TRACE METAL SPECIATION AND TOXICITY IN PHYTOPLANKTON CULTURES

F.M.M. Morel, N.M.L. Morel, D.M. Anderson,
D.M. McKnight and J.G. Rueter, Jr.
Division of Water Resources
and Environmental Engineering
Civil Engineering Department
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

In: Advances in Marine Environmental Research
F.S. Jacoff, Ed. EPA-600/9-79-035. 1979

ABSTRACT

The toxicity of trace metals to phytoplankton has been demonstrated to depend on metal ion activities. The various chemical processes that control metal speciation, and thus activities in aquatic systems, are inorganic complexation, chelation, precipitation and adsorption. For example, the activity of metals such as mercury, cadmium or lead are controlled in saline waters of low organic content by the formation of chloride and bromide inorganic complexes. For mercury, this is also the case in typical phytoplankton culturing media. Artificial chelating agents permit convenient manipulation of metal ion activities in algal toxicity experiments. However, kinetic phenomena can result in transient peaks in metal ion activities and lead to large overestimations of toxicity. The release of metal complexing agents by algae is not expected, in general, to affect markedly the chemistry of metals in highly chelated artificial media except in cases of high specific affinity. The greatest complication in interpretation of photoplankton toxicity experiments arises from the presence of solids in the culture medium. These can precipitate during the preparation of the medium, or as a result of the pH increase due to photosynthetic carbon uptake. The kinetics of precipitation of these solids, their aging and the adsorption of trace metals on their surface, lead to variations in metal activities that are difficult to quantify, and do not permit proper assessment of the toxic effects. Understanding the global aquatic chemistry of trace metals in algal culture media, is a *sine qua non* prerequisite to proper design and interpretation of toxicity experiments.

INTRODUCTION

Using copper as the principal example, this study aims at establishing a chemical framework for the study of laboratory and natural processes involving trace metal toxicity to phytoplankton. If it is a reasonable assumption that the mechanisms of metal toxicity are similar in many phyla, this framework should be useful for toxicity studies with many aquatic organisms, and can serve as the general basis for design and interpretation of such studies.

Although the importance of both organic complexing agents and trace metals in phytoplankton cultures has been recognized for some time, the critical role played by the speciation of trace metals in controlling their toxicity and availability to algae has just begun to be understood. It has now been established that it is the activity of the free ions, rather than the total metal concentrations, which determine the toxicity of metals to phytoplankton (46, 2). The study of the chemical processes which govern the activity of a given trace metal in culture media becomes then a prime area of concern to phycologists interested in metal toxicity experiments. An enumeration of these processes includes inorganic complexation, chelation, precipitation and adsorption. In addition, indirect chemical effects involving several interacting chemical species in the medium can influence trace metal activities in unobvious ways. In this study, these basic principles of Aquatic Chemistry (43) that apply to metals in phytoplankton cultures will be discussed systematically.

There are but a few free metal activities that can be experimentally measured in the range and under the conditions of interest. There are also few metallic complexes which can be analytically determined in chemical systems as complex as culturing media. Henceforth, theoretical equilibrium calculations will be used throughout this paper to assess metal speciation and activities. The assumption of equilibrium is a reasonable one when the proper precautions are taken during medium preparation. Thermodynamic calculations provide, then, a convenient means of illustrating the critical chemical principles, even if they have inherent uncertainties. Complications introduced in the chemistry of the system by kinetic phenomena, or by the influence of the algae, will be discussed for each of the examined processes.

Copper has been the metal of choice in studies of metal toxicity of phytoplankton because it has been postulated that cupric ion toxicity might play a role in the ecology of phytoplankton in some natural waters (6, 37, 7, 10). In keeping with this situation, this paper will focus, albeit not exclusively, on copper which provides a rather good example for metal speciation and toxicity in phytoplankton cultures.

EQUILIBRIUM SPECIATION OF METALS IN CULTURING MEDIA

Before studying in detail the role of chemical processes in controlling metal speciation, it seems useful to examine what metal species are expected to be important in typical culturing media.

In artificial media where the analytical concentrations of the components are precisely known, the exact composition of the system, including all soluble and insoluble species of the various metals and their activities, can be computed if a state of equilibrium or partial equilibrium is established (43). It should be underlined that such calculations of chemical speciation depend on correct identification of the principal species, and knowledge of the corresponding equilibrium constants. The calculations presented here were performed with the computer programs, REDEQL (25, 21) and MINEQL (49), which contain a list of possible species, and a selection of the necessary thermodynamic constants from a variety of sources (36, 38, 33).

