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Physico-Chemical Limitations in Experimental Investigations [and Discussion]

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Physico-chemical limitations in experimental investigations

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An element or compound in a natural water system is usually distributed between a variety of physico-chemical forms, both dissolved and particulate. The distribution is determined by the properties of the ion or molecule in question and by a number of major variables, including ionic strength, the nature and concentrations of major dissolved elements, particulate matter and organic complexing material, pH and the electron activity (pE); it may thus vary widely between different environments. The design of experiments to study sublethal effects of pollutants in sea water ideally requires that the test medium is closely matched to the environment for which information is needed, with respect to the ranges of concentration and activity, and the chemical speciation, of the pollutant and of any other constituents which may influence its effects. This in turn requires either that the pollutant can be added in the appropriate forms, implying a knowledge of the existing speciation, or that the added material rapidly exchanges with the forms already present. The implications of these requirements are most apparent for those pollutants that show complex chemical behaviour in sea water. This account concentrates on metals of toxicological significance.

Consideration of particulate associations, redox speciation, and complex formation in the dissolved state with inorganic and organic ligands, suggests that physico-chemical factors limit the usefulness, in terms of environmental predictions, of experimental studies of biological effects of metals, both inherently and through inadequate knowledge of environmental speciation and the mechanisms and rates of interconversion between species. Of particular importance are non-equilibrium features in speciation, such as the presence of thermodynamically unstable oxidation states and of kinetically non-labile associations. Interpretation of the nature of these associations is complicated by the presence of colloidal and organic macromolecular material in dissolved fractions as conventionally defined. While the chemical behaviour of some substances in sea water is considerably less complicated than that of the trace metals, there is a need with all types of pollutants for greater attention to physico-chemical factors in both the design and interpretation of experiments to investigate biological effects.

Introduction

An element or compound present in a natural water system will generally be distributed between a variety of physico-chemical states. The distribution among these states, as well as the total concentration, varies between different environments, for example between the river and sea waters which form the end-members of estuarine mixing series, between coastal seas and the open ocean, surface and deep oceanic waters, sediments with their associated pore waters and the overlying waters. Furthermore, there will usually be major differences in physico-chemical forms between pollutant inputs and the receiving waters and sediments.

These facts place inherent limitations upon the validity of conclusions, drawn by extrapolation from findings on experimental systems, regarding the biological consequences of the presence of pollutants in natural environments and the biological implications of variations in base-line concentrations. Many studies on bioaccumulation and toxicity have been carried out with the use of concentrations which are so high as to be irrelevant to environmental conditions. The need for investigations of effects at environmentally realistic levels has become increasingly recognized. Correct matching of test media to environmental conditions involves, however, additional consideration of the activity and speciation of the element concerned and of other elements present which may influence biological responses. The purpose of this paper is to provide a brief overview of the physico-chemical factors relevant to the design of experiments on biological effects. While differences in physico-chemical state affect the environmental impact of any substance, the implications are perhaps most clearly demonstrable in relation to inorganic inputs, such as micronutrients, heavy metals and radioactive nuclides; the present account concentrates on metals of toxicological significance.

Chemical speciation is often used as a term referring to the various forms of occurrence of elements in a given system. The nomenclature adopted here is as follows: *species* is used for those entities (ions, molecules, complexes) which can be described in terms of a well defined chemical stoichiometry; *form* is employed to cover both species and less well defined entities or groups of entities (e.g. a metal associated with uncharacterized organic material); *fraction* is used to describe the groups of forms resolved by, and thus operationally defined by, particular analytical techniques. A major limitation in this field is that, for trace constituents, analytical techniques rarely resolve individual species and there is often considerable uncertainty as to the relation between the operationally defined analytical fractions and the various species the presence of which may be postulated by theoretical models.

In the following discussion, consideration is given first to particulate forms and the resolution of particulate and dissolved fractions. Subsequently the questions of redox speciation and the complexation of dissolved elements are considered.

Particulate material in natural waters and the resolution of particulate and dissolved fractions

The concentration and composition of suspended particulate material varies greatly in natural waters. An average concentration of 1 mg/l has been reported for coastal water, while that for open oceanic surface waters is of the order of 100 μg/l (Chester & Stoner 1972); open oceanic waters below 200 m, excluding nepheloid layers, generally contain less than 20 μg/l (Lal 1977). In some estuarine environments concentrations may rise to 1 g/l or higher, often as a result of extensive resuspension of bottom sediments. The suspended material in natural waters consists of a mixture of components of diverse origin, principally lithogenous (e.g. clay minerals, quartz, continentally derived carbonates), biogenous (living and detrital organic and skeletal material) and inorganic authigenic (e.g. hydrous iron oxide, which often forms a coating on other inorganic particles). Material may also be held more or less firmly on the surfaces of particles by adsorption and ion exchange.

