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Metal Speciation. Effects on Aquatic Toxicity

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■ Most water quality criteria for metals are presently based on total metal concentrations. However, for a series of algal bioassays in which zinc and various chelators were added, toxicity was not related to total metal but was related to the predicted free metal concentration.

Recent investigations regarding the chemistry of trace metals in natural waters have demonstrated that the metals exist both as the free metal ions and in various combined chemical forms. Physicochemical speciation schemes have been developed to separate the metals by size and to fractionate them into organic and inorganic forms (1). Florence (2) has shown that, in freshwaters, copper is predominately associated with organic colloidal matter, lead is divided between inorganic and organic forms, cadmium is present as the free ion, and zinc is present in both ionic and colloidal inorganic forms. Biological availability of a trace metal as either a required nutrient or a toxicant is dependent on chemical form (3). Many toxicological studies on trace metals are designed and conducted in order to evaluate the toxic effects of the metal to aquatic organisms. Such studies should be designed to measure and report the critical parameters that may affect the chemical form of the metal. This approach would facilitate bioassay interpretation in terms of those forms of the metal which are biologically available. Sunda and Guillard (4) found that, in highly chelated seawater media, the growth rate and the copper content of algal cells are related to cupric ion activity and not the total copper concentration. Other studies also suggest that free metal ions are the toxicant chemical species (5-11). In addition, it has been suggested that the stronger the metal complex, the lower the toxicity of a given concentration of total metal (12, 13).

In defined media, chemical forms of trace metals can be predicted using computer programs to solve simultaneous equations describing the chemical equilibria (14-16). The computed concentrations of the chemical forms of the metals should be useful in interpreting the biological effects of the metals. To demonstrate the applicability of this approach, algal assay experiments were conducted in a medium which contained a toxic concentration of zinc. Several levels of different chelators were added to alter the chemical speciation of the zinc as well as the growth response. Under these defined conditions there was a significant correlation between the calculated free zinc concentration and the number of cells present after 5 days of growth.

This research was undertaken as a portion of a program to evaluate the effects of chelation in aquatic environments. The materials tested have been considered for use as detergent builders in substitution for polyphosphates. A number of

questions regarding the addition of chelators to the aquatic environment have been expressed (17). Of biological concern is increased toxicity due to possible mobilization of metals from sediments. Any mobilized metal would be expected to be a complex. It has been recently shown that any mobilization by nitrilotriacetic acid (NTA) at expected environmental concentrations would be exceedingly small (18). We conducted the following experiments to demonstrate that algal toxicity is related to the free metal ion and not to the total soluble metal concentration. Extrapolation of the results of these experiments to either more complex defined media or to natural aquatic systems would require additional information about chemical speciation and biological response.

Experimental

A series of algal assays was conducted in which zinc (at a toxic concentration of 4.8×10^{-7} M) and a series of chelators at various concentrations (Table I) were added to the media. The chelators used were the sodium salts of nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), oxydisuccinic acid (ODS), carboxymethylloxysuccinic acid (CMOS), and carboxymethyltartronic acid (Builder M). We used algal assay procedure: bottle test (AAP:BT) media (19), with a 1:1 molar Fe:EDTA ratio, but with none of the other specified trace metals added. This permitted assessment of effects of test materials at submicromolar concentrations. The test organism, *Microcystis aeruginosa* Kutz emend Elenkin, was selected because of its high sensitivity to unchelated metals. Bioassays were conducted at a temperature of 20 °C, pH 8.0, fluorescent illumination of 200 ft-c, and with an inoculum of 4×10^4 cells/mL.

Results and Discussion

Growth curves for the various test concentrations of the chelator NTA (Figure 1) are representative of the algal assay results. Zinc toxicity was progressively reduced by increasing concentrations of NTA. Addition of 1.0×10^{-6} M NTA re-

Table I. Concentrations of Chelators (Moles/Liter) Added to the Algal Assay Media

NTA	EDTA	ODS	CMOS	Builder M
1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}
2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}
4.0×10^{-7}	4.0×10^{-7}	4.0×10^{-7}	4.0×10^{-7}	4.0×10^{-7}
6.0×10^{-7}	6.0×10^{-7}	6.0×10^{-7}	6.0×10^{-7}	6.0×10^{-7}
1.0×10^{-6}		1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}
5.0×10^{-6}		5.0×10^{-6}	5.0×10^{-6}	5.0×10^{-6}
1.0×10^{-5}		1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}
		1.0×10^{-4}	1.0×10^{-4}	1.0×10^{-4}
		1.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}

