

STUDIES ON ORGANO-METALLIC INTERACTIONS IN THE MARINE ENVIRONMENT

Part 1. Interaction of Some Metallic Ions with Dissolved Organic Substances in Sea-Water

ELIZABETH KOSHY, M. V. M. DESAI AND A. K. GANGULY

Health Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay-85 (AS), India

ABSTRACT

The role of dissolved organic matter in the marine environment in complexing the metallic elements present at tracer levels has been studied. Dissolved organic matter obtained from a filtrate from a marine culture solubilized Mn, Zn, Fe and Zr-Nb. Mn and Zn formed cationic complexes or were attached to the organic complex as exchangeable cations, while Fe and Zr-Nb, in part, were either 'non-ionic' or 'anionic'.

INTRODUCTION

MANY dissolved elements occur in trace quantities in sea-water. At the pH of sea-water and in the presence of major inorganic chemical constituents, however, bi-, tri- and tetravalent elements, such as zinc, iron and zirconium, precipitate.

Sillen,¹ while discussing the different chemical states of trace elements dissolved in sea-water, noted that organic compounds at concentrations of 10^{-5} M to 10^{-4} M may form strong complexes (cf. also Goldberg²) with certain metal ions, and that these organic complexes might contribute largely to some of the elements present at the very low concentrations of 10^{-6} M to 10^{-7} M. Goldberg² speculated on the possible complexing of the heavier lanthanides, whose concentrations are of the order of 10^{-10} M to 10^{-11} M, by soluble organic debris. In discussing the recovery of vanadium by precipitation with $\text{Fe}(\text{OH})_3$, Burton³ noted the possibility that vanadium, in the form of organic complexes, may not be recovered by the procedure. The presence of a non-dialyzable, yet ultrafilterable, fraction of manganese and zinc in sea-water indicates a complexed form (probably organic) for these elements (Rona et al.⁴). In analyzing sea-water for iron (Laevastu and Thompson⁵) and copper (Alexander and Corcoran;⁶ Slowey et al.⁷) higher concentrations of these elements were observed in the dissolved state when the water was oxidized than when determinations were carried out without the oxidation step. Hood⁸ stated that it has not been clearly demonstrated in any of the investigations that the inorganic elements in sea-water are chelated with organic compounds.

ZoBell⁹ defined the dissolved organic matter as that which is not removed with a high speed centrifuge. Fox et al.,¹⁰ Jeffrey and Hood¹¹ and Hayer et al.,¹² have reported the soluble organic matter as the one which passes through a $0.45\text{-}\mu$ Millipore membrane filter. Duursma¹³ attributed the following properties to the dissolved organic matter:

1. Not precipitable at pH 1 within 24 hours in a half-litre bottle,
2. Not retained by a membrane filter,
3. Not readily separated by centrifugation, and
4. Not co-precipitable with $\text{Al}(\text{OH})_3$.

Of the various experimental definitions, the simplest, of course, is the one that uses the property of filterability of the solute in the medium. The $0.45\text{-}\mu$ Millipore membrane filter has been widely used to separate dissolved substances and in this laboratory, as a lower limit, $0.22\text{-}\mu$ membrane filters have been used for filtration throughout this work.

SOLUBILIZATION

It has not been possible, in the present experiments, to establish the chemical states of the trace elements dissolved in the medium; therefore, the rather loose term 'solubilization' was used to mean the process of keeping the elements in a state of dispersion or solution such that they passed through a $0.22\text{-}\mu$ Millipore membrane filter.

The following laboratory experiments were conducted to provide direct evidence of the role of dissolved organic matter in solubilizing and complexing trace metallic ions. As representatives of di-, tri-, and tetravalent elements, manganous manganese, zinc, ferric

iron and zirconium-niobium were chosen for the preliminary experiments.

MATERIALS AND METHODS

Culture.—An algal culture (*Microcystis littoralis*, a common littoral Cyanophyceae from west coast of India) was grown in sea-water (collected from near-shore regions of Bombay, India) for six months and allowed to decay in dark for two years at constant pH(8.2), at ambient temperature (27° to 29° C.), and with constant concentration of major inorganic constituents. The supernatant liquid was then filtered. This culture filtrate and a sample of near-shore filtered sea-water were used for the investigations.