The distribution of the total concentration of a metal between the particulate and dissolved fractions clearly varies widely according to the concentration and nature of the suspended material. Individual elements are preferentially associated with particular components as has been shown by Gibbs (1973) for riverborne particulates, and with particular size fractions (de Groot, Salomons & Allersma 1976). When metals are discharged in waste to aquatic systems fractionation between dissolved and particulate components can clearly modify the environmental consequences to a great extent. Thus high concentrations of iron in acidic wastes will be almost entirely converted to particulate ferric hydroxide on mixing with natural waters, and such precipitates are capable of scavenging other ions from solution. Mercury is an example of an element which shows dominant associations with particulate phases in waters with high concentrations of suspended material; Smith, Nicholson & Moore (1971) found that in brackish and saline waters of the tidal Thames, 96–98 % of the total mercury was associated with particulate material. It appears that the processes of uptake onto particles are often only partly, if at all, reversible in terms of response to changes in dissolved concentration. Major changes in physico-chemical conditions, e.g. in pH or pE, often led to redistribution between phases, however, and in some circumstances desorption from solid phases may arise through large changes in the ionic strength of the solution phase.

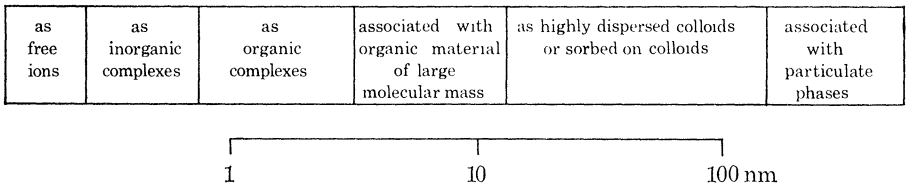

Figure 1. Forms of metals in natural waters. Size values refer to the diameters of the molecules or particles. Adapted from Stumm & Brauner (1975).

The extent of association with particulate material influences biological availability and may change the pattern of transference through food webs in a natural ecosystem. Because of the variables discussed above, these effects are inherently difficult to simulate in experimental studies. Other practical problems arise in experimental systems as a result of the tendency of some materials to be adsorbed on the solid surfaces. When a metal is added in solution to an enclosed vessel containing organisms, the reservoir concentration of the dissolved metal may diminish rapidly as a result of uptake on the container, the external surfaces of tissues, and faecal material. In studies of the bioaccumulation of radioactive nuclides, regular adjustment of the dissolved concentration to compensate for losses due to these processes, and to others such as volatilization, is often a feature of experimental design but because of the greater analytical problems this practice has not been generally extended to work on stable species.

Important limitations on the interpretation of both environmental data and experimental findings arise from the fact that the resolution of particulate and dissolved fractions achieved by conventional procedures is operational rather than fundamental. In environmental aquatic chemistry the use of cellulose ester membrane filters with an average pore diameter of about 0.5 μm has been widely adopted on the basis of its practicality. The actual cut-off in particle size for such a filter will vary with filter characteristics, the volume filtered and the particulate load. While particles much smaller than the specified pore size will be retained (Sheldon 1972) the filtrate will contain material that is not in true solution, including colloidal material and organic macromolecules. This is apparent from figure 1 which shows the size range of various forms present in natural waters. The fact that the so-called dissolved fraction is in fact heterogeneous in terms of the phases present leads to considerable difficulties in work on speciation.

The implications of the arbitrary operational definition of dissolved fractions are best illustrated by reference to the behaviour of iron. Recent work on 'dissolved' iron in river waters (Boyle, Edmond & Sholkovitz 1977; Moore 1978) suggests that it is present mainly as colloidal material. Ultrafiltration experiments (Moore 1978) show that in river water with concentrations of *ca.* 100 μg/l in the fraction filtered through a 0.45 μm membrane filter, at least 90 % of the iron occurs in the fraction with an apparent molecular mass greater than 10^5, probably as an organically stabilized colloid. Much of this material becomes flocculated during estuarine mixing. The form of the 'dissolved' iron in sea water has not been investigated but it may be present in part as residual colloidal material. When truly dissolved Fe III is added to a natural water, hydrolysis to precipitated or colloidal ferric hydroxide occurs. This is a slow process in terms of laboratory experimentation, a stationary state being attained in sodium chloride solution after a minimum period of some 20 days, the period increasing with decrease in pH (Biedermann & Chow 1966). Experiments on the uptake of iron by sediment particles (Aston & Chester 1973) demonstrate that the ageing of iron into colloidal forms reduces the extent of removal from solution. This decrease in geochemical reactivity is paralleled by well documented biological effects. Lewin & Chen (1971) found that the iron present in coastal sea water was essentially unavailable for growth of phytoplankton in culture experiments. Variations in the biological availability of iron from different sources have been strikingly shown by findings (Jenkins 1969) on the uptake of radioactive ^{55}Fe, freshly introduced by long-range fallout into surface oceanic waters. The radioactive nuclide was taken up by organisms more readily, by many orders of magnitude, than the stable element already present; similar effects were found with radioactive isotopes of cobalt and zinc.