Results of chemical equilibrium computations of three media recipes, the freshwater medium WC (11) and the seawater media F/2 (12) and Aquil (26), are shown in Table 4-1. Possible adsorption processes are not considered in these calculations. Note that some heavy metals (Pb, Cd, Hg, Ni, Co, Cr) which are not part of the recipes, have been added in trace amounts (10^{-9}M) to illustrate how they would be speciated if they were present as contaminants in the media. Such low metal concentrations affect the rest of the chemical systems negligibly. Heavy metal speciation in all media is completely dominated by the chelation with ethylenediaminetatraacetate (EDTA) which is included in the recipes for the very purpose of chelating metals. An important exception is mercury, which, according to the computations, is present entirely as chloride complexes in F/2 and Aquil and half as hydroxide species in WC. For all metals, the free ion activities are several orders of magnitude smaller than their total concentrations. In all media, iron and manganese oxides and calcium phosphate (hydroxylapatite) are computed to precipitate at equilibrium. Calcium carbonate (calcite) is also shown to be saturated in the seawater media. Actual precipitation of these various solids is dependent on kinetic processes as will be discussed later.

The trace metal chemistry of such culturing media can be grossly affected by the presence of algal cells due to metal uptake. For example, typical values for the uptake of copper by phytoplankton are in the range 10^{-16} to 10^{-14} moles/cell (44, 35, 16). With the algal densities and the copper concentrations commonly used, a sizeable part of the total concentration of copper in the medium can thus be taken up by the cells. This underlines the necessity of "buffering" metal ion activities in toxicity experiments in order to render these activities relatively insensitive to total metal concentrations. The use of various chelating agents for this purpose will be discussed later.

INORGANIC COMPLEXATION

It has been observed in several instances that the toxicity of metals such as lead, cadmium, mercury or silver to a variety of organisms, from bacteria to

Table 4-1. Equilibrium Trace Metal Speciation in Typical Algal Growth Media

	WC(EDTA= $1.2\ 10^{-5}$ M)			F/2(EDTA= $1.2\ 10^{-5}$ M)			Aquil (EDTA = 5×10^{-6} M)		
	Analytical Concentration M	Computed Activity of free ion. M (-log)	Major Species	Analytical concentration M	Computed Activity of free ion. M (-log)	Major Species	Analytical Concentration M	Computed Activity of free ion. M (-log)	Major Species
Iron	1.2×10^{-5}	21.5	FeEDTA 22% $Fe(OH)_3(S)$ 78%	1.2×10^{-5}	21.5	$Fe(OH)_3(S)$ 99%	4.5×10^{-7}	21.5	FeEDTA 6% $Fe(OH)_3(S)$ 94%
Manganese	8.9×10^{-7}	14.7	$MnO_2(S)$ 100%	8.9×10^{-7}	14.7	$MnO_2(S)$ 100%	2.3×10^{-8}	14.7	$MnO_2(S)$ 100%
Copper	4.0×10^{-8}	14.8	CuEDTA 100%	4.0×10^{-8}	13.3	CuEDTA 99%	1.0×10^{-9}	14.4	CuEDTA 100%
Cadmium	1.0×10^{-9}	14.8	CdEDTA 100%	1.0×10^{-9}	13.3	CdEDTA 99%	1.0×10^{-9}	12.8	CdEDTA 98% $CdCl^+$ 1% $CdCl_2$ 1%
Zinc	7.9×10^{-8}	12.2	ZnEDTA 100%	7.9×10^{-8}	10.7	ZnEDTA 100%	4.0×10^{-9}	11.5	ZnEDTA 100%
Nickel	1.0×10^{-9}	16.8	NiEDTA 100%	1.0×10^{-9}	15.3	NiEDTA 100%	1.0×10^{-0}	14.8	NiEDTA 100%
Mercury	1.0×10^{-9}	20.4	HgEDTA 49% $Hg(OH)_2$ 51%	1.0×10^{-9}	23.6	$HgCl_4^{2-}$ 87% $HgCl_3^-$ 13%	1.0×10^{-9}	23.6	$HgCl_4^{2-}$ 87% $HgCl_3^-$ 13%
Lead	1.0×10^{-9}	16.3	PbEDTA 100%	1.0×10^{-9}	14.8	PbEDTA 100%	1.0×10^{-9}	14.3	PbEDTA 99%
Cobalt	5.0×10^{-8}	13.9	CoEDTA 100%	5.0×10^{-8}	12.3	CoEDTA 100%	1.0×10^{-9}	11.9	CoEDTA 99%
Chromium	1.0×10^{-9}	23.1	CrEDTA 100%	1.0×10^{-9}	21.5	CrEDTA 100%	1.0×10^{-9}	21.1	CrEDTA 100%

fish, decreases with increasing salinity of the water (45). One possible explanation for such a phenomenon is the decrease in metal ion activity resulting from the formation of ion pairs with the major anions of seawater. Figure 4-1 shows, for example, how the speciation of mercury and the activity of the mercuric ion vary in function of salinity in the medium F/2. As salinity increases the bromide complexes of mercury replace the EDTA chelate as the major species, followed by the chloride complexes as the salinity approaches that of seawater. In natural systems, in the absence of strong chelating agents, the same phenomenon would extend to other metals such as lead and cadmium. Table 4-2 illustrates this point by giving the major species of the various metals in Aquil where EDTA has been reduced to 10^{-8}M. Besides the chloride complexes, a number of carbonate (Cu, Pb), sulfate (Zn, Mn, Co) and hydroxide (Zn, Pb, Co, Cr) complexes become significant. Because the kinetics of formation of the various inorganic complexes of metals are typically fast (43), equilibrium is a good assumption in this instance, and the thermodynamic calculations should give accurate values of metal activities.