Dissolved Organic Matter.—Dissolved organic constituents in the filtered sea-water (20 l) and the culture filtrate (2 l) were concentrated on $\text{Fe}(\text{OH})_3$ by the addition of FeCl_3 (5 mg./ml. of sea-water and culture filtrate) to the acidified medium followed by dropwise addition of ammonia while stirring. Stirring of the ammoniacal slurry was continued for 5 hours, $\text{Fe}(\text{OH})_3$ was allowed to settle overnight, and the supernatant liquid was siphoned off; the $\text{Fe}(\text{OH})_3$ was centrifuged, dispersed once in distilled water, recentrifuged, the supernatant liquid was discarded, the precipitate was dissolved in concentrated HCl, and the molarity was adjusted to 8. Iron was removed by repeated extractions with isopropyl ether equilibrated with 8 M HCl. During the process, 13% of organic matter was removed by the ether. The aqueous layer was then passed through an anion exchange column (20 cm. \times 1 cm.) of Dowex 1- \times 8 (50–100 mesh, conditioned with 8 M HCl) to remove last traces of iron, and the column subsequently was washed with two column volumes of 8 M HCl. The effluent and washings were concentrated to 5 ml. by evaporation under partial vacuum. The amino-acids in these concentrates were identified by descending paper chromatography using an *n*-butanol : acetic acid : water (4 : 1 : 4) solvent system. Amino-acids detected, by the comparison of R_f values of the ninhydrin reactive spots of the culture and sea-water with those of standard amino-acids, were cystine, lysine, arginine, serine, glycine, proline, glutamic acid, threonine, phenylalanine, tyrosine, methionine, valine, isoleucine, leucine and an unidentified amino-acid with a higher R_f (0.84) than that of Leucine (0.73). It may be noted that under the condition of low pH adopted in the procedure, the peptides

or polypeptides that may be present in the media could have hydrolysed. The amino-acids identified in the chromatogram are all not necessarily present in the monomeric state. Since $\text{Fe}(\text{OH})_3$ does not quantitatively remove the organic matter (see also Jeffrey and Hood,¹¹ Koshy¹⁵) no attempt was made to estimate the relative abundances of the different amino-acids in the two media. From the similarity of the nature of the amino-acids present in the culture filtrate and in sea-water, we have assumed that the nature of the organic matter present in the culture filtrate resembles those usually present in sea-water. The culture filtrate as well as the sea-water also gave an indication of the presence of the organic compounds of the quinonoid and phenolic character.

The carbohydrate content of the filtered sea-water and the culture filtrate was determined by use of anthrone reagent in H_2SO_4 (Lewis and Rakestraw¹⁴). Sea-water and culture filtrates were found to contain 0.3 mg./l. and 1.77 mg./l. of carbohydrates, respectively. Carbohydrates were not analysed for the individual components.

The dissolved organic matter of the culture that passed through the 0.22- μ Millipore membrane filter was caused to interact with added tracers such as Mn^{54} as MnCl_2 , Zn^{65} as ZnCl_2 , Fe^{59} as FeCl_3 and Zr^{95} - Nb^{95} as $\text{ZrO}(\text{NO}_3)_2$.

EXPERIMENTAL

Experiments with each element were conducted with 10 ml. of filtered sea-water and 10 ml. of culture filtrate. In each case, 100 λ of the tracer activity was added while pH was maintained at 8 by stirring with a glass rod previously dipped in dilute alkali. Solutions were stored for a week at room temperature (25° C.) with intermittent shaking, and then filtered again. Aliquots of the filtrates (5 ml.) were counted. As a blank, 100 λ of each respective tracer was dissolved in 5 ml. of distilled water. Since MnCl_2 was highly soluble in sea-water and in the culture filtrate, the effect of organic matter on solubilization of manganese was studied with the use of the insoluble manganese compound— $\text{Mn}(\text{OH})_2$. Equal volumes of manganese chloride solution labelled with Mn^{54} were placed in two tubes and the hydroxide was precipitated by dilute ammonia, the solution was centrifuged, the supernatant rejected, and the precipitate repeatedly washed with doubly distilled water (DDW) until free of alkali. Equal volumes

(10 ml.) of filtered sea-water and culture filtrate were added to the precipitates, the solutions were stored for a week as above and filtered. Counting was conducted on 5 ml. aliquots of this filtrate. An equivalent amount of manganese, precipitated as hydroxide and washed free of alkali, was dissolved in 5 ml. of dilute HCl and counted to estimate the added activity. The specific activities and the results obtained are shown in Table I.

of the bi-, tri-, and tetravalent ions present in the influent.