Redox speciation of dissolved elements

A number of elements can exist to significant extents in more than one oxidation state in oxic sea water. Given the pE and pH of a particular system and the appropriate equilibrium constant for the redox reaction concerned, the equilibrium ratio of the activities of the redox species may be calculated.

This type of thermodynamic calculation indicates a boundary condition towards which the system tends. There are several difficulties, however, about equilibrium calculations for redox species in natural waters. The main one is that the characteristic redox potential and the related parameter, the electron activity (pE), are not known for oxic natural waters. Sillén (1961) assumed that the pE corresponds to the equilibrium value for the O_2/H_2O couple and is therefore 12.5 at pH 8.1; the pE is relatively insensitive to variations in oxygen concentration until it falls into the sub-oxic range. Breck (1972, 1974) has suggested, however, that the pE may be as low as 8.5 and may be determined by the O_2/H_2O_2 couple, depending on the presence of a very low steady-state activity of hydrogen peroxide. This controversy cannot be resolved from present evidence. Another important aspect is that the correct speciation for the element in each oxidation state must be identified. It should be emphasized also that the result of most equilibrium calculations relates to the activities of the species, while analytical observations on natural waters generally give information on concentrations.

Despite these difficulties in equilibrium modelling and the consequent uncertainties as to the basis for comparisons with observed speciation in natural environments, it is apparent that in many cases the distribution of an element between different oxidation states corresponds to a steady-state, rather than an equilibrium, condition. Reduced forms of a number of elements may be either introduced directly into the marine environment or formed *in situ* by biological processes. If the rate of oxidation is slow relative to that of input or formation, the redox speciation may differ from that corresponding to equilibrium in the sea water medium.

Such phenomena are well illustrated by the data of Billen (1975), shown in figure 2, on nitrification in the polluted Scheldt estuary. Figure 2*b* gives the thermodynamic stability diagram for the system NH_4^+–NO_2^-–NO_3^-, and figure 2*a* shows the existence of two estuarine zones in one of which NH_4^+ is the only form of the three which is detectable while in the other all three forms are present; the boundary between these zones is drawn parallel to the thermodynamic boundary in figure 2*b* but its position is determined by the observed values. While there is a quantitative discrepancy in redox potential between the boundaries in the model and environmental systems, which probably reflects the fact that the measured potential is an empirical rather than a thermodynamic parameter, the zone in which nitrification occurs broadly corresponds to the conditions under which NO_2^- and NO_3^- are the thermodynamically stable forms, in accordance with the fact that the process of nitrification is mediated by bacteria that obtain energy therefrom. The observation that NH_4^+ is still present in the zone where it is thermodynamically unstable illustrates the importance of kinetic factors.

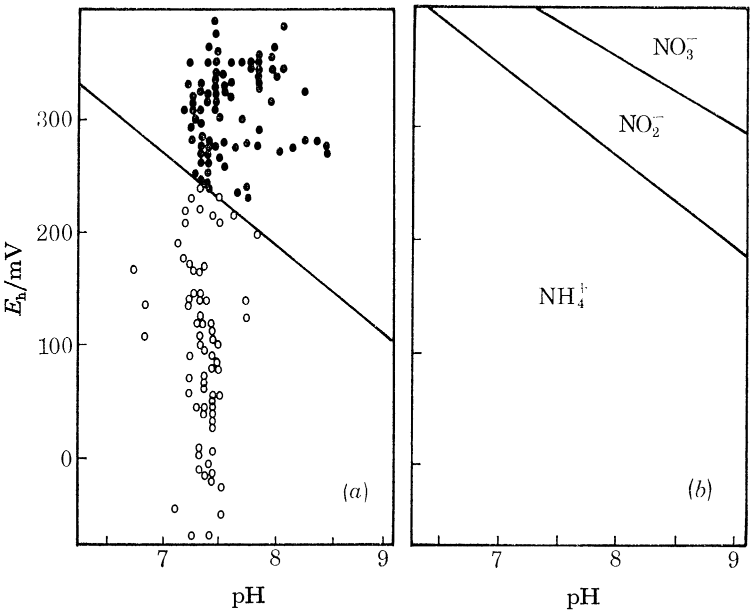

FIGURE 2. (*a*) Variation in speciation of dissolved combined nitrogen in the Scheldt Estuary. Each point represents a sample plotted according to its measured redox potential (E_h) and pH. Open and full circles are used respectively, for samples in which nitrite and nitrate were absent (less than 2 µmol/l) and for those in which they were present. The line separating the zones is drawn parallel to the thermodynamic boundary in (*b*). (*b*) Corresponding thermodynamic stability diagram for the system NH_4^+–NO_2^-–NO_3^-. Adapted from Billen (1975).