The role of carbonate complexation in decreasing the toxicity of metals in unchelated media has been verified for copper on *Daphnia magna* (3), for

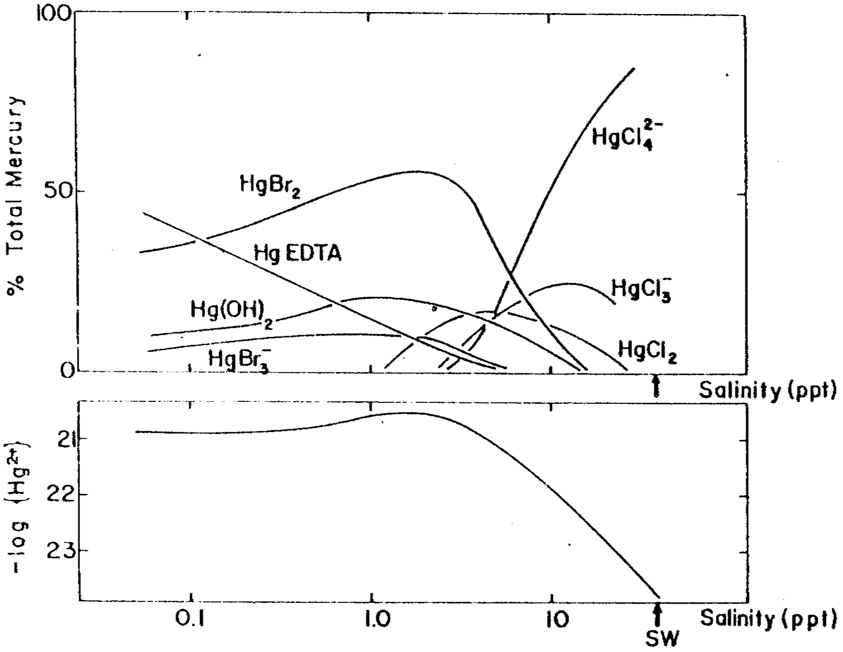


Figure 4-1. Chemical speciation of mercury in F/2 medium as a function of salinity at pH = 8.1.

Note: Top: mercuric complex as a percent of the total mercury (10^{-9}M); Bottom: the activity of the mercuric ion. All other trace metals remain bound to EDTA throughout the salinity range. SW represents seawater, salinity 33ppt.

Table 4-2. Speciation of Trace Metals in Aquil with EDTA Concentration Reduced to 10^{-8}M.

	Major Species	Percent of Total Metal
Iron	$FE(OH)_3(S)$	100%
Manganese	$MnO_2(S)$	100%
Copper	Cu^{2+}	1%
	CuEDTA	87%
	$CuCO_3$	12%
Cadmium	Cd^{2+}	3%
	$CdCl_3^+$	39%
	$CdCl_2$	42%
	CdEDTA	6%
Zinc	Nz^{2+}	60%
	$ZnSO_4$	8%
	ZnCl	3%
	$ZnCl_3^-$	1%
	ZnEDTA	27%
	$ZnOH^+$	1%
Nickel	NiEDTA	99%
Mercury	$HgCl_4^{2-}$	87%
	$HgCl_3^-$	13%
Lead	$PbCO_3$	21%
	$PbCl_3^-$	50%
	$PbCl_2$	5%
	$PbCl^+$	3%
	PbEDTA	20%
	$PbOH^+$	1%
Cobalt	Co^{2+}	51%
	$CoSO_4$	11%
	$CoCl^+$	21%
	CoEDTA	15%
	CoOH	2%
Chromium	CrEDTA	57%
	$Cr(OH)_4^-$	40%
	$Cr(OH)_2^+$	3%

copper on some fishes (31), and for cadmium on a grass shrimp (45). Although there has been no experiment reported to date that provides direct evidence for the importance of inorganic complexation in controlling metal toxicity to phytoplankton, this result can be inferred from data with these other organisms, and from the general demonstration that metal ion activities are the important parameters of toxicity to algae.

