

ORGANIC MATERIALS IN THE MARINE ENVIRONMENT AND THE ASSOCIATED METALLIC ELEMENTS*

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ABSTRACT

Dissolved organic matter obtained from an algal culture grown in marine medium solubilizes trace elements. The rate at which the solubilized elements change their ionic properties with the growth of the culture and the relative abundance of cationic, anionic and non-ionic species (at different periods of culture growth) of these elements were studied at elemental concentration upto $500 \mu\text{g/l}$. The probable role of PO_4 and of the organic constituents in the solubilization or fixation of trace elements on the organic matrices has been indicated.

Influence of organic constituents on the sorption of trace elements by marine sediments has been investigated for some elements such as Zn, Mn and Co. The sorption of Zn, Mn and Co by a sample of coastal marine sediment was observed to be many times higher than by the same sediment freed of organic matter by H_2O_2 treatment whereas sorption of alkali and alkaline earth elements such as Cs, K, Ca and Sr did not show any significant difference.

Complete hydrolysis of the humic and fulvic acids extracted from marine sediments could not be established and as such amino-acids, purines, pyrimidines and carbohydrates were qualitatively identified. Trace element analyses showed that the humus materials were high in the content of trivalent elements such as Al and Fe and of bivalent elements such as Cu and Zn. Of the associated metallic elements Ca and Mg are found to be mostly exchangeable cationic in their behaviour on Dowex-50 column, major fractions of Al, Fe, Cu and Zn escape through the cation column—indicating different states of combination of the metallic elements with the humus complex.

Studies on the interaction of humic acid with added solutions of trace elements show that alkali and alkaline earth elements such as Sr, Ba, Ra exist in exchangeable cationic, transition elements such as Zn, Mn, Cu and Co exist in cationic and non-cationic, while trivalent and heavy elements exist mostly in non-cationic forms in the humic acid. Similar were the observations with fulvic acid. The ionic behaviour of humic and fulvic acid complexes of the elements are compared and their characteristics are discussed.

INTRODUCTION

MODEL of distribution of trace elements, existing entirely as inorganic species in the marine environment, needs revision in view of new data being accumulated on the influence of organic constituents on the physico-chemical state of trace elements. Most of the global organic matter is present in the ocean in dissolved form and in the sediments as humus material (cf. Table I). The final resting place for organic materials in sea-water is in the sediment as humus substances—complex of organic monomers of high biochemical stability.²

Sea-water is known to contain in dissolved state,† a number of organic (ligands) com-

TABLE I¹

Carbon content of natural exchange reservoir

Carbon reservoir	Carbon content (g/cm ² of earth's surface)
Atmosphere	0.125
Humus	0.19
Biosphere (land)	0.06
Biosphere (marine)	0.002
Oceanic dissolved carbon (organic)	0.533
Oceanic inorganic carbon (above thermocline)	0.20
Oceanic inorganic carbon (below thermocline)	7.25

pounds such as amino-acids, peptides, carbohydrates, etc.³ Hydrolytic products of the humus material have also been found to contain carbohydrates, amino-acids, etc.^{4,5} The complexing properties of these leading to solubilization/fixation of a host of trace elements in organic compounds or matrices is a complicated process, progressive elucidation of which will lead to better understanding of the

* Adapted from the paper presented at the IAEA Symposium on Distribution of Chemical Species in Marine Environment held in Tokyo in September, 1970

† Material passing through 0.45μ membrane filter is usually considered as 'dissolved'. We have used in the present work 0.22μ membrane instead, for filtration.

distribution of these metallic species in oceanic waters.

Solubilization and complexation of elements, such as Ba, Zn, Mn, Fe and Zr-Nb in sea-water culture filtrate containing higher amount of dissolved organic constituents than in natural sea-water, was reported recently by Desai *et al.*⁶ and Koshy *et al.*⁷ The concentrations obtained for a number of trace elements in natural sea-water exceed the amount one can predict from basic principles of solubility product and inorganic compound-metal ion interactions.⁸⁻¹⁰ Solubilization of trace elements in sea-water has been observed by many investigators.⁴⁻⁹ Presence of dissolved organo-metallic complexes in sea-water was postulated from experimental evidences for Fe, Cu, Zn, Mn and V by earlier workers¹¹⁻¹⁷ but *in situ* formation and progressive evolution of the chemical state of the metallic elements in the complexes is not dealt with at length in the literature.

