possible that the bacteria may only release some copper on extraction with diethyldithiocarbamate.

It is, however, difficult to believe that bacterial action will account for much of the observed concentration changes, and evidence for this view appears in Figs. 11 and 12. Bacteria multiply extremely rapidly, and if bacterial action were the cause of the loss, one would expect an initial rapid decrease in metal concentration followed by a constant level of concentration when the surface was "saturated" with bacteria. This is not apparent in the figure. Manganese concentration does not change appreciably at all, and the copper and iron results show a steady decrease.

A slow precipitation of iron as the hydrated oxide may occur on standing, as a large surface area is exposed on which reactions may take place. The precipitate may carry down some copper as well. The process of shaking samples well before removing an aliquot for iron and manganese determination seems to rule out the possibility of losses due to precipitation.

The process of shaking should suspend all the precipitated material which would then either be brought into solution in the iron determination, or precipitated in the usual way with magnesium hydroxide in the manganese procedure. Precipitation processes may, however, account for the losses of copper as the reagent does not react with particulate copper.

The most likely source of removal of ions is by sorption on the walls of the polythene container. The containers used had an internal surface area of about 200 cm^2 exposed to the sample.

In the following discussion, the scatter in the experimental results made it difficult to reach accurate conclusions as to the process concerned.

Application of the Freundlich adsorption isotherm(57) showed that the decrease in concentrations of copper and iron ions apparently did not obey the empirical equation. This indicated that a simple sorption process was not likely.

Next, the rate of decrease in concentration of copper and iron was tested to see whether 1st or 2nd order kinetics(57) were followed (Figs. 19, 21). It was uncertain whether the rate of decrease followed either 1st or 2nd order kinetics.

The sorption of ions on polythene may, to a first approximation, be regarded as an ion exchange process. The polythene may be analogous to a highly cross-linked ion exchanger of very low exchange capacity. Possible sites(87) in the polythene where "exchange" might possibly occur are -

(i) Polyethner linkages formed from the molecular oxygen used to initiate polymerization.

(ii) Trapped oxygen atoms, from the initiation reaction, bound in the lattice.

(iii) Double bonds, resulting from disproportionation during the ethylene polymerisation.

(iv) As polythene is 80-85% crystalline, surface "crystal" defects may be present and these might sorb ions.

(v) Hydroxyl groups may be present in small quantities in the polymer.

The preliminary washing of the containers with acid would most probably attach hydrogen ions to these active sites, and it may be expected that these ions would only partially be removed by the rinsing process, due to slow diffusion through the lattice. (The containers were allowed

87/...

to soak in acid for some time, whereas rinsing only occupied a few minutes).

It was possible, therefore, that an exchange of hydrogen ions for ions of copper and iron took place. The results for iron were tested by the procedure of Boyd et al.,(12) which was originally developed to investigate the rate-determining step in ion exchange sorption. Film diffusion through a Nernst film was not expected to be limiting here as the reaction was slow. The form of the plotted function (Fig. 22) indicated that diffusion through the "particle" (in this case, one large "particle" - the container wall) was apparently the rate determining step. This procedure assumed that the chemical process of exchange was almost instantaneous and that the rate was only limited by physical diffusion processes. These assumptions would also apply to the case of polythene, if the sites mentioned above were responsible for exchange.

The scatter of the results for copper made a plot for checking the rate determining step quite inconclusive and this was not drawn.

Carrying the assumption of ion exchange properties of polythene further, it is to be expected that the affinity of the polythene for ions would be dependent on the relative sizes of the ions and their ability to form covalent bonds.(48) From section 3.3.2, it may be predicted that copper should be taken up more readily than iron, which again has a higher affinity than manganese. Divalent manganese ions are larger than either cupric or ferrous and ferric ions. On an ion exchange affinity basis, therefore, copper would readily be accepted, iron less so, and manganese would only be taken up in lesser amounts.

Examination of the results (Figs. 11,12) shows that the manganese concentration remains fairly constant, and so manganese was apparently not taken up by the polythene. Comparing the mean rate of change of the copper and iron concentrations over the first three days shows that for copper the decrease was <u>c</u>. 2.8 µg/day whereas for iron a decrease of <u>c</u>. 2.1 µg/day was observed. The copper decreased more rapidly during the first two days, whereas the iron concentration decreased steadily during the first three days.

The experimental results approximate to predicted results on the assumption of ion exchange properties of the polythene, and it is reasonable to assume that a diffusion of ions through the sea water to the polythene surface, followed by sorption and ion exchange on impurities and defects in the polymer was largely responsible for the decrease in concentration observed.

Freezing would prevent sorption of metals, as the ions cannot then diffuse through the solution to the polythene surface, but the fact that freezing preserved the samples better would also be true if bacterial or precipitation processes were responsible for the losses.