Through uptake of carbon dioxide for photosynthesis, algae can modify the inorganic species of metals by decreasing the total concentration of carbonate in the system and increasing the pH. As pH increases, the hydroxyl ion activity increases and so does the importance of metal hydroxide complexes. The effect on the carbonate ion activity and on the metal carbonate complexes is less straightforward, and depends on the original pH of the medium. In seawater media (pH 8), and in freshwater media around neutral pH, the carbonate complexes will increase with CO_2 uptake due to the predominance of the resulting pH increase over the total carbonate decrease. Such variations in metal chemistry can be alleviated by bubbling air in the cultures, thus insuring a steady concentration of carbonate in the medium.

CHELATION

The history of the development of artificial culturing media for algae is in part that of the replacement of "growth factors" and "soil extracts" by chelating agents (17). The exact role of these chelating agents in promoting algal growth has been a subject of some controversy (6, 14). It is now well established that they do control the toxicity of various heavy metals – copper in particular (46). Whether they also increase the availability of some metallic nutrients – chiefly iron – is yet unproven. Figure 4-2 shows the percentage of chelated metal and the metal activities in Aquil (with contaminant metals) as a function of the concentration of EDTA, by far the most widely utilized chelating agent in algal media. Note that the order in which the metals are chelated by EDTA is not simply related to either the metal ion activities or their affinities for EDTA (FE>Cr>Cu>Ni>Pb>Zn, Cd, Co).

Other chelating agents which are commonly used include nitrilotriacetate (NTA), citrate and various amino acids. "Tris" (tris(hydroxymethyl)amino methane) commonly used as a pH buffer for biological experiments has received much use in recent studies of copper toxicity to phytoplankton (44). Used in conjunction with EDTA which chelates the other metals at a very low concentration, Tris permits a convenient manipulation of the cupric ion activity. Figure 4-3 illustrates this point by comparing how the cupric ion activity varies with total copper in Aquil (EDTA = $10^{-5.3}$M) and in a modified Aquil recipe containing Tris (EDTA = $10^{-6.3}$M) and Tris = 10^{-3}M). Around $[Cu_2^+]$ = 10^{-10}M, where many toxicity studies are run, the cupric ion activity in the Tris medium is less sensitive to variations in total copper concentration than in the EDTA medium. However, with the proper precautions, both media yielded the same results in a study of copper toxicity to *Gonyaulax tamarensis* (2).