The sorption of radioelements by sediments in the sea-water system is, in general, guided by the basic physico-chemical interactions with the sediment such as adsorption, isomorphous replacement and double decomposition. A parameter that has not been well investigated is the role played by organic matter present in the sediment in its sorptive properties.¹⁸

The organic matter in the sediments is principally composed of the humus material. Soil²⁰⁻²² and marine⁴⁻¹⁰ humus substances are known to form complexes with a large number of metal ions and these materials in the sediments can thus influence the distribution of such elements in the marine environment. Koshy *et al.*¹⁹ studied the sorption of Cs, Zn, Mn, Co, Cu and Y by a marine humic acid in sea-water medium. Koshy *et al.*^{4,19} and Desai *et al.*^{5,23} demonstrated the capacity of the humic acid to form soluble and insoluble complexes of elements such as Mn, Zn, Co, Fe, Y, La, Ac

and Zr-Nb in sea-water and in ammoniacal medium.

There is a lack of information about the interaction of heavy elements with humus substances (humic and fulvic acids).

COMPLEXATION OF TRACE ELEMENTS BY DISSOLVED ORGANICS

In order to study the formation of metal-organic complexes in dissolved forms in sea-water, cultures of a phytoplankton (*Microcystis littoralis*) were grown in sea-water medium in presence of organic matter free (by H₂O₂ treatment) sediment separately for some radioactive elements containing the inactive carriers of these elements (upto 500 µg. of each per litre). The ionic nature of the elements in dissolved form was determined after 3 days and onwards after the addition of the nutrients containing ¹⁴CO₂ and raising the culture. The supernatants were filtered at intervals of time and an aliquot of each of the filtrates was counted for gamma activity. Two similar aliquots of each were passed through a cation (Na⁺) and an anion (Cl⁻) column (5 cm. × 1 cm. at the rate of 0.2 ml./min.) and the effluents and washings were counted as before. The detailed experimental procedure is given elsewhere.⁵ Results are given in Tables II, III and IV.

Results indicate that amphoteric Zn with time changes from the cationic form of 100% to about 35%, while anionic form completely disappears in course of time—thus indicating the formation of about 65% non-ionic species of Zn organic complex in 16 weeks. Similar behaviour is observed with Mn which is transformed from cationic (100%) form to non-ionic form (70%) in 16 weeks. Co cationic initially changes over to 71% non-ionic form in about 4 weeks and remains so thereafter. Behaviour of Ru is somewhat erratic, but indications are

TABLE II

Ionic states of Zn, Mn and Fe in presence of dissolved organic constituents at different time intervals

	Dissolved form (%)									
	3 days			21 days			42 days		112 days	
	Zn	Mn	Fe	Zn	Mn	Fe	Zn	Mn	Zn	Mn
Cationic	100	100	100	100	93.6	75.5	82.7	88.2	35	28.9
Non cationic*	0	0	0	0	6.4	24.5	17.3	11.8	65	71.1
Anionic	79	0	15.5	6.4	7.5	50.0	0	0
Non-anionic†	21	100	84.5	93.6	92.5	50.0	100	100

The elements were added in the chloride form. * Per cent of activity passing through the cation column. † Per cent of activity passing through the anion column.

TABLE III

Ionic states of Co^* in presence of dissolved organic constituents at different time intervals

		Dissolved form (%)			
		3 days	16 days	28 days	55 days
Cationic	..	100	36	28.3	28.4
Non-cationic	..	0	64	71.7	71.6
Anionic	..	0	0	0	0
Non-anionic	..	100	100	100	100

* Added in the CoCl_2 form.