The large scatter of the copper results required explanation. If the copper was strongly held on the polythene, it is possible that variations in the structure of the polymer in individual containers may have a large effect on the rate of exchange of copper. On adding diethyldithiocarbamate reagent,copper is extracted as the complex from those exchange sites which are readily available, and the number of available sites may well vary between different containers made from different batches of polythene. The overall effect would be to produce variable results when the same batch of sea water was stored for a day or so in different containers.

No ready explanation can be offered for the decrease of iron in the frozen samples. The samples froze about 3 hours after they were placed in the refrigerator, and were thawed within ½ hour. It is highly unlikely that sorption losses could occur to any extent in this short time, and it is difficult to see how losses could occur in the frozen samples. The determination of iron was carried out by the normal procedure in which losses were negligible.

From the above discussion it appears that the preliminary determinations of iron (Table 5) must be treated with some caution, as samples were frozen until analysed, and the results may be low. In the case of copper and manganese, freezing apparently preserved the samples quite well.

It is of interest to note that in the determination of copper, iron and manganese in sea water by atomic absorption spectroscopy,(29) the authors attributed a large scatter in the results to experimental error. They further stated that no loss of metals on the polythene storage vessels (unfrozen) were observed. It is considered that the variable losses of elements on the containers would account for a large part of the experimental scatter.

Glass vessels are not suitable for storing sea water samples as sorption of ions occurs(36,85) (up to 23% of the metal present in solution may be sorbed on the walls). Glass further releases metals such as aluminium and iron into samples.(28)

11. BOWLES, P.F., NICKS, P.F. *Analyst* 1961, *86*, 483.

12. BOYD, G.E., ADAMSON, A.W., MYERS, L.S. *J.A.C.S.* 1947, *69*, 2836.

13. BROOKS, R.R. *Analyst* 1960, *85*, 745.

14. CHATT, J., DUNCANSON, C.A., VENANZI, L.M. *Nature* 1956, *177*, 1042.

15. CHOW, T.J., THOMPSON, T.G. (a) *J. Mar. Res.* 1952, *11*, 124.
    (b) *J. Mar. Res.* 1954, *13*, 233.

16. CLOWES, A.J. *Fish.and Mar. Biol. Survey Div. Invest. Rep.* 1950, No. 12.

17. COLLINS, P., DIEHL, H. *J. Mar. Res.* 1960,*18*,152.

18. CONWAY, D.E., GREEN, J.H.S., REICHENBERG, D. *Trans. Faraday Soc.* 1954, *50*, 511.

19. COOK, G.B., CRESPI, M.B.A., MINCZEWSKI, J. *Talanta* 1963, *10*, 917.

20. COOPER, L.H.N. *J. Mar. Res.* 1958, *17*, 128.

21. CROXTON, F.E., COWDEN, D.J. "Applied General Statistics", Pitman and Sons, London, 1955.

22. DAVANKOV, A.B. *et al.* *Ordzhonikidze, Isv., V.U.Z. Tovetnaye Metallurgiye* 1962, *2*, 118.

23. DEFANT, A. "Physical Oceanography", Vol. I, Pergamon Press 1961.

24. DEGTYARENKO, A.P., LIBINA, R.I., MILLER, A.D. *Gidrokhim. Materialy* 1959, *29*, 264.

25. DINSEL, D.L., SWEET, T.R. *Anal. Chem.* 1963, *35*, 2077.

26. DOBRZHANSKAYA, M.A., PSHENINA, T.I.
    (a) *Doklady Akad. Nauk. SSSR.* 1958, *123*, 895.
    (b) *Tr. Sevastopol'sk. Biol. St. Akad. Nauk. SSSR* 1959, *11*, 316.