In considering the environmental redox speciations of trace metals, some uncertainties arise from the fact that the analytical techniques measure operationally defined fractions which may not correspond to the postulated species. It nevertheless appears that the behaviour of some important metals is probably also explicable in terms of a steady-state model. For chromium, Elderfield (1970) found that, in contrast to the equilibrium prediction that Cr VI should be the only significant form of the element in sea water, from 12 % to over 99 % of the dissolved chromium in coastal sea water was present apparently as Cr III, suggesting a high kinetic stability for the reduced state. Measurements by Shigematsu, Gohda, Yamazaki & Nishikawa (1977) also demonstrate the occurrence of Cr III in natural waters and give some indication that the proportion of this form decreases in the open sea. A selective and sensitive method for the measurement of Se IV in natural waters has been used to show that some river inputs to the ocean contain detectable amounts of selenium in the lower oxidation state (Measures & Burton 1978*a*). Although the thermodynamically preferred form, Se VI, is dominant in estuarine and surface oceanic waters, Se IV becomes a substantial fraction of the total in deep oceanic waters (Measures & Burton 1978*b*), probably as a consequence of biological cycling and transport of the element. Recent measurements (Andreae 1978) of dissolved arsenic in waters off southern California have shown that As III is detectable, particularly in, or around the base of, the photic zone. The oxidation of As III to As V, which was the dominant form at all depths, is known to occur at a measurable rate in sterile sea water (Johnson & Pilson 1975) and may be accelerated by bacterial activity. In zones where reduction to As III occurs as a result of biological activity, however, the rates of reoxidation are evidently slow enough to allow the persistence of the thermodynamically unstable oxidation state.

Since the toxicity of metals such as arsenic, selenium and chromium varies according to the oxidation state, information on environmental redox speciation is essential for the assessment of biological implications of inputs, whether these are of the thermodynamically stable or unstable forms. Information is necessary also on the mechanisms and rates of both the oxidation of unstable reduced forms and the formation of the latter by biological processes.

In studies of bioaccumulation or toxicity in experimental systems, the initial oxidation state of the added element is known but changes in the redox speciation may occur during the course of the experiment. There is thus an inherent limitation on the extent to which such a system simulates natural conditions. Particular problems may be anticipated with algal culture experiments with an element such as arsenic which may be reduced to a greater extent than it would with natural population numbers. Such an effect could lead to misleading conclusions as to the environmental impact of the element.

Considerations similar to those discussed above arise in relation to the biological transformation of inorganic species into organometallic species. Low concentrations of methylated arsenic species have been detected in the photic zone of waters off California and in some fresh waters (Andreae 1978). Despite the well established rôle of methylated forms in the biogeochemical cycle of mercury, the existence of a significant standing concentration of such species in natural waters has not been demonstrated. Knowledge of mechanisms and rates of conversions between the inorganic and organic forms are again necessary for interpretation of environmental situations and for the design of experiments to assess effects.

Chemical forms of dissolved elements

In solutions of high ionic strength, such as sea water, the activities of the ionic components, both the major constituents and the trace elements, are significantly influenced by the presence of other ions. These effects can be modelled by two principal approaches, each of which makes use of single-ion activity coefficients:

(*a*) Ion association models, which have been developed from Bjerrum's treatment of ionic interactions in terms of the formation of ion pairs by electrostatic association of ions of opposite charge. In this approach the single-ion activity coefficient can be considered as being determined by two kinds of process, one arising through effects of ionic strength independently of the nature of the ions present and the other arising through specific associations. These are envisaged as temporary associations arising from electrostatic interactions but their formation can be represented by a conventional association constant. Two activity coefficients may be defined on this basis, the free ion activity coefficient which relates the activity of the ion to its free (unassociated) concentration and a total activity coefficient which relates the activity of the ion to its total concentration in free and associated forms. These models provide a description of speciation in terms of particular ion associations. The physical existence of these species is not, however, a requisite of the model.