TABLE IV

Ionic states of Ru^* in presence of dissolved organic constituents at different time intervals

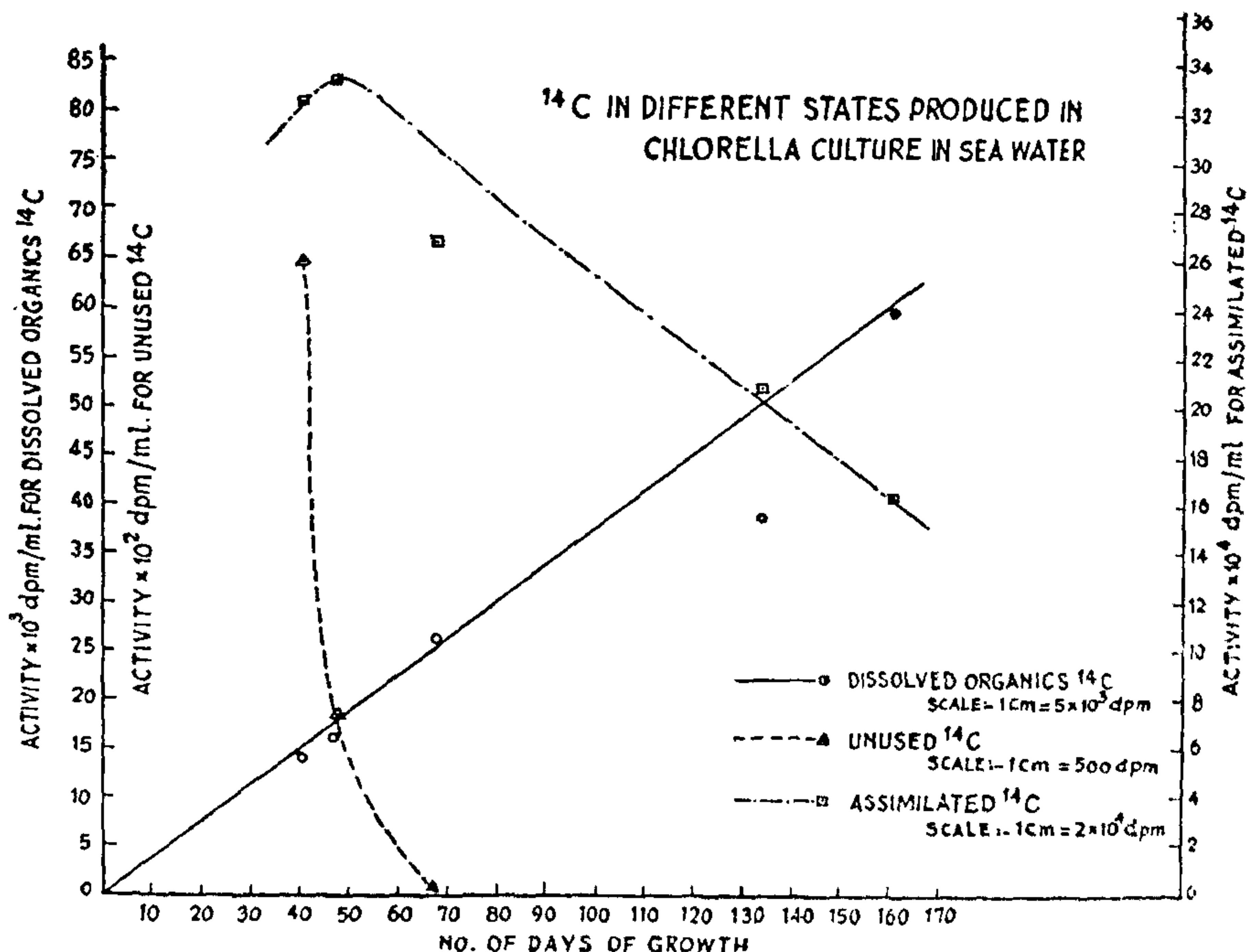
		Dissolved form (%)			
		7 days	42 days	98 days	112 days
Cationic	..	32.8	52.6	31	2.5
Non-cationic	..	67.2	47.4	69	97.5
Anionic	..	37.9	..	59	0
Non-anionic	..	62.1	..	41	100

* Added in the RuCl_3 form.

that this element is also progressively transformed to non-ionic form, Fe appearing some-

what amphoteric after 3 days develops into at least 25% non-ionic form after 3 weeks.