27. EDGE, R.A. Anal. Chim. Acta 1961, 24, 143.

28. EREMENKOV, V. Ya. Gidrokhim. Materialy 1959, 29, 242.

29. FABRICAND, B.P., SAWYER, R.R., UNGAR, S.G., ADLER, S.
Geochim. Cosmochim. Acta. 1962, 26, 102

30. FERNANDEZ, A.A., SOBEL, C., JACOBS, S.L.
Anal. Chem. 1963, 35, 1721.

31. FONSELIUS, S.H., KOROLEFF, F.
Bull. Inst. oceanogr. Monaco 1963, 63, 1.

33. GJESSING, E.T. Limnol. Oceanogr. 1964, 9, 273.

34. GOLDBERG, E.D. (a) Geol. Soc. Am. Mem. 1957, 67, 345.
(b) Limnol. Oceanogr. 1962, 7, lxxii.

35. GOLDBERG, E.D., ARRHENIUS, G.O.S.
Geochim. Cosmochim. Acta. 1958, 13, 15

36. GUBIN, A., HASS, E., HOFFMAN, H., REINMUTH, W.
Mitt. Ver. Grosskesselbesitzer 1955, No. 33, 435.

37. HARIYA, Y., KIKUCHI, T.
Nature 1964, 202, 416.

38. HASHITANI, H., YAMAMOTO, K.
Nippon Kagaku Zasshi 1959, 80, 727.

39. HERDMAN, H.F.P. in "The Sea" Vol. II, Interscience Publishers, 1963. pp 124-127.

40. HOOD, D.W., Oceanogr. Mar. Biol. Ann. Rev. 1963, 1, 129.

41. HOOD, D.W., RONA, E., MUSE, L., BUGLIO, B. Am. Chem. Soc Div. Water Waste Chem. Preprints 1961, 44.

42. HOSAIN, M., WEST, T.S. Anal. Chim. Acta 1965, 33, 164.

43. IRVING, H., COX, J.J. Analyst 1958, 83, 526.

44. ISHIBASHI, M., SHIGEMATSU, T., NISHIKAWA, Y. Rec. Oceanogr. Wks. Japan 1960, 5, 63.

45. JOHNSTON, R. (a) J. Mar. Biol. Assoc. U.K. 1963, 43, 427.
(b) J. Mar. Biol. Assoc. U.K. 1964, 44, 87.

46. JOYNER, T. *J. Mar. Res.* 1964, 22, 259.

47. KRAUSKOPF, K.B. *Geochim. et Cosmochim. Acta* 1956, 9, 1.

48. KUNIN, R. "Ion Exchange Resins", J. Wiley, New York, 1958.

49. LAEVASTU, T., THOMPSON, T.G. *J. Mar. Res.* 1958,16,192.

50. LEWIS, G.J., GOLDBERG, E.D. *J. Mar. Res.* 1954,13,183.

51. LOVERIDGE, B.A., MILNER, G.W.C., BARNETT, G.A. THOMAS, A.M., HENRY, W.M. *At. Energy Res. Estab. (Gt. Brit.)* 1960, R3323, 36 pp.

52. MAGEE, R.J., RAHMAN, A.K.M. *Talanta* 1965, 12, 409.

53. MARCZENKO, Z., KASIURA, K. *Chem. Anal.* 1960, 6, 37.

54. MARTELL, A., SILLEN, L.G. "Stability Constants of Metal-ion complexes", The Chemical Society, London, Special publication 17, 1964.

55. MENZEL, D.W., SPAETH, J.P. *Limnol. Oceanogr.* 1962, 7, 151.

56. MILLER, A.D., LIBINA, R.I. *Zhur. Priklad. Khim.* 1959,32,2624.

57. MOORE, W.J. "Physical Chemistry" Longmans Green and Co. 1958.

58. MORITA, Y. *Rec. Oceanogr. Wks. Japan* 1953,1,49.

59. MUSTAFIN, I.S., FRUMINA, N.A., KOVALEVA, V.S. *Zavodsk. Lab.* 1963, 29, 782.

60. NAKANO, S. *Yakugaku Zasshi* 1962, 82, 1256.

61. NATELSON, S., LEIGHTON, D.R., CALAS, C. *Microchem. J.* 1962, 6, 539.

62. NEMODRUK, A.A., STASYUCHENKO, V.V. *Zhur. Anal. Khim.* 1961, 16, 407.

63. NICHOLAS, D.J.D. *Analyst* 1952, 77, 629.

64. ORADOVSKII, S.G. *Zh. Analit. Khim.* 1964, 19, 864.

94/...

65. ORREN, M.J. (a) *Invest. Rep. Div. Seafish. S. Afr.* 1963, No. 45.

(b) *Invest. Rep. Div. Seafish. S. Afr.* 1965, No. 54.