(*b*) Specific interaction models which express the interactions between ions in terms of equations from which total activity coefficients may be derived directly.

These approaches have been reviewed in detail by Whitfield (1975*a*). For the purposes of the present discussion it is convenient to use the ion association model.

TABLE 1. SPECIATION OF MAJOR ANIONS IN SEA WATER

anion	percentage as				
(X)	free ion	NaX	MgX	CaX	KX
SO_4^{2-}	39	37	19	4	0.4
HCO_3^-	81	11	6.5	1.5	—
CO_3^{2-}†	8	16	44	21	—

After Pytkowicz & Hawley (1974). The model is for 25 °C and 1 atm (*ca.* 10^5 Pa) total pressure. It has been assumed in all ion pair models that chloride has a negligible tendency to associate with the major cations.

† Significant amounts of carbonate are associated as $Mg_2CO_3^{2+}$ (7 %) and $MgCaCO_3^{2+}$ (4 %).

In a natural water system, a trace metal such as copper will be associated to a significant extent with hydroxyl ions and the major dissolved anions. The activities of the free major anions, with the exception of chloride, are considerably modified by association with major cations as is shown by the data in table 1. This stresses the importance in experimental studies with artificial media of reproducting closely the major composition of the medium which it is intended to simulate.

Ion association models for the inorganic speciation of a number of trace metals, generally at 25 °C and 1 atm (*ca.* 10^5 Pa) total pressure, have been developed for sea water by several workers (Zirino & Yamamoto 1972; Dyrssen & Wedborg 1974; Ahrland 1975) and for global average river water by Millero (1975). The distribution of a metal between the various species is insensitive to the variations of the trace metal concentrations over the normal range encountered in natural waters, because of the large excess concentrations of the important anionic ligands. The detailed conclusions from such models often disagree because of differences as to which ion associations are considered and in the ion association constants adopted. A further limitation of these models is that the possible formation of organic complexes was not taken into account because of a lack of information as to the stability constants for relevant organic complexes. This in turn reflects in part the fact that much of the dissolved organic material in sea water, the fraction generally described as humic material, has not been characterized in terms of its molecular composition. Stability constants for a number of trace metals with humic material isolated from various natural waters have been used by Mantoura, Dickson & Riley (1978) in an equilibrium model which includes the changes in speciation along a model estuary. Their data for copper are shown in figure 3.

These data illustrate the major alterations in speciation to be expected with changes in salinity in estuarine environments, with their associated variation in pH and activities of individual anions. They suggest that in average river water, dissolved copper is almost entirely complexed with humic material but these organic complexes become less important as the salinity increases, because of the displacement of copper by calcium and magnesium ions. The stability constants for the complexes formed by these latter ions are comparatively low, but this is offset by the high concentrations of the elements, so that in sea water they essentially saturate the complexing capacity of the humic material. Corresponding to the decreasing proportion of humic complexes with increasing salinity there is a marked increase in the importance of the inorganic species, particularly $Cu(OH)_2^0$. The fraction of unassociated cupric ion is maximal in the salinity range of *ca.* 5–10‰ but over the whole salinity range the proportion of the unassociated ion is very low. The activity coefficient of the free cupric ion decreases with increase in salinity. The change in activity of the cupric ion between river and sea water is therefore less marked than the change in the concentration of the free ion.

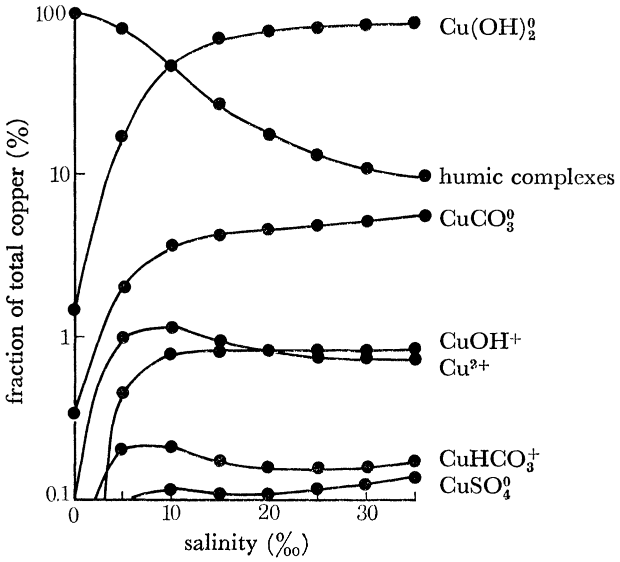

FIGURE 3. Equilibrium speciation of copper as a function of salinity in a model estuary. From Mantoura *et al.* (1978), with some minor species and alternative calculations omitted.