RESULTS

Sea-water did not significantly solubilize Mn from $\text{Mn}(\text{OH})_2$, whereas 11.6% of the Mn which was solubilized in presence of dissolved organic matter was in the cationic form (Tables I and II). About 29% of the zinc plated or precipitated out in sea-water at pH 8,

TABLE I

Solubilization of Mn, Zn, Fe^{3+} and Zr-Nb in sea-water in presence of dissolved organic matter

	Mn^{54}	Zn^{65}	Fe^{59}	* $\text{Zr}^{95}\text{-Nb}^{95}$
Specific activity in the system (counts per 5 min. per $\mu\text{g.}$)	15.85	1064	44370	678100
	Activity in counts per 5 minutes of			
	Mn^{54}	Zn^{65}	Fe^{59}	$\text{Zr}^{95}\text{-Nb}^{95}$
Total in the system	.. 7928	4042	4526	6433
Solubilized in 10 ml. sea-water	.. 26 (0.3%)	2866 (70.9%)	158 (3.5%)	1816 (28.7%)
Solubilized in 10 ml. culture filtrate	.. 916 (11.6%)	4132 (100%)	2126 (47%)	5300 (82.4%)

Figures in parentheses indicate the percentage of the added activity. *Values of Zr concentration in sea-water are from Sarma *et al.* (1968).²¹

TABLE II

Behaviour of Mn, Zn, Fe^{3+} and Zr-Nb on Dowex 50W- \times 8 cation column in presence of dissolved organic matter in sea-water

		Activity in counts per 5 min.							
		Mn^{54}		Zn^{65}		Fe^{59}		$\text{Zr}^{95}\text{-Nb}^{95}$	
		*S.W.	Culture	S.W.	Culture	S.W.	Culture	S.W.	Culture
Influent	..	10	366	1146	1653	55	744	738	2120
Effluent and washings	..	Nil	Nil	Nil	Nil	Nil	259	306	1815
							(34.8%)	(41.4%)	(85.6%)

Figures in parentheses indicate the percentage of the influent activity. * $\text{Mn}^{54} \text{Cl}_2$ in sea-water was also observed to be completely cationic.

To investigate the state of the tracer activity in the sea-water and in the culture solution, 4 ml. aliquots (3.5 ml. for Fe^{59}) of each filtrate was passed through Dowex 50W- \times 8 (50-100 mesh) cationic exchange columns (5 cm. \times 1 cm.) conditioned to the Na^+ form by neutral 3.5% NaCl. Each column was washed with 6 ml. of 3.5% NaCl, and the total effluent (10 ml.) from each concentrated to 5 ml. and counted. The activity recovered in the effluents is shown in Table II. The quantity of cationic resin taken was in overwhelming excess in its exchange capacity compared to the equivalents

but in the presence of dissolved organic matter all of the added zinc remained in solution or apparent solution. However, the solubilized zinc, in sea-water as well as in culture solution, was all in the cationic form as is evident from the cation exchange column experiment (Table II). While 96.5% of the added iron precipitated from sea-water, that solubilized (3.5%) was in the cationic form (Table II). Dissolved organic matter solubilized added iron to the extent of 47% of which at least 35% (corresponding to 16% of that added) was in the 'non-ionic' or 'anionic' complex

form. Zirconium-niobium precipitated to the extent of about 71% at pH 8, whereas only 18% precipitated in the presence of dissolved organic matter. This indicates that at least 53% of zirconium-niobium was solubilized by the organic matter of the culture filtrate. Of the 29% of zirconium-niobium in sea-water, 41% passed through the cationic column (corresponding to complexing of about 12% of initial); of the 82% solubilized in the presence of dissolved organic matter in the culture filtrate, 85% passed through the cationic column (corresponding to complexing of about 70% of initial charge). This indicates the formation of very stable 'non-ionic' or 'anionic' complexes of zirconium-niobium.

The molar concentrations, readily calculated from the specific activity values, of the trace elements in solubilized state in sea-water and in the culture filtrate are about, 3×10^{-6} M and 1×10^{-4} M for Mn; 4×10^{-6} M and 5.8×10^{-6} M for Zn; 6.4×10^{-9} M and 8.6×10^{-8} M for Fe; and 3×10^{-9} M and 8.5×10^{-9} M for Zr-Nb, respectively. Sillen¹ noted that organic complexes contribute to some of the elements present at the very low concentration of 10^{-6} M to 10^{-7} M and it is striking that the concentrations of elements solubilized by the dissolved organic constituents have been observed to lie generally in the range of 10^{-6} M to two orders of magnitude below that speculated by Sillen (1961).