67. PARKER, P.L., GIBBS, A., LAWLER, R. *Publ. Inst. Mar. Sci. Univ. Tex.* 1963, *9*, 28.

68. PROVASOLI, L. in "The Sea" Vol. II, Interscience Publishers, 1963, pp. 165-210.

69. RANKAMA, K., SAHAMA, T.G. "Geochemistry", University of Chicago Press, 1950.

70. RICHARDS, F.A. in *"Physics and Chemistry of the Earth"* Vol. II, Pergamon Press 1957, pp. 77-128.

71. RILEY, J.P., SINHASENI, P. *Analyst* 1958, *83*, 299.

72. RONA, E., HOOD, D.W., MUSE, L., BUGLIO, B. *Limnol. Oceanogr.* 1962, *7*, 201.

73. SAMUELSON, O. "Ion Exchangers in Analytical Chemistry", J. Wiley, N.York 1953.

74. SANANMAN, M., LEAR, D.W. *Chesapeake Sci.* 1961, *2*, 207.

75. SANDELL, E.B. "Colorimetric Determination of Traces of Metals", Interscience Publishers, 1959.

76. SCHAEFER, M.B., BISHOP, M.M. *Limnol. Oceanogr.* 1958, *3*, 137.

77. SCHULEK, E., REMPORT-HORVATH, Z., LASZTITY, A. *Talanta* 1964, *II*, 941.

78. SCHUTZ, D.F., TUREKIAN, K.K., *Geochim. Cosmochim. Acta* 1965, *29*, 259.

79. SEBBA, F. "Ion Flotation" Elsevier Publishing Co. 1962.

80. SILLEN, L.G. in "Oceanography" edited by Mary Sears, *Amer. Assoc. for Adv. Sci.* Washington 1961.

5. ACKNOWLEDGEMENTS.

I would like to express my sincere thanks to my supervisor, Professor E.C. Leisegang, for his most helpful support and valuable advice given to me throughout this work, especially in the circumstances of my inability to attend at the University on a full-time basis during this project.

I should like to thank Mr. B.v.D. de Jager, Director of Sea Fisheries, both for much encouragement during this project, and for the generous facilities accorded to me, which enabled this work to be carried out.

My thanks are due to Mr. M.B. Hanley for much assistance and advice, especially in respect of the use of ion exchangers, during the early stages of this work.

Finally, I would like to thank those colleagues of mine at the Division of Sea Fisheries who willingly assisted me with preparation of the illustrations for reproduction.

6. APPENDIX.

6.1 Washing Procedure.

All glassware used, with the exception of sintered glass filters, was rinsed with aqueous detergent ("Teepol") solution and then with water to remove grease etc. The articles were then soaked in hot (50° to 60°C) hydrochloric acid (70%, v/v)(84) for a few minutes to remove traces of metals and thoroughly rinsed with water. Subsequent washing was carried out by rinsing with the 70% acid followed by repeated rinses (at least six) with water. This procedure was carried out before each experiment to ensure freedom from metallic contamination. Precautions were taken to prevent dust from entering clean glassware. Pipettes were stored in the 70% acid and washed with water when required. Separating funnels were cleaned between each batch of samples by shaking vigorously with three separate amounts of water, allowing to drain and then rinsing well with acetone to remove the last traces of organic reagents and solvents. Sintered glass filter plates used for filtering silver halides were washed with concentrated aqueous ammonia and rinsed with water. This was followed by a wash with concentrated nitric acid, and repeated rinses with water. Soaking in chromic acid followed by repeated rinses with water was used to clean the sintered glass filters used for filtering sea water samples.

Polythene articles were soaked in hot 70% hydrochloric acid for about 30 minutes with frequent shaking and were then repeatedly rinsed with water. The sea water sampler was cleaned by soaking in cold 70% hydrochloric acid for about 10 minutes and was then washed thoroughly with water. Rubberware was heated briefly to about 60°C in the 70% acid and was then boiled in water to remove traces of

98/...

impurities and finally rinsed well.

6.2 Procedure for copper determination.

6.2.1 Four hundred ml samples of sea water.

Place 400 ml sea water (measuring cylinder) in a polythene bottle and pipette in 2 ml DDC reagent (6.10) and 5 ml xylene. Screw the bottle cap on tightly and shake to mix contents. Shake on an automatic shaker for one hour, then let the bottle stand in the absence of strong light for about an hour. Transfer the contents of the bottle into a separating funnel, discard the aqueous layer and run the xylene layer into a 1 cm path length spectrophotometer cuvette. Measure the optical density of the xylene layer against the pure solvent at a wave length of 435 mμ. Carry out a reagent blank by shaking 2 ml DDC reagent with 5 ml xylene.

Note (1) Emulsions sometimes form in sea water and are probably due to the presence of organic matter. These may usually be broken by running off the aqueous layer and gently swirling the xylene solution in the separating funnel.

(2) Xylene may dissolve tap grease from the separating funnels and this causes cloudiness.

(3) The cuvettes should be rinsed with dilute hydrochloric acid between each set of samples in order to remove the film of copper diethyldithiocarbamate formed on the glass.

6.2.2 Strongly acid copper samples.

Add about 50 ml water to an aliquot of the copper solution and add 10 ml (pipette) of citrate reagent (6.10). Add ammonia solution until the pH lies in the range 8.2 to

8.5, transfer quantitatively to a 100 ml volumetric flask and make up to the mark with water. Pour into a polythene bottle and proceed as from the addition of 2 ml DDC reagent in 6.2.1.