The equilibrium calculations by Mantoura *et al.* (1978) indicate that organic complex formation is significant also for mercury at salinities below about 10‰ but that humic material has only trivial effects on the speciation of zinc, cadmium, manganese, nickel and cobalt. With these latter elements, the unassociated cations assume greater importance in the speciation with an overall tendency for the proportions to decrease at higher salinities, especially with cadmium. The inorganic speciation of mercury is strongly dominated by associations with chloride and bromide ions and at all salinities the proportion of the unassociated mercuric ion is negligible. Comparisons of the inorganic speciation of copper, zinc, cadmium and lead in sea water and average river water by Millero (1975) indicate a greater importance of the unassociated ions in the former medium. There are thus major differences between these speciation models which probably reflect differences in the stability constants which have been selected for use in their derivation. Such differences are illustrated by the comparison in table 2 of various models for the inorganic speciation of zinc in sea water.

Regardless of the physical significance of the species in terms of which ion association models are expressed, it is apparent that the activities of the free ions of many trace metals in natural waters are very much lower than the total dissolved element concentrations, a fact which is indicated more directly by specific interaction modelling (Whitfield 1975*b*). Moreover, major differences in ionic interactions arise with variations in composition and ionic strength. The detailed implications of these phenomena in terms of biological processes are far from clear, but for any biological response which is related to the activity of free ions they must be considerable. Experiments with copper-deficient cultures of freshwater algae have shown a relation between growth and the activity of cupric ions (Manahan & Smith 1973), and culture experiments with an estuarine diatom indicated a dependence of growth rate inhibition on the same parameter (Sunda & Guillard 1976). However, where, biological responses are mediated through uptake of an element from solution into cells, the association of copper in *labile* complexes, such as are considered in equilibrium models, will not in theory reduce the availability of the element, since the whole labile pool is potentially available through readjustment of equilibrium.

TABLE 2. PERCENTAGE DISTRIBUTION OF ZINC BETWEEN ITS PRINCIPAL INORGANIC SPECIES IN SEA WATER

Zn^{2+}	$Zn(OH)^0_2$	$ZnCl^+$	$ZnCl^0_2$	$Zn(OH)Cl^0$	$ZnCO^0_3$	$ZnSO^0_4$	reference
17	62	6	4	—	6	4	1
16	—	44	15	13	3	2	2
47	—	20	10	—	—	10	3

Models are for a salinity of 35 ‰, a pH of about 8, a temperature of 25 °C and a pressure of 1 atm (*ca.* 10^5 Pa). References: 1, Zirino & Yamamoto (1972); 2, Dyrssen & Wedborg (1974); 3, Mantoura *et al.* (1978).

The matter is further complicated by the fact that the chemical speciation of some metals, in the conventionally defined dissolved fraction in natural waters, cannot be adequately described by equilibrium models because of the rôle of non-labile associations. The existence of forms which do not exchange rapidly with the labile pool of dissolved metal has been demonstrated in the case of zinc by radiochemical experiments; this and related work on zinc has been reviewed by Bernhard, Goldberg & Piro (1975). The response to analytical separation procedures of the radioactive isotope ^{65}Zn, when added as the ionic form to sea water, indicates that it does not exchange freely with all the forms of stable zinc. In some experiments, exchange between the added radioactive isotope and complexed forms of stable zinc occurred to the extent of only 10 % after 90 days at pH 6, whereas exchange between ionic zinc and the zinc–EDTA complex was complete within 30 min. At a characteristic pH for seawater of 8, exchange with the naturally occurring complexed forms was still slower.

Additional evidence for the occurrence of analytically non-labile fractions of certain metals has been obtained by comparing, for a particular sample of natural water, the fraction which is directly available to a particular analytical procedure with that available after an initial treatment designed to release the metal from strongly complexed forms. The principal evidence comes from the use in this way of ultraviolet photooxidation to decompose dissolved organic material, in conjunction with various methods of trace metal determination, and the use of acidification to render available otherwise non-electroactive fractions for measurement by anodic stripping voltammetry. Such operationally defined non-available fractions, when measured by different methods, are not generally equivalent but the analyses have shown them to form significant, although often highly variable, proportions of the total dissolved concentration of a number of metals in estuarine and coastal environments. There is some evidence that the significance of such fractions decreases in open oceanic waters for mercury (Fitzgerald 1975) and copper (Moore & Burton 1976).