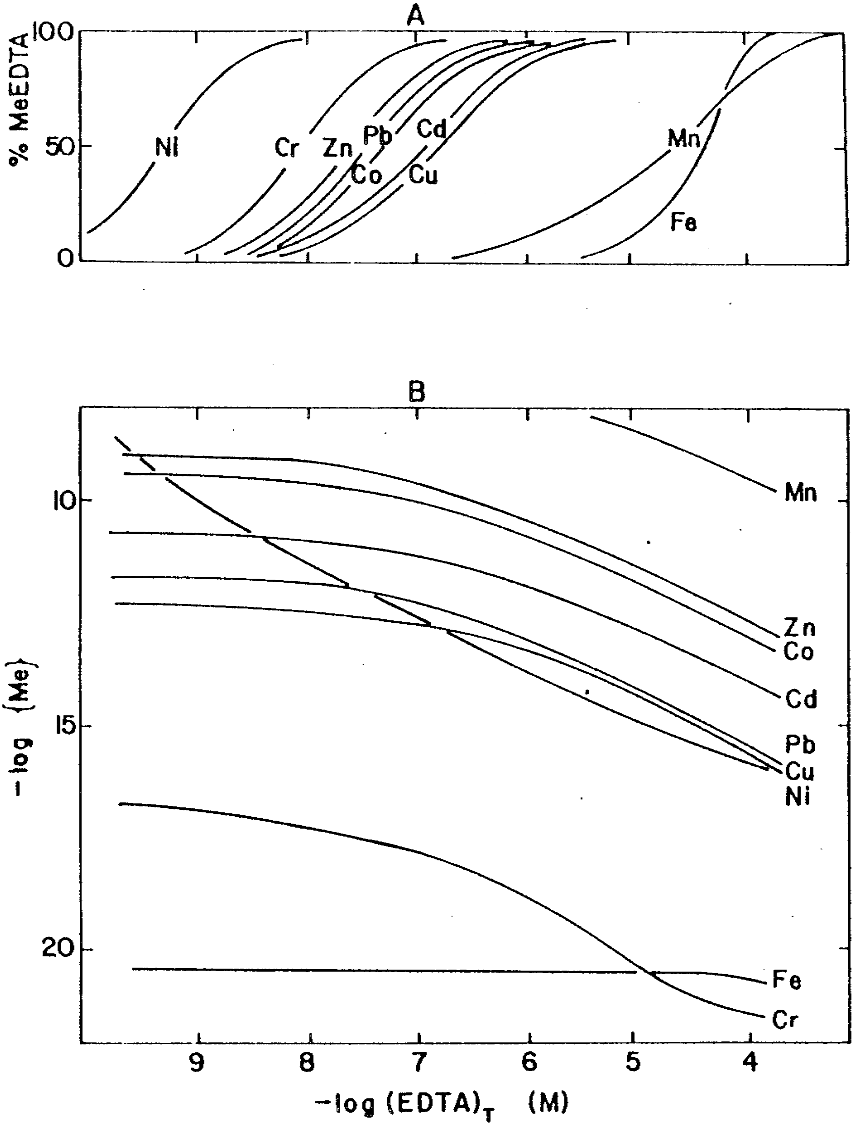


Figure 4-2. The effect of EDTA on the speciation of metals in Aquil.

Note: Total metal concentrations are given in Table 4-1, with Pb, Cd, Hg, Ni, Co, and Cr added as contaminants (10^{-9}M). A) The percent of each metal that is chelated (MeEDTA) versus total EDTA concentration, (M); B) Metal ion activity (M) versus total EDTA concentration (M).

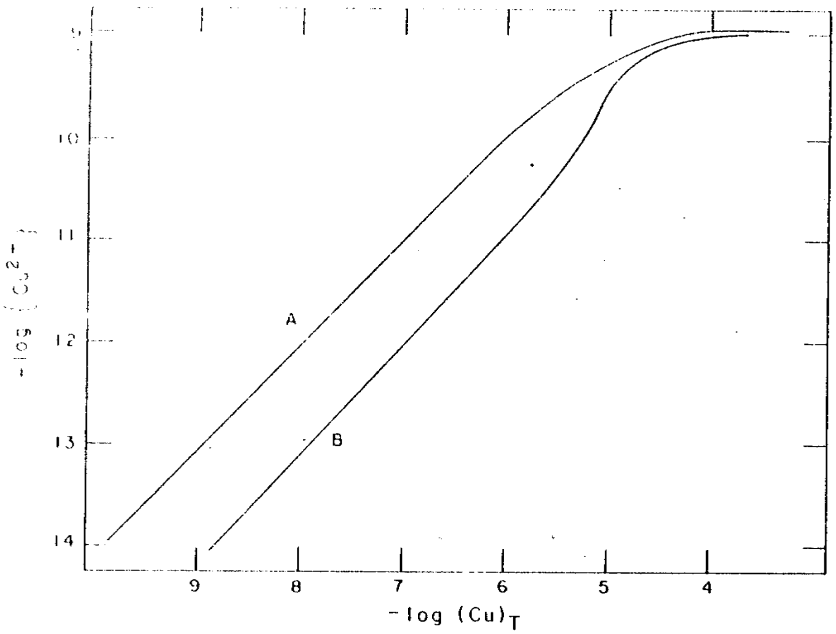


Figure 4-3. Computed activity of the cupric ion (M) versus total copper concentration for two Aquil recipes.

Note: Chelated with: A) $10^{-6.3}$M EDTA plus 10^{-3}M Tris and B) $10^{-5.3}$ EDTA.

Although the forward kinetic constants of chelate formation are invariably very large, resulting in quasi instantaneous kinetics in simple systems, the situation can be very different in systems as complex as culturing media. For example, when copper was spiked in Aquil cultures of *G. tamarensis*, a dramatic short term toxic response was observed much above that expected for the calculated equilibrium activity of the cupric ion (2). This phenomenon which was not observed when Tris replaced EDTA as the major copper chelating agent, was attributed to the slow kinetics of the metal exchange reaction:

$$Cu^{2+} + CaY \rightleftharpoons CuY + Ca^{2+}$$

This appears as a reasonable explanation, since the calcium chelate is the major form of EDTA in Aquil and the dissociation is slow. No such phenomenon can occur with Tris, whose major species in culturing media are the various protonated forms of the ligand. This can be checked directly by monitoring the cupric ion activity with a mixed sulfide electrode (34, 13) in chemical systems similar to the culturing media. Figure 4-4 presents the results of such an experiment, and leaves no doubt as to the slow kinetics of copper reaction with