Build-up of dissolved organic constituents with time in the culture medium was studied earlier by Koshy et al.⁴ (cf. Fig. 1). The nature of the organic constituents building up with time was not investigated. The column behaviour of dissolved organic matter also changed with time as observed by ^{14}C counting of the influent and effluent in column studies. However, the correlation of the amount of associated organic matter or the nature of the organic compounds involved in different complex formation could not be followed. As will be evident in sequel that several metallic species are solubilized or complexed simultaneously by the highly complex organic molecular species such as humus substances, such correlation remains a difficult problem. The non-ionic effluent from all the experiments in this series can be taken to be of very similar composition. It is also not clear that the ionic and non-ionic species that are observed during the early days of culture growth are similar or



different (probably latter) from the ones observed towards the end of the experiment.

SORPTION OF TRACE ELEMENTS BY MARINE SEDIMENTS

Influence of organic matter on the sorption of trace elements by marine sediments is followed from the distribution of the elements in the trace element equilibrated sea-water and the added sediment.

Fresh coastal marine sediment (containing 3.76% organic matter and 24% acid-soluble CO_3) slurried with filtered sea-water (1:10 sediment: sea-water, weight: volume) and passed through 70 mesh sieve was taken for the experiment. The particle size was fairly uniform—less than 5μ , 80%. Organic matter free sediment was obtained from this by repeated digestion of a part of this sieved slurry with H_2O_2 in presence of 0.05 N HCl at slightly elevated temperature ($50-60^\circ\text{C}$). The digested material was centrifuged, washed free of acid, equilibrated by repeated suspension in filtered sea-water and finally slurried in sea-water to give the same sediment: sea-water ratio as in the sieved original sediment slurry. ^{65}Zn , ^{54}Mn and ^{60}Co in chloride forms containing $100\mu\text{g}$ of each of the trace element were used to find out their distribution coefficient, K_d , as described by Ganapathy *et al.*¹⁸ Results are given in Table V.

The K_d values obtained are quite demonstrative of the profound influence of organic matter on the sorptive properties of the natural sediment. Similar experiments done with K, Ca, Sr did not indicate any difference in the K_d values between the original and organic matter free sediment. Earlier, Ganapathy *et al.*¹⁸ also observed the influence of the organic matter in similar experiments with air-dried sediment sample. Their results are also given in the same table for comparison. Further, Ganapathy *et al.*¹⁸ demonstrated that organic matter in the sediment has some small influence in Cs sorption. This is understandable in the light of observation that marine humic acid does not pick up Cs significantly in sea-water medium as observed by Koshy *et al.*⁴

It was thus of interest to extract the organic matter from different sediments to study the trace elements associated with the organic matter and the interactions of extract with metal ions.

HUMUS FROM MARINE SEDIMENTS

In the absence of a method for the isolation of humus substances in native form from sedi-

TABLE V
Sorption of trace elements by coastal marine sediments in sea-water

Element	K_d^\dagger			
	Fresh sea-water sediment slurry		Air-dried sediment*	
	Original sediment	Organic matter free sediment	Original sediment	Organic matter free sediment
Zn	4453	18.5	6508	1868
Mn	28340	3.3	90	25
Co	8461	3.7	689	23

* Ganapathy *et al.*¹⁸.

Carriers added: $100\mu\text{g}/50\text{ ml.}$ for all elements in fresh sediment slurry,
 $200\mu\text{g}/50\text{ ml.}$ for Zn and Mn for air-dried sediment.

Activity sorbed by 1 g. of the sediment

$$\dagger K_d = \frac{\text{Activity sorbed by 1 g. of the sediment}}{\text{Activity in 1 ml. of sample filtrate}}$$

ments, alkali extraction procedure was adopted. A known quantity of a marine sediment was extracted with 0.2 N ($\text{NaOH} + \text{Na}_2\text{CO}_3$) mixture (1:10 solid: solution) at 80°C . under constant stirring condition. The extract was allowed to stand and the supernatant was siphoned off, centrifuged (at 5500 G) and decanted. An aliquot of the extract was dialysed in cellophane bags till the dialysate was free of alkali. The product was made ammoniacal, filtered and dialysed again till the dialysate was free of ammonia. It was taken up in ammonia, filtered and stored as purified humus extracts stock (solid content 0.46 mg./ml.).