The formation of non-labile associations by certain trace metals is particularly well demonstrated by measurements with anodic stripping voltammetry. Data summarized by Whitfield (1975*c*) show that, for example, the electroactive forms of dissolved copper in various coastal sea waters amount to some 30 % of the total. In this method the electroactive fraction of a trace metal is initially concentrated from solution onto an electrode by reduction at a controlled potential. The voltage is then altered in the anodic direction and at a characteristic potential a current peak is obtained the height of which is proportional to the concentration in the solution. The electroactive fraction comprises the free ionic metal and the fraction present in labile associations. The definition of labile and non-labile associations in this way remains operational and is not entirely sharp but it has a better theoretical basis than is provided by other operational definitions, the labile associations being those which are directly reduced at the electrode or which dissociate rapidly relative to the cell constant (Matson 1968).

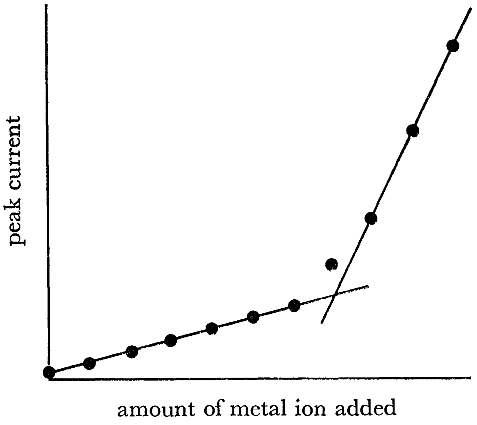

FIGURE 4. Generalized representation, with arbitrary units, of the amperometric titration of the complexing material in a sample of natural water by a metal ion, with the use of anodic stripping voltammetry. The point of intersection of the two lines gives the complexing capacity of the sample for the metal ion used as titrant.

This technique also enables quantitative determinations to be made of the complexation capacity of a water sample by titrating the sample with successive aliquots of the ionic metal and measuring the peak current. Such titrations often give results conforming to the generalized pattern shown in figure 4. The marked change in slope in the increase in peak current with increasing concentration corresponds to the saturation of the complexing capacity. Examples of the application of this method include studies on lead in river water (Matson 1968) and sea water (Clem 1973, cited by Whitfield 1975*c*), and copper in lake water (Chau, Gächter & Lum-Shue-Chan 1974) and sea water (Duinker & Kramer 1977). Davey, Morgan & Erickson (1973) have demonstrated complexation capacity in sea water by an analogous method using a biological response to increases in added concentration of ionic copper, namely the inhibitory effect on growth of *Thalassiosira pseudonana*.

Particularly with the use of photooxidation of organic material as a means of rendering additional fractions of dissolved elements analytically available it has often been assumed that the increase in available concentration is a result of release from organic complexes. The presence in natural waters of organic ligands capable of forming non-labile complexes with metals has not, however, been established. It is possible that some metals become associated in kinetically stable forms with organic molecules in the cells of organisms and are released thus to solution. The fact, previously discussed, that the fractions conventionally defined as 'dissolved' in natural waters can contain a wide range of polymeric and colloidal forms, must be reemphasized in this context. It is possible that some of the processes underlying the analytical phenomena of complexation capacity and unreactive fractions arise through the binding of metals to aged colloidal material, such as ferric hydroxide, which may be stabilized by adsorbed organic material.

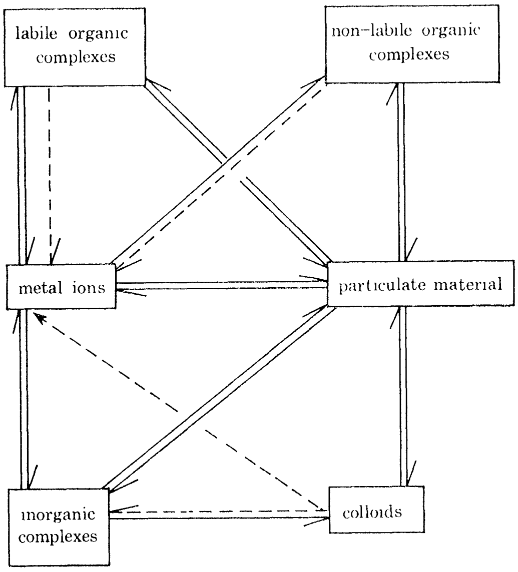

FIGURE 5. Principal physico-chemical interrelations between the forms in which trace metals occur in natural waters. The dotted lines indicate conversions which may be brought about in the dissolved fraction, as conventionally defined, by acidification or destruction of organic matter. Adapted from Whitfield (1975*c*).

An outline of the relations between the different fractions among which a trace metal may become distributed in natural waters is given in figure 5. This picture follows that used by Whitfield (1975*c*) to categorize the electrochemically active and inactive forms which appear respectively in the left and right columns.