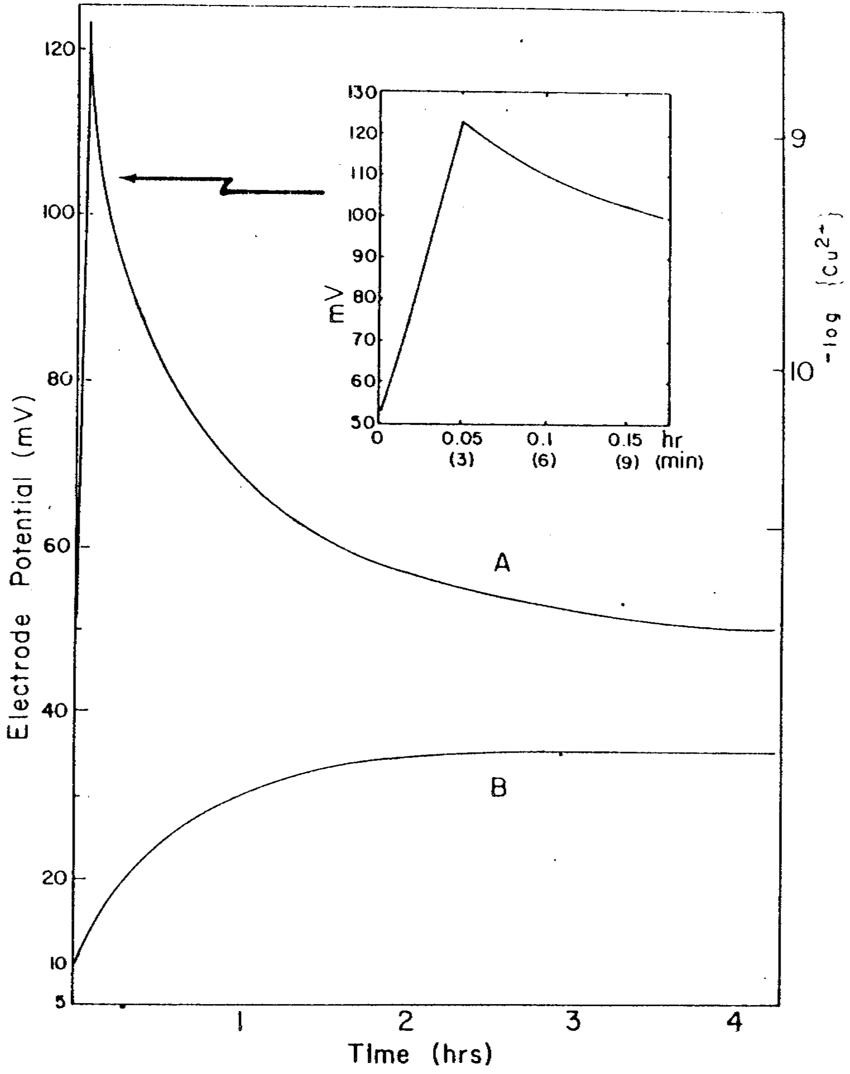


Figure 4-4. Effects of dissociation of Ca-EDTA on short term cupric ion activity after addition of $10^{-4.5}$M $Cu(NO_3)_2$.

Note: Background electrolyte 0.5 m KNO_3, pH 8.25 (2 x 10^{-3}M $NaHCO_3$ bubbled with air), $10^{-4.3}$M EDTA, temperature 23°C. A Radiometer selectrode (F 3000), an Orion d/j reference electrode, and an Orion pH electrode were used. In both experiments, the cupric ion activity (M) was calculated using the Nernst equation and data from 10^{-4}, 10^{-5} and 10^{-6}M $Cu(NO_3)_2$ solutions at pH 4:00 in 10^{-3}N KNO_3 background electrolyte. A) Calcium (10^{-2}M) in equilibrium with EDTA prior to copper addition; B) No calcium present.

EDTA in the presence of an excess of calcium: an initial peak in cupric ion activity is measured by the electrode, and it takes about four hours to approach the equilibrium value. Such phenomena have to be taken into account when studying the toxicity of metals to any aquatic organism, as transient effects can lead to large overestimations of toxicity.

The release of chelating metabolites has been widely assumed as a conditioning mechanism for culture media (39). As is the case for natural waters, most of the chemically quantitative work on this topic has focused on the synthesis and exudation of iron chelating agents, particularly hydroxamates (19, 30). What seems often overlooked is that hydroxamic acids do not chelate exclusively iron, and that their binding of other metals can result in sizable decrease of these metals' activities (1).

By direct potentiometric techniques, extracellular metabolites of algae have been characterized in terms of copper complexing capacity and affinity (48). According to this work, the ligand produced by the algae under the conditions of the experiments is characterized by a constant of approximately unity for the reaction:

$$Cu^{2+} + HY^{-} = H^{+} + CuY$$

If one assumes the ligand to be copper specific, the effect of its release in Aquil and Aquil with Tris is shown in Figure 4-5. Note that a significant decrease in the cupric ion activity does not begin until the total ligand concentration reaches 10^{-4}M, an upper limit for the measured ligand releases. Although $[Cu^{2+}]$ start decreasing at a slightly lower ligand concentration when the copper concentration is elevated, the release of such relatively weak complexing ligand has little overall effect on the cupric ion activity in a well chelated medium. Ligands, with higher affinity for copper, appear to be released by some blue green algae (22).