STATUS OF TRACE ELEMENTS IN HUMUS SUBSTANCES

An aliquot of the purified humus extract was passed through Dowex-50 $\times 8$ (10 cm. \times 1 cm.) NH_4^+ column first at the rate of 1 ml./min. and a part of the effluent was again passed through another similar column at the rate of 0.1 ml./min. The extract and the two effluents were wet-ashed with concentrated HNO_3 and analysed for metallic elements and Table VI gives the results obtained.

The extract contained significant quantities of PO_4 , Al, Fe, Cu, Mn, Co, Zn, Mg and Ca. The ion exchange experiment showed that of the associated metallic elements, Ca was mostly exchangeable cationic, while Mg, Zn, Co and Mn were associated in cationic and non-cationic forms. Fe and Al are bound to the humus complex mostly in non-cationic forms.

The metallic elements are thus observed to be associated with the purified humus complex

TABLE VI
Trace elements in mildly purified humus extract

Element	Concentration in ppm		
	Before passing through Dowex-50	After first pass at 1 ml./min.	After second pass at 0.1 ml./min.
P as PO ₄	10240	10240	10240
Al ..	1479	1479	1479
Fe ..	1395	1246	1246
Cu ..	2431	2304	2071
Mn ..	249	249	133
Co ..	132	120	60
Zn ..	748	302	207
Mg ..	1727	1727	617
Ca ..	8035	1127	337

Stability order : Al > Fe > Cu > Mn > Co ≥ Mg > Zn > Ca.

in different states of chemical combination and the relative abundance of cationic species and stability. The stabilities of Al, Fe and Cu complexes are the highest, the order of stability being Al > Fe > Cu > Mn > Co ≥ Mg > Zn > Ca.

The purified humus extracts from different sediments were treated with HCl to pH 2 and centrifuged. The humic acid residue (dispersed in ammonia) and the supernatant fulvic acid fractions were further purified by prolonged electrodialysis raising the voltage progressively to 200 v till the breakdown of humic and fulvic acids was apparent from the appearance of colouration in the anion compartment.^{4,5} Throughout the progress of electrodialysis PO₄ and amino-acids continued to appear in the anion compartment declining to very low values and then increased again when the visual breakdown of the acids were apparent. The product in the electrodialysis cell was taken up in ammonia and filtered. The products were wet-ashed as before and were observed to contain 1-3% ash. Results of trace metallic element analyses are given in Table VII.

It is significant to note that in spite of drastic electrodialysis that was continued for three months, neither the humic nor the fulvic acid fractions were observed to be ash-free. It seems inescapable to conclude that the trace elements present therein form integral part of the humic and fulvic acids. The acids breakdown (or decompose) on progressive removal of the metallic components. It is obvious that though humus substances are ion exchange materials, they hold many elements, specially bi- (Cu and Zn) and trivalent (Fe and Al),

TABLE VII
Ash and trace elements in humic and fulvic acids

Element	Concentration				
	Humic acid			Fulvic acid	
	Coastal	SS I*	SS II†	SS I	SS II
Ash (%)	1.0	2.94	2.99	2.6	..
SiO ₂ ..	Nil	Nil	Nil	Nil	Nil
SO ₄ ..	ND	ND	ND	ND	ND
PO ₄ (ppm)	3226	3676	1248	10720	7754
Al ..	3481	9864	2850	2454	13730
Fe ..	179	1654	5920	ND	22390
Cu ..	1380	1430	815	223	ND
Zn	2520	1061	..	ND
Mg ..	22	205	337	429	1395
Mn ..	ND	23	408	178	779
Ca ..	ND	ND	ND	515	ND
Sr ..	ND	ND	ND	ND	ND
Cr ..	83	15	..	ND	..
K ..	81	132	ND	357	698

* 21 miles away from shore at 50 m depth.