The distinction made between electrochemically active and inactive forms might be regarded as an approximate guide to biological availability in such cases where uptake by organisms occurs from solution. There has been, however, no general agreement as to the way in which organic complexation influences biological activity. Thus, Barber & Ryther (1969) explained their observations on changes with time in the productivity of newly upwelled water by invoking organic chelation of trace metals as a factor increasing their availability, whereas Steemann Nielsen & Wium-Andersen (1970) attributed these effects to a reduced toxicity of copper as a result of its chelation. While more recent work on the concentrations of copper in oceanic waters (Boyle & Edmond 1975; Moore & Burton 1976) throws doubt on the latter interpretation, the inherent uncertainty remains as to the effects of organic complex formation on availability. It may not be possible to make valid generalizations on this aspect since the effects may differ between organisms according to the mechanism of action or uptake, just as association with particulate material may increase availability of a metal to some organisms while decreasing it to others.

Concluding remarks

This paper has emphasized the difficulties in relating closely the fractions determined analytically in natural waters and the species that theoretical models suggest to be present. Some of these difficulties arise through the adoption of analytically convenient but fundamentally inappropriate separations such as those normally achieved by filtration. Others stem from the fact that it is usually impossible with present techniques to measure individual species directly at concentrations within the picomolar to micromolar range in complex media; in this area electrochemical approaches almost certainly offer the greatest scope for advancement. Another type of problem arises through the little understood nature and rôle of uncharacterized polymeric and colloidal forms.

In view of these difficulties it is hardly surprising that the relations between the physicochemical form of an element and its impact on biological systems are very poorly understood, despite their obvious importance. Consideration of natural water systems in terms of even so general an outline as that shown in figure 5 indicates, however, that it would be simplistic in the extreme to suppose that the effects of trace metals added, in only one of the forms actually present, to laboratory or field systems in relatively short-term experiments will generally provide a close simulation of effects in a natural environment. Extrapolation over a range of environments with different chemical characteristics is still more uncertain. Closer simulations may be attained for the localized effects of specific pollutant discharges but it is again necessary to consider the kinetics of the reactions into which the discharged species may enter and the variable characteristics of different receiving environments.

A great deal remains to be learned about the chemical species in natural waters and the mechanisms and rates of their transformations. More investigation in this area is of evident importance in the context of the behaviour of pollutants. The urgent need to assess sublethal effects of pollutants in the sea means, however, that some experimental studies of these effects have to be pursued without the fundamental knowledge ideally required for their design. It is important that these studies should be undertaken with full regard to such information on physico-chemical factors as is available and interpreted with due consideration for the limitations which are imposed both by gaps in knowledge and inherently. This implies the need for particularly close collaboration between marine biologists and chemists in the design and interpretation of effects of pollutants at low concentrations.

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Discussion

M. Waldichuk (*Pacific Environment Institute, West Vancouver, B.C., Canada*). We have a copper–molybdenum mine on the northwest coast of Vancouver Island which discharges mine tailings (38000 t/day) into Rupert Inlet. The tailings contain of the order of 700 mg/kg of copper, but there appears to be very little copper above background in the overlying water or in the benthic organisms. The bottom water is quite well oxygenated. In contrast to the situation in Rupert Inlet, another copper mine on the west coast of Greenland, also discharging tailings into an inlet (the water likewise has high dissolved oxygen concentration from surface to bottom) leads to concentrations of copper and lead near to 1 mg/l. This shows up in moderately high concentrations of these metals, as well as cadmium, in tissues of marine organisms, including fishes.

Why should there be this difference?

J. D. Burton. Given the apparent similarity of chemical characteristics in the two environments, and assuming that there is no obvious difference in circulation leading to very different rates of dispersion, it seems possible that the difference might lie in the properties of the discharged solids in respect of the ease with which metals are leached.

A. R. D. Stebbing (*Institute for Marine Environmental Research, Plymouth, U.K.*). If one considers the relative toxicities of some metals, cadmium for example, in fresh and salt water one finds that in the marine environment these metals are significantly less toxic. Is this because fresh water species are inherently more sensitive, or is it due to some of the factors Dr Burton has mentioned that influence metal availability to organisms?

J. D. Burton. Since it is unclear in many cases whether a particular change in speciation, as such, increases toxicity or reduces it, the answer must be uncertain on these grounds quite apart from any uncertainties regarding the nature of speciation changes between media. Even with a single organism, responses in different salinities may be affected by physiological factors as well as those relating to chemical speciation. The findings from recent equilibrium models of speciation are contradictory. Those of Mantoura *et al.* (1978) indicate a large decrease in the proportion of unassociated cadmium ions in sea water relative to that in river water, but this was not indicated in the model of Millero (1975).