In principle, phytoplankton could modify the trace metal chemistry of the medium by assimilating artificial chelating agents. However, this potential problem is avoided by using EDTA or NTA which have been shown not to be assimilated by algae (23). Although photodegradation of EDTA and NTA has been reported (40), the light intensities normally used for culturing phytoplankton are insufficient to promote it in the laboratory.

PRECIPITATION

According to the computations of Table 4-1, the precipitation of several solids is calculated to be thermodynamically favorable in typical culturing media. Visible precipitates are indeed a common observation of users of algal

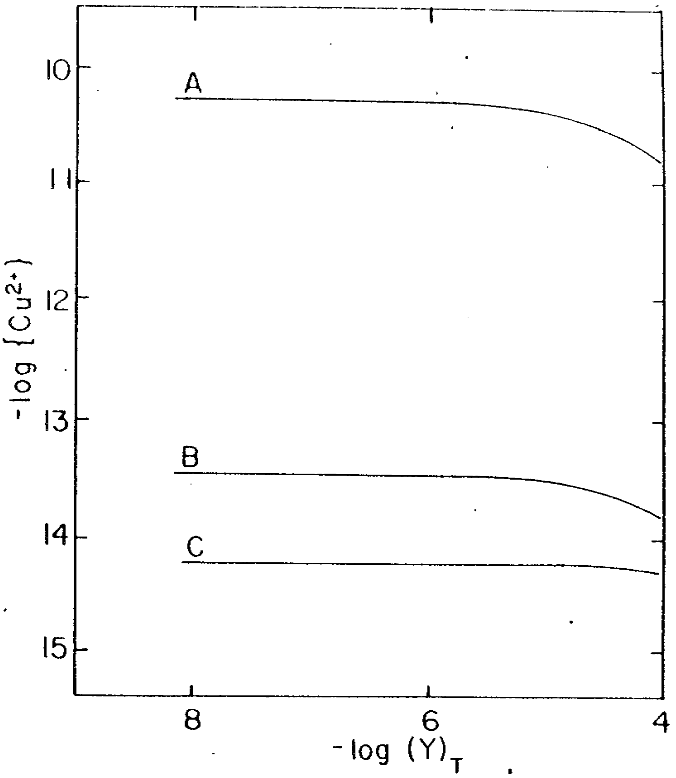


Figure 4-5. The effect of different molar concentrations of copper specific metabolite (Y) on the activity of the cupric ion for three variations of Aquil medium.

Note: A) $10^{-5.3}$M EDTA plug 10^{-6}M Cu; B) $10^{-6.3}$M EDTA plus 10^{-3}M Tris; C) $10^{-5.3}$M EDTA.

media. This is especially true following autoclaving, which brings about a large pH increase by eliminating carbon dioxide from the system. This problem has been studied by researchers involved in the design of culturing media (32, 8, 12, 18, 26). The increase in temperature and pH during autoclaving decreases the solubility of calcium carbonate, and results in the precipitation of a magnesium rich solid (this suggests the solid to be magnesium calcite, although aragonite has been identified in such precipitates). Hydrous oxides of iron and manganese can also precipitate under such conditions, depending on the chelating agent concentration and the pH reached during autoclaving. When such precipitates occur, phosphate becomes largely associated with the solid phase, presumably in some calcium precipitates (apatite or $CaHPO_4$), or as an adsorbate on the various solids. Depending on the initial concentration of silicic acid and on the nature of the container, which can increase the silicate concentration of the solution by dissolution, some amorphous or crystalline form of SiO_2 can form in the medium.