† 70 miles away from shore at 90 m depth.

at different reactive sites with strong bonds not easily dissociable. Discussion by earlier workers on the nature and properties of humic acid centres mostly around the organic monomeric constituents and the entire structures are sought to be built on the basis of the concept of polymerization reactions amongst the organic monomers. However, it appears particularly important to take cognizance of the metallic ions present in the humus structure as participants in the build-up of the polymeric humus complex. The possibility of covalent binding of metallic ions with monomers in the humus complex needs to be investigated in greater detail.

Phosphate content is high in all the samples and of the total PO₄ content in the purified extract, only about 10% was observed to be associated with the humic acid fraction; and further, of the total weight of organic matter in the humus extract, only about 10% by weight was accounted for as fulvic fraction. Thus in the extract where treatments were mild, the PO₄ content per unit weight of fulvic acid was about 80 times of that in humic acid. We could not correlate the trace element content in the extracts with the phosphate content. The equivalence of the totality of the metallic elements present and the relative number of the atoms of metals are very much higher than the total PO₄ or P. That PO₄ is not in ionically bound state to the humus complex is also apparent from the high PO₄ content in the drastically purified (electrodialysed) humic and fulvic

acids. It appears that PO_4 is also involved in the polymerization giving rise to the organic complex. No silica or sulphate were detected in the samples analysed.

INTERACTION OF TRACE ELEMENTS WITH HUMUS SUBSTANCES

Solubilization of trace elements by humic and fulvic acids in ammoniacal medium was studied using $^{65}\text{Zn}^{+2}$, $^{54}\text{Mn}^{+2}$, $^{60}\text{Co}^{+2}$ and $^{59}\text{Fe}^{+3}$ in chloride forms containing respective inactive carriers and also using $^{231}\text{Th}^{+4}$, Natu^{+6} and $^{239-240}\text{Pu}^{+6}$ in nitrate forms.⁵ The sample solutions with tracers were kept for a week with intermittent shaking, filtered and an aliquot of the filtrate was analysed for the trace element. Another aliquot of the filtrate was passed through a Dowex ($-50 \times 8 \text{ NH}_4$, -0.2 ml./min.) column, the effluent and washings were also analysed for the element. Results are presented in Table VIII. Behaviour of humus bound metallic ions on passing through an anion column could not be investigated since the host materials, viz., humic and fulvic acids were themselves anionic.

Humic and fulvic acids solubilize the elements significantly and fulvic acid solubilizes more than the humic acid. The nature of the trace element complexes with humic and fulvic acids appears to be similar. Bivalent elements form cationic and non-cationic complexes while tri- and tetravalent ions form mainly non-cationic complexes.

DISCUSSION

Quantitative analyses of the monomeric constituents of humic and fulvic acids are not yet possible and in the present work it was not possible to effect complete degradation or hydrolysis of the humus material. Presence of amino-acids and sugars (including pentose) in the hydrolysates of humus substances was confirmed. The presence of purines and pyrimidines has also been qualitatively indicated. Our attempts to estimate the amino-acids and sugar constituents in the material did not succeed.

Understanding of the nature of the binding of trace elements with different reactive groups in the humus complex and quantitative assessment of the stability and their variation with the source of the material shall have to wait further careful investigation. In our investigations we could only observe that alkaline earth elements such as Sr, Ba, Ra and possibly Ca are quantitatively ion-exchangeable

TABLE VIII
Solubilization of certain metal ions by and the nature of the metallic complexes of marine humic and fulvic acids

Element	Solubilization (mg./g.)		% Non-cationic in solubilized fraction	
	Humic acid	Fulvic acid	Humic acid	Fulvic acid
Zn	5.97	41.51	12.4	0
Mn	..	85.7	100†	66
Co	33.8*	22.8*	64	35
Fe	15.4	34.22	100	90
Zr-Nb	95	94
Th	2.85	8.72	100	100
U	6.0	..	70	..
Pu	0.042	0.14	100	100
	$\times 10^{-3}$	$\times 10^{-3}$		

Humic acid used: 0.95 mg. Fulvic acid used: 0.11 mg.

Trace element used:

H.A.: Zn, 21.92 μg ; Co, 50 μg ; Fe, 53.3 μg ; Th, 3 μg ; U, 9.8 μg .

F.A.: Zn, 14.62 μg ; Mn, 10 μg ; Co, 10 μg ; Fe, 21.3 μg ; Th, 1.83 μg .