In a similar series of experiments, the authors (Desai *et al.*¹⁵) reported solubilization of *in situ* precipitated BaSO_4 , as well as precipitated BaSO_4 , in sea-water in the presence of dissolved organic matter. The average values obtained were 168 μg . Ba/l. in sea-water and 621 μg . Ba/l. in the presence of dissolved organic matter for *in situ* precipitated BaSO_4 . Solubility of precipitated BaSO_4 was 132 μg . Ba/l. in sea-water and 497 μg . Ba/l. in presence of dissolved organic matter. Solubilized Ba was quantitatively retained on a sodium exchanged, Dowex 50 W- \times 8 (50-100 mesh) cation exchange column (5 cm. \times 1 cm.). Similar behaviour was observed with solubilized BaSO_4 in dilute ammoniacal EDTA. Reported values for the concentration of Ba in sea-water cluster around 30-50 μg . Ba/l. (Krauskopf;¹⁶ Chow and Goldberg;¹⁷ Turekian and Johnson;¹⁸ while Burton *et al.*¹⁹ obtained a saturation value of 48 μg . Ba/l. (81 μg . BaSO_4 /l).

Although the total organic content in the culture solution was comparable to that in some of the sea-waters, it is not yet clear as to what extent the above data can be quantitatively applied in the marine environment. The solubilization of all the ions observed in sea-water used in the experiment may also be caused, in part at least, by the dissolved organic matter already present in the system. However, in these demonstration experiments this point was not investigated further. Further work is in progress.

No attempts have been made to study the stability constants of the solubilized material since it has not been possible to establish the individual concentrations of the dissolved organic constituents of the culture filtrate.

1. Sillen, L. G., In *Oceanography*, Ed. by Mary Sears, Amer. Ass. Adv. Sci., 1961, p. 549.
2. Goldberg, E. D., In *Chemical Oceanography*, Ed. by Riley, J. P. and Skirrow, G., Academic Press, 1965, 1, 163.
3. Burton, J. D., *Nature*, 1966, 212, 976.
4. Rona, E., Hood, D. W., Muse, L. and Buglio, B., *Limnol. Oceanogr.*, 1962, 7, 201.
5. Laevastu, T. and Thompson, T. G., *J. Mar. Res.* 1958, 16, 192.
6. Alexander, J. E. and Corcoran, E. F., *Limnol. Oceanogr.*, 1967, 12, 236.
7. Slowey, J. F., Jeffrey, L. M. and Hood, D. W., *Nature*, 1967, 214, 377.
8. Hood, D. W., In *Oceanography and Marine Biology*, Ed. by Barnes, H., George Allen & Unwin Ltd., 1963, 1, 129.
9. ZoBell, C. E., *Marine Microbiology*, Waltham, Mass., U.S.A., 1946.
10. Fox, D. L., Oppenheimer, C. H. and Kittredge, T. S., *J. Mar. Res.*, 1953, 12, 233.
11. Jeffrey, L. M. and Hood, D. W., *Ibid.*, 1958, 17, 247.
12. Hayer, D. W., Slowey, J. P. and Hood, D. W., *TID-23295*, 1966.
13. Duursma, E. D., In *Chemical Oceanography*, Ed. by Riley, J. P. and Skirrow, G., Academic Press, 1965, 1, 433.
14. Lewis, G. J. Jr. and Rakestraw, N. W., *J. Mar. Res.*, 1958, 14, 253.
15. Desai, M. V. M., Koshy, E. and Ganguly, A. K., *Curr. Sci.*, 1969, 38, 107.
16. Krauskopf, K. B., *Geochim et Cosmochim Acta*, 1954, 9, 1.
17. Chow, T. J. and Goldberg, E. D., *Ibid.*, 1960, 20, 192.
18. Turekian, K. K. and Johnson, D. G., *Ibid.*, 1966, 30, 1153.
19. Burton, J. D., Marshall, N. J. and Phillips, A. J., *Nature*, 1968, 217, 834.
20. Koshi, E., *M.Sc. Thesis*, University of Bombay, 1968.
21. Sarma *et al.*, BARC/HP/BS/4, 1968.