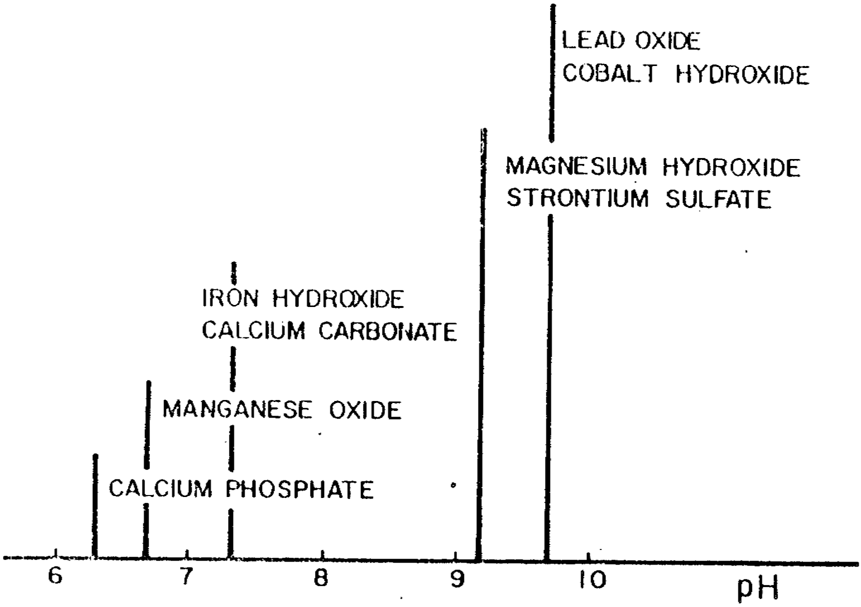


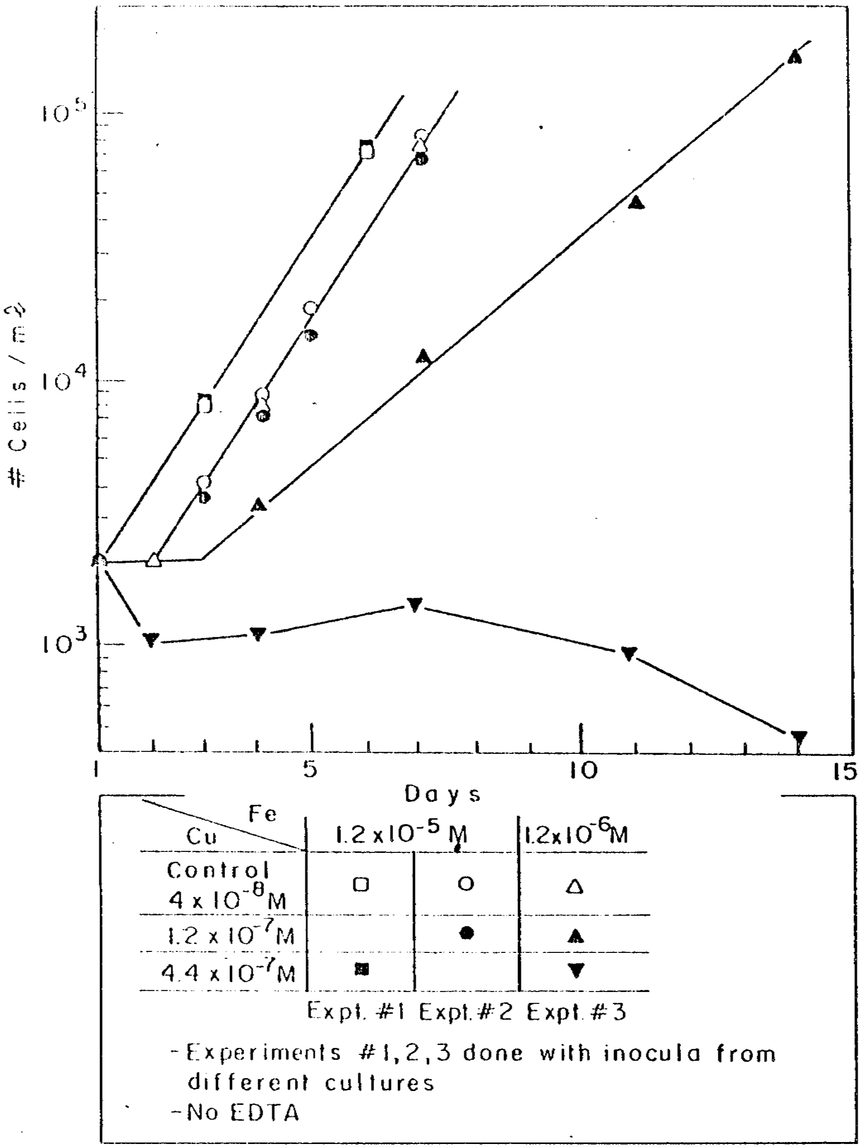
Figure 4.6. Saturation pH for various metal solids in Aquil.

These various solid formation processes are dependent on kinetic factors which are controlled by the particular temperature and pH regime of the medium. These, in turn, depend on the conditions and duration of autoclaving, as well as on the size of the containers and the mixing conditions. Precipitates are rarely seen with filter sterilization. Figure 4-6 shows the onset of saturation for various solids as pH is increased in the medium Aquil, normally designed to avoid precipitates. None of the four solids that are saturated at pH = 8 in Aquil are actually seen to precipitate, even after autoclaving if the volumes are kept smaller than 100 ml. If larger volumes are autoclaved, immediate bubbling with carbon dioxide prevents precipitation. Avoidance of calcium carbonate precipitate is very important for maintaining iron and manganese in solution, as the presence of $CaCO_3(s)$ will catalyze the formation of hydrous oxides of manganese and iron (43). For other trace metals the formation of these precipitates creates difficulties mostly through adsorption processes (see next section).

For toxicity studies, trace metals are sometimes introduced in algal cultures in excess of the chelating agent concentration. Precipitates are then often expected to form mostly oxides, hydroxides and carbonates, depending on the metal. For example, a hydroxide ($Cu(OH)_2$), an oxide (CuO, tenorite) when a carbonate ($