* Non-cationic fraction only, † Sorbed on humic acid in sea-water.

and fractions of the trivalent elements present in the dialysed humus extract and in sea-water soluble material* are ion-exchangeable.^{4,5,7,23} Other bivalent elements are present in the humus and in the dissolved material in different states of combination, viz., cationic, anionic and non-ionic. Presence of PO_4 is very significant and there are indications that pentoses and purine-pyrimidines are present in the material besides the amino-acids, hexose sugars, etc. Pillai *et al.*²⁴ have produced evidence of the presence of dissolved nucleic acids in sea-water. Role of this component in the organo-metallic interactions observed in sea-water is still to be investigated.

It is also very significant that it was not possible to remove the trace elements from humic or fulvic acid without degrading them to low molecular weight fragments. There are statements in the literature²⁵ regarding ash-free humic acid. However, this could not be confirmed in the material extracted by the procedure adopted in the present work. No investigation was undertaken on the humus material.

* Dissolved organic matter and soluble humus in sea-water.

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VIRAL ANTIGENS IN THE BAI STRAIN A (AVIAN MYELOBLASTOSIS) VIRUS ASSOCIATED MYELOBLASTS AND CHICK EMBRYO FIBROBLASTS

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CHICKEN leukemia viruses are released like myxoviruses by a budding process from cellular membranes and have been shown to contain cellular material in their constitution. Avian myeloblastosis virus (AMV) which causes leukemia in chickens picks up ATPase of cellular origin as it buds from myeloblast cell surface and when this same virus is produced by chick embryo fibroblasts (CEF), the viruses do not show ATPase activity.¹ Beauer² has also shown that in addition to ATPase, an RNA digesting enzyme associated with myeloblast virus is lacking in fibroblast virus. Differences in the sedimentation behaviour during CsCl density gradient centrifugations were also observed between myeloblast and fibroblast virus of AMV.³ It was then of interest to see if there will be an antigenic variation of viral components present in fibroblast and myeloblast cells producing this virus. To study this we have analysed the viral components of the myeloblast host cell using the Ouchterlony technique of double diffusion with antisera produced against detergent split AMV and recently shown avian tumor virus group specific (GS) hamster antiserum.⁴ A similar analysis was done on chicken fibroblast cells infected with AMV to see if identical antigens are present in these two different host cells.

Myeloblast cells collected from leukemic chickens⁵ and washed twice with 0.01 M phosphate buffered saline (PBS), pH 7.4, were stored at -20° C. until needed. Primary cul-

tures of RIF-free⁶ chick embryo fibroblast were infected with a multiplicity of 100 virus particles per cell. After five days incubation at 37° C., cells were collected, washed twice with PBS and stored as were the myeloblasts. 1 ml. of frozen and thawed myeloblasts or CEF diluted to 10 ml. with PBS was homogenized with a glass pestle. The homogenate was centrifuged to remove nuclear, mitochondrial and microsomal fractions.⁷ The supernatant obtained after these centrifugations is the soluble fraction used in this study.

Purified AMV pellets⁸ containing 10¹² particles as determined by particle⁹ count were dissolved in 1 ml. of PBS with sodium dodecyl sulfate (SDS) or sodium deoxycholate (SDC) in 0.5% concentration. Excess SDS was removed by adding a drop of KCl and sedimenting the precipitate. Rabbits were inoculated with these virus split products according to Eckert et al.¹⁰ A part of each serum was absorbed twice for 1 hr. at 37° C. and finally at 4° C. for 16 hr. with a mixture of normal chick liver, kidney and spleen. Hamster serum pool used in this study was obtained from hamsters bearing transplanted Schmidt-Ruppin tumours¹¹ and did not react with normal chick tissue. All sera were inactivated at 56° C. for 30 min. Diffusion studies were made with immunoplates (Pattern B, Hyland Laboratories, Los Angeles, U.S.A.). Plates prepared with antigen and antisera were incubated at 37° C. in a moist chamber for 48-72 hr., washed for 1-2 days and stained with Amido-black. The stained preparations were then photographed.

* This work was done while the author was at Duke University Medical Centre, U.S.A.