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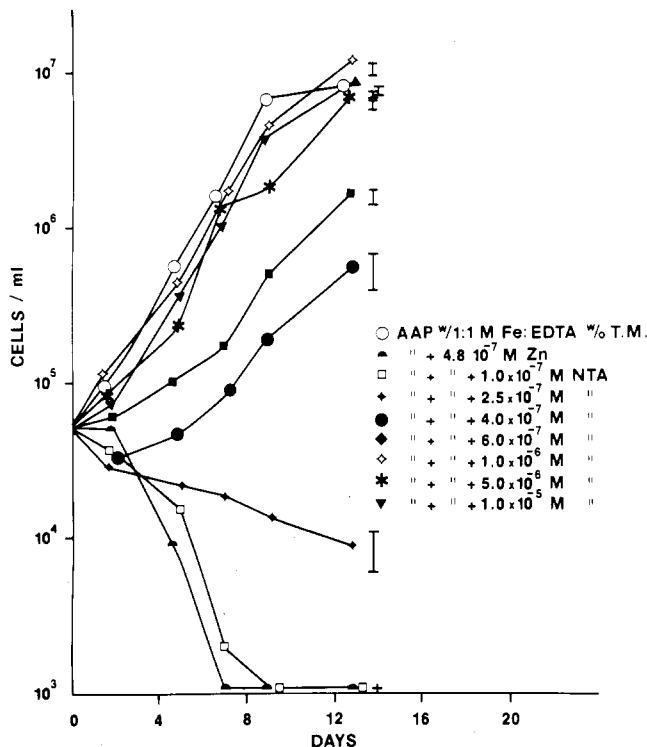


Figure 1. Growth of *Microcystis aeruginosa* in modified algal assay procedure media containing added zinc and the chelator NTA

stored algal growth to that of the control. Although all five of the chelators tested reduced the toxicity of zinc, the concentration required to reduce the toxicity to a given level differed for each compound. The lowest test concentrations of each chelator that allowed growth in the presence of 4.8×10^{-7} zinc were: EDTA, 2.5×10^{-7} M; NTA, 4.0×10^{-7} M; ODS, 1.0×10^{-5} M; CMOS, 1.0×10^{-5} M; and Builder M, 1.0×10^{-4} M.

To relate algal growth to the chemical forms of zinc present, stability constants for the reaction of each of the chelators with hydrogen, calcium, and magnesium ions, as well as with zinc ions, were required. Experimentally determined stability constants for the complexes of calcium with ODS and CMOS were compared to literature values to validate the method (20). Good agreement was obtained between experimentally determined stability constants and available literature values. As most constants required for ODS, CMOS, and Builder M were not available, the acid dissociation constants were determined by potentiometric titration (21), and the stability constants for complexation by the metal ions were measured by the ion exchange equilibrium method (22).

The stability constant and concentration data for the chelators and for the other constituents in the media were used in the computer program developed by Perrin and Sayce (14) to calculate the concentrations of the various zinc species in each test media. In every case, the only principal forms of zinc, in addition to the complex formed with the added chelator, were the ions Zn^{2+} and $ZnOH^+$, which have both been considered to be free zinc. Furthermore, the ratio of these two ions was fixed by the pH of the media.

For all cultures in which there was an increased number of algae after 5 days, the logarithm of the number of cells was plotted vs. the calculated concentration of free zinc (Figure 2). In addition, data were included for a series of controls in which no additional chelator was added to the media, but in which the concentration of added zinc was varied. The correlation coefficient for the line is 0.947 and the equation is $\log \text{cells} = 5.44 - 0.053 (\text{free zinc})$, where the concentration of free zinc is expressed in moles/liter. This linear relationship was

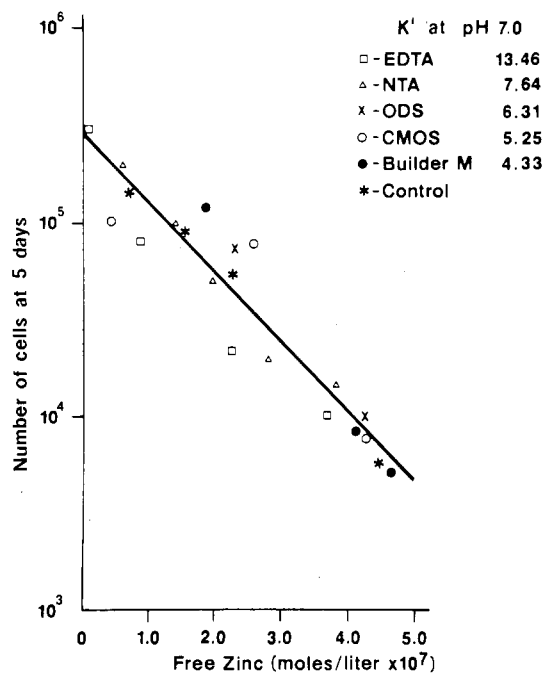


Figure 2. Algal growth as a function of free zinc concentration. For the samples containing test chelators, the total zinc concentration was 4.8×10^{-7} M. Control samples had no added test chelator, but the zinc concentration was varied

obtained even though the conditional stability constants varied by nine orders of magnitude and the ligand concentrations varied by three orders of magnitude.