6.3 Procedure for iron determination.

6.3.1 One hundred ml samples of sea water.

Place 100 ml sea water (measuring cylinder) in a separating funnel and pipette in 10 ml dilute hydrochloric acid (6.10) and 2 ml hydroxylamine reagent. Shake to mix the contents and allow to stand for 5 minutes. Pipette in 2 ml acetate reagent and 5 ml bathophenanthroline reagent, shake and allow to stand for 10 minutes. Measure out 15 ml isoamyl alcohol into a dry, stoppered 50 ml measuring cylinder, drain this into the separating funnel and restopper the cylinder. Extract the contents of the funnel vigorously for one minute and then allow the layers to separate for at least 15 minutes. Discard the aqueous layer, run the alcohol layer back into the cylinder and adjust the volume to exactly 15 ml by adding acetone. Swirl gently to mix (Note 1) and allow to stand for a few minutes to allow any large water droplets to settle out. Transfer the alcohol extract to a 5 cm path length spectrophotometer cuvette and measure the optical density of the alcohol extract against water at a wavelength of 533 mμ. Run a reagent blank on 100 ml water.

Note

(1) Excessive agitation of the mixture entrains water droplets in the solvent and these impart a turbidity to the organic layer. If the solution is not absolutely clear, allow to stand for about 30 minutes to enable the water to settle out.

6.3.2 Strongly acid iron samples.

Make an aliquot of the iron solution up to 100 ml with water in a volumetric flask. Pour into a separating funnel, pipette in 2 ml hydroxylamine reagent, shake and allow to stand for 5 minutes. Pipette in 5 ml acetate reagent and adjust the pH to between 4 and 4.5 with sodium hydroxide solution. Add 5 ml (pipette) bathophenanthroline reagent and proceed as from the addition of bathophenanthroline in 6.3.1 above.

6.4 <u>Procedure for manganese determination.</u>

Add 50 ml aqueous 0.2N potassium hydroxide, slowly and with stirring, to a 1000 ml sample of sea water in a beaker. Allow the precipitate to settle for an hour, and then stir thoroughly once again. Allow to settle for at least 15 hours (usually overnight). Siphon off the supernatant liquid and discard. Transfer the precipitate quantitatively to 100 ml centrifuge tubes with water. Centrifuge at 4,000 rpm for 10 minutes. Discard the supernatant liquid and add 1 ml (pipette) concentrated sulphuric acid to dissolve the precipitate. Wash the solution into a 100 ml beaker with water and evaporate gently down to a volume of about 12 ml or less. Add aqueous 2N silver nitrate to precipitate the halides, and add a slight excess (± 0.1 ml) of silver nitrate. Warm and stir the solution to coagulate the silver halides, and then allow it to stand in the dark for at least one hour. Filter off the precipitate with a sintered glass filter (5 to 10 micron pores) and wash with the minimum volume of dilute aqueous nitric acid (1%, v/v). Transfer the solution to a 20 ml pyrex test tube, and add 0.5 ml syrupy orthophosphoric acid (88%) followed by about 30 mg potassium metaperiodate. Heat the solution to near boiling and maintain it at 100°C on a boiling water bath for 1½

101/...

hours. Cool the solution in ice and encourage precipitation of silver sulphate by frequent stirring and "scratching" the sides of the tubes with a glass rod. After cooling for about 30 minutes, centrifuge the sample at 2000 rpm for 5 minutes. Measure the solution volume (measuring cylinder) and transfer rapidly to a 5 cm path length spectrophotometer cuvette. Read the optical density of the solution immediately at a wavelength of 525 mμ against water. Reduce the optical density value to that of a 10 ml sample for the purpose of comparison.

Note

(1) A precipitate of calcium sulphate (formed from the action of sulphuric acid on calcium carbonate precipitated from the sea water by the alkali treatment) may form on adding sulphuric acid to the precipitate. This disappears on warming and does not interfere.

(2) If the optical density is not read quickly, further precipitation of silver sulphate may take place in the cuvette and the apparent optical density increases.

6.5 Treatment of frozen samples.

Frozen samples in 400 ml polythene containers were brought to room temperature as rapidly as possible by shaking well in hot tap water. Before opening the container, the accessible underside part of the cap was rinsed with a stream of water to prevent tap water reaching the sample on opening. Care was taken not to heat the samples appreciably above room temperature, as this might hasten the loss of metal by sorption or bacterial action. Samples at -18°C could generally be brought to within a degree or two of room temperature in 20 minutes. The samples were analysed as soon as possible after thawing.

102/...

6.6 Procedure for determining losses on storage in polythene.

Known amounts of standard copper, iron and manganese solution were added to 18 l filtered (20 to 30 micron pore size sintered glass filter) sea water in a large polythene container to give approximate concentrations of 20 µg Cu/l, 20 µg Fe/l and 10 µg Mn/l. The stock solution was well mixed by shaking. Samples (three of 400 ml, three of 100 ml and one of _c._ 900 ml) were taken immediately for determination of copper, iron and manganese respectively. As soon as possible, twelve approximately 400 ml samples in polythene containers were taken from the stock solution and placed in a refrigeration at -18°C. Twenty four more approximately 400 ml samples in polythene containers were allowed to stand at room temperature (20° ± 2°C) in the absence of strong light. All storage sample containers were filled within one hour. After one day, a set of six samples was taken from those standing at room temperature and analysed for copper, iron and manganese. Three approximately 400 ml samples were used for the determination of copper, and 100 ml samples were taken from each of the three remaining samples (after shaking) for iron determination. The volume of sea water used for the determination of copper was measured (after extraction) with a measuring cylinder, and the results corrected to µg copper/l. The remaining 700-800 ml sea water was combined for determination of manganese.

The volume was measured by measuring cylinder. Morning and afternoon room air temperatures were noted daily during the experiment.

A similar set of analyses were completed after two, three and seven days. Similarly, sets of frozen samples were thawed (6.5) and analysed for copper, iron and manganese after eight and forty days respectively. The results were tabulated in Table 11.

6.7 Ion exchange column procedures.

6.7.1 Preliminary work.

Ordinary burettes of 1 cm internal diameter were used as columns. A glass wool plug was used to support the resin. The glass wool was cleaned by the standard glassware procedure (6.1). Approximately 6 g amounts of dry sodium form IRC50 resin were slurried into the columns with water, and allowed to soak overnight.(18) The resin bed was about 90 mm in length and 10 mm in diameter, decreasing to about 75 mm in length when converted to the hydrogen form. The columns prepared as above had a theoretical exchange capacity of 40 to 50 meq. (Total capacity of dry sodium form resin is 8.4 meq/g.)(48)

400 ml (measuring cylinder) samples of sea water of known metal concentration were filtered through the columns from polythene constant-head devices at flow rates of 2 to 4 ml/min. (The columns decreased in length during sorption as sodium exchange took place). The effluents were collected in 400 ml polythene containers, and the metal present was determined by the usual procedure.

Elution was performed with 15 ml (pipette) hydrochloric acid (6.10) in the case of the copper samples, and with 5 ml (pipette) hydrochloric acid followed by 5 ml (pipette) water in the case of iron. Manganese was not determined in the eluted solution as the chloride ion concentration was too high (2.4.6).

In the preliminary experiment, on copper, the eluted ions were collected in 400 ml sea water of known copper content. The acid sea water was then titrated to pH 8-85 by sodium hydroxide solution (6.10), and the copper in the sample determined in the usual way. The increase in copper content was assumed to be due to the eluted copper. This procedure was adopted since at this stage a suitable copper-free buffer had not been developed. Sea water was accordingly used as a natural buffer solution low in copper. In later work, ammonium citrate buffer solution was used (see 2.2.6).

Iron sorbed on the columns was eluted into 100 ml beakers, and the contents of these were made up to 100 ml (graduated flask) and determined as in section 2.3.6.

A sample of sea water containing known amounts of both copper and iron was sorbed on the resin as above. The columns were eluted with 10 ml hydrochloric acid (6.10) and 10 ml water. Aliquots of the acid effluent were analyze d for copper and iron.

Normally, six columns were operated. Four were used for samples, and two columns were used as blanks. In these, 400 ml of water were filtered through the resin and the metal in the effluent was determined. This values was subtracted from the sample effluents obtained during the sorption process, and represented the amounts of metal impurity introduced during the ion exchange process.

The blank columns were eluted in the same way as the samples, and the effluents analysed. The average value obtained from the two blanks was subtracted from the eluted sample values to allow for impurities introduced by the procedure, including traces of metal present in the columns.

In the initial tests on copper, blank determinations were only carried out for the elution stage as no procedure had been developed at this stage for determining copper in distilled water (again as no suitable buffer had been developed). Later work showed that the effluent blank after sorption of copper was equal to the reagent blank, so omission of this determination in the preliminary work would not alter the results obtained.

The percentage of metal retained by the resin and the percentage of metal recovered were calculated from the results.

Regeneration was performed by passing 20 ml sodium hydroxide solution (6.10) through the hydrogen form resin at a rate of about 1 ml/min. The column was then washed with about 30 ml water (see 3.3.2).

6.7.2 Experimental test of the ion exchange method.

Two small columns (about 110 mm long by 20 mm diameter) were cleaned and fitted with clean glass wool plugs. 18 ml hydrogen form resin (c. 63 meq. capacity)(48) were slurried in with water, and the columns allowed to stand overnight.(18) The resin beds were about 90 mm long in the sodium form and 65 mm long in the hydrogen form.