We believe this demonstrates that the toxicity of trace metals to algae is directly dependent on the concentration of free metal ion rather than the total metal concentration. This method of predicting biological toxicity could be extended to natural waters provided adequate information on organism response to and equilibrium relationships for the metal are available. An understanding of the relationship of an organism's response to the specific chemical form of a toxicant in the environment rather than to its total concentration is an important consideration in the development of water quality criteria (23, 24).

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Trace Metals in Humic and Fulvic Acids from Lake Ontario Sediments

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■ The distribution of trace metals in humic acid, fulvic acid, and the associated sediments from Lake Ontario has been determined. The principal metal components of the humic acids are copper (1000–1600 $\mu\text{g/g}$) and iron (1000–2000 $\mu\text{g/g}$). Metals enriched in the humic and fulvic acids compared to the entrapping sediments include Cu, Pb, Ni, and Cr, whereas the concentrations of Co, Cd, Mn, and Zn are much less in these acids. It is estimated that nearly all the Cu, about 5–10% of the Pb, Cr, and Ni, and <5% of the Co, Cd, and Zn in the sediments are bound to the organic matter. Evidence is presented to show that the humic and fulvic matter have acquired most of their trace metal burden in the overlying water.

It is now widely believed that humic and fulvic acids play a prominent role in the cycling and accumulation of trace metals in soils and sediments (1–5). Few of the studies, however, have addressed the binding of metals by organic phases in lake sediments. This report describes the distribution of trace metals in the humic acid, fulvic acid, and associated sediment samples from Lake Ontario and considers the relative importance of organic matter as metal carriers in the sediments. Lake Ontario receives substantial quantities of pollutant metals annually (6, 7), and the humic and fulvic acids may be expected to exercise a strong influence on the fate of these metals in the lake.

Methodology

Sediment samples were collected (using a Benthos gravity corer) from close to the deepest point in the lake (Station 64) and from the shallow Kingston (eastern) basin (Station 78). The deep water sample is typical of the fine-grained sediments in the main basin of the lake, whereas Station 78 is characterized by a high sedimentation rate and a large deposition of organic matter, made up primarily of blue-green algae. The physical and chemical characteristics of sediments at the two locations have been described (6, 8, 9).

The humic acids (HA) and fulvic acids (FA) were extracted by continuous shaking of a mixture of sediment sample and 0.5 N NaOH for 24 h under nitrogen. The HA and FA samples used in the present study were obtained from two extraction passes which lasted for 48 h. The leachate was acidified to pH 1.0 to precipitate out the humic acid. The HA was collected by centrifugation, washed with 0.1 N HCl, and oven-dried at 40 °C. Concentrated lead chloride solution was added to the acid supernatant to precipitate out the FA (e.g., see ref 10), which was then rinsed with distilled water and dried.

Aliquots of the HA, FA, and freeze-dried sediment samples were digested with a nitric-perchloric acid mixture (11), and the metal contents of the extract determined by atomic ab-

sorption spectrometry. The organic carbon contents of acid-leached sediment samples were determined by the dry combustion method using a Leco induction furnace (12).

Results and Discussion

The concentrations of organic carbon, HA, and FA at different depths in the sediments are shown in Table I. There is a marked enrichment of the surficial (0–5 cm) sediments with organic carbon. As expected, the levels of both organic carbon and HA are significantly higher at Station 78 compared to Station 64. From the approximate relation, *organic matter* = $1.8 \times$ *organic carbon*, the fraction of organic matter in sediments in the form of humic acids has been estimated (Table I). Humic acids account for about 9 and 10–16% of the organic matter in the surficial sediments and deeper layer deposits, respectively. The HA fractions are roughly the same at the two stations sampled. The FA extractable by lead precipitation constitutes 1–4% of the organic matter compared to the 12–21% obtained by Kemp (12, 13), using the method of Kononova (14).

The concentrations of metals in HA, FA, and sediments at different depths are shown in Tables II and III. The ratios of the metal contents of HA to those of the entrapping sediments (referred to as the enrichment factor in this report) are displayed in Table IV.

Table I. Organic Carbon, Humic Acid, and Fulvic Acid Contents of Lake Ontario Sediments

station and depth below interface, cm	observed concentrations			org matter assoc with HA, %
	org C, %	HA, $\mu\text{g/g}$	FA, $\mu\text{g/g}$	
Station 64				
0–5	2.26	3810	1030	9.4
5–10	1.83	4860	1080	15
10–15	1.72	4540	690	15
15–20	1.82	4240	710	13
20–25	1.59	4040	860	15
25–30	1.72	3120	1050	10
30–35	1.59	3670	1140	13
35–40	1.70	3790	830	12
Station 78				
0–5	4.24	6950	1770	9.1
5–10	2.61	5390	1310	12
10–15	2.45	4840	1160	11
15–20	2.15	5040	1050	13
20–25	2.16	5780	1440	15
25–30	2.42	6830	1060	16