The resin beds were well washed with 20 ml dilute hydrochloric acid (6.10) followed by 20 ml water to remove the last traces of metals from the resin and apparatus. 90 ml 2N sodium hydroxide solution (obtained by diluting the 4N solution made up as described in 6.10) were passed through the column at a rate of about 3 ml/min to convert the resin to the sodium form (see 3.3.2). The column was then washed with 90 ml water (at the same flow rate) before use.

The filter apparatus, (3.3.2) column and sampler were set up as sketched in Fig. 18. The air space between the top of the resin bed and the millipore filter was partially filled with water to prevent the resin bed running dry when filtration started. The tap of the sampler was well rinsed with water before attaching the clean plastic tube.

One litre of a stock batch of filtered sea water was "spiked" to the required levels of copper and iron by adding standard metal solutions, and was well stirred. 500 ml were then rapidly transferred to the sampler by measuring cylinder, and the filtration started by opening the screw clip. The flow rate was adjusted to 2-3 ml/min. (Flow rates were estimated by counting the number of drops falling in a given time, and were checked by measuring the overall rate). As soon as possible, the remaining 500 ml of spiked sea water was divided into 400 and 100 ml samples which were then used for copper and iron determination respectively.

The flow rate was maintained at 2-3 ml/min by the flow through the filter under gravity and suction was not necessary.

After 500 ml had passed through (measured on the graduated receiver), the flow was stopped and the millipore filter was removed and stored, folded face to face, between filter paper. The last few ml of sample were now run through the resin at 2-3 ml/min. The wet column was then stored in an upright position until eluted.

The column was washed with about 20 ml water to displace the solution in the resin bed, and was then eluted with 10 ml (pipette) hydrochloric acid (6.10) followed by 10 ml (pipette) water, both at a flow rate of 0.2 to 0.3 ml/min. The acid effluent was collected in a 100 ml beaker and aliquots were taken for determination of copper and iron. The total volume of the effluent was measured. The total concentration of metal present in the effluent was then calculated.

A blank value for the procedure was determined by eluting a column of sodium form resin, prepared as above.

The concentrations obtained were corrected for blanks and tabulated in Table 14. These concentration values were then plotted against the amount of metal added (Fig.24).

A 500 ml quantity of water was run through the procedure exactly as above to obtain the value for establishing the zero on the "metal added" scale.

The columns were washed with 20 ml dilute hydrochloric acid and 20 ml water and were regenerated as described above for conversion to the sodium form.

6.8 Treatment of micropore filters.(84)

The filter (millipore HA filter, 0.45 micron pores) was placed in a stoppered 50 ml measuring cylinder containing 11 ml dilute hydrochloric acid reagent solution. Cleaned perspex-tipped forceps and a clean glass rod were used for handling the filter to avoid the introduction of metallic contamination. The filter was completely immersed in the acid, by use of the glass rod. The loosely stoppered cylinder was then placed in a boiling water bath for ten minutes and then allowed to cool. 5 ml aliquots were pipetted out and treated as described for "acid" copper and iron samples. (6.2.2, 6.3.2). A blank determination was made by extracting clean filters from the packet in triplicate and calculating a mean blank value for the copper and iron contributed by the filter. This amounted to about 3 µg copper and 1 µg iron respectively.

~~6.9 Instruments.~~

108/...

6.9 Instruments.

Optical density measurements were carried out on a Unicam SP 600 spectrophotometer, using matched 1 cm. glass cuvettes, and on a Zeiss M4Q spectrophotometer using matched 1 and 5 cm. glass cuvettes. A Photovolt Model 180 omni-range glass electrode pH meter was used.

6.10 Reagents.

All chemicals used were of reagent grade quality and were not specially purified unless otherwise stated. Double glass distilled water was used throughout. Sea water used for calibration purposes was collected in polythene containers from flushing rock pools, filtered through a porosity 3 (20 to 30 micron) sintered glass filter to remove seaweed, plankton and detritus, and then stored in polythene vessels.

DDC reagent: 2 g sodium diethyldithiocarbamate (B.D.H.) was made up to 200 ml with water, *c*. 100 mg anhydrous sodium carbonate were added and the solution filtered (Whatman 40) in order to remove copper impurity as insoluble copper diethyldithiocarbamate. This reagent is unstable below a pH of seven(2.2.1), but with the addition of sodium carbonate, the solution was stable for up to two weeks. The solution was discarded when a strong, unpleasant odour developed.

Xylene: Xylene was redistilled in an all glass apparatus.

Hydrochloric acid: About 750 ml of concentrated acid was diluted with about half its volume of water and slowly distilled from an all glass apparatus. The first 100 ml and the last 400 ml were rejected. The acid was standardized gravimetrically as silver chloride.(93) The laboratory facilities were such that this method was rapid and more convenient than the standard volumetric procedure.

Sodium hydroxide solution: Approximately 4N aqueous sodium hydroxide was passed at about 5 ml/min through a column (130 mm long; 25 mm diameter) of sodium form IRC 50 resin (0.3 to 0.5 mm bead size) to remove metal traces, and was then stored in polythene.

Standard copper sulphate solution: A solution of hydrated cupric sulphate (B.D.H. micro-analytical reagent grade) of approximate concentration 50 µg copper/ml was prepared and diluted as required. The solution was standardized by the thiocyanate method.(93)

Dilute hydrochloric acid solution: 30 ml of the redistilled hydrochloric acid reagent (see above) were made up to 400 ml. This solution was <u>c</u>. 0.55 N.

Hydroxylamine reagent: Hydroxylamine hydrochloride (B.D.H.) was recrystallized(84) from 0.4 times it~~e~~s weight of 10% v/v hydrochloric acid, then again from 0.4 times its weight of water. The crystals were washed with a little cold water and dried at 110°C for an hour. 40g crystals were dissolved in 400 ml water, 5 ml bathophenanthroline reagent added followed by 10 ml isoamyl alcohol and the whole extracted in a separating funnel for 1 minute. The organic layer was then discarded. The extraction was repeated until the organic layer was colourless, the organic layer separated off, and the reagent stored in polythene.

110/...

Acetate reagent: 300 g hydrated sodium acetate were added to 400 ml water and 2 ml hydroxylamine reagent added, followed by 5 ml bathophenanthroline reagent and 10 ml isoamyl alcohol, and the whole extracted in a separating funnel for 1 minute.(84) The organic layer was then discarded. Extraction was repeated until the organic layer was colourless, the organic layer was separated off, and the reagent stored in polythene.

Bathophenanthroline reagent: 140 mg 4,7-diphenyl-1,10-phenanthroline (B.D.H. commercial grade) was dissolved in 200 ml absolute ethanol and 200 ml water added.(84) The reagent was stored in polythene.

Standard iron solution: A solution of ferrous ammonium sulphate ($6H_2O$) (B.D.H.) containing approximately 50 µg iron/ml was diluted as required. 2 ml hydrochloric acid were added to each litre to stabilize the solution. The solution was standardized for total iron (Fe^{2+} plus Fe^{3+}) by the permanganate method.(93)

Standard manganese solution: Approximately 0.1 N aqueous potassium permanganate (c. 2 N in sulphuric acid) was standardized by arsenic trioxide,(93) and appropriate dilutions were reduced by a slight excess of sodium sulphite and the sulphur dioxide boiled off.(75)

Ammonia solution: 200 ml ammonia solution (S.G. 0.88) were isothermally distilled(43) into 100 ml water for 5 days, and the solution stored.

Citrate reagent: Triammonium citrate, ("for copper determinations", B.D.H.) of stated copper content "less than 1 ppm", was purified by adding 2 ml DDC reagent and 5 ml xylene to a 1 M aqueous solution

111/...

and extracting in a polythene vessel for 1 hour. Two more extractions were required to reduce the copper content sufficiently (indicated by a colourless organic layer). (The salt originally contained c. 0.6 ppm copper).

IRC 50 resin: Amberlite IRC 50 (hydrogen form) ion exchange resin (analytical grade, bead size 0.33 to 0.5 mm) was cycled from the hydrogen form to the sodium form repeatedly to remove polyelectrolytes and metal traces, and was washed to remove fines. The final cycle involved the use of redistilled hydrochloric acid and purified sodium hydroxide solution (both diluted with water to c. 2N) to give sodium form resin of high purity. The resin was air-dried at 50°C using an infra-red lamp for 8 hours to provide a dry weight reference. The resin was subsequently allowed to soak overnight(18) in water before use.

6.11 Statistics.

The statistical terms used were as follows:

Mean value $\bar{x} = \sum^{i} x_i/n$

n = number of results

x_i = individual results

Standard deviation of a single determination $s = \left[\sum(x_i-\bar{x})^2/n-1\right]^{\frac{1}{2}}$

For a regression equation $y_c = a + bx$,

$$a = \sum x \sum xy - y \sum x^2/(\sum x)^2 - n\sum x^2$$

$$b = \sum x \sum y - n \sum xy/(\sum x)^2 - n\sum x^2$$

The standard error of estimate, or the standard deviation of the y values around the estimating equation $y = a + bx$ is(21

$s_{y,x} = \left[\Sigma y_s^2/n-1\right]^{\frac{1}{2}}$ where

$\Sigma y_c^2 = a\Sigma y + b\Sigma xy$

$\Sigma y_s^2 = \Sigma y^2 - \Sigma y_c^2$

The correlation coefficient $r = \left[\Sigma y_c^2/\Sigma y^2\right]^{\frac{1}{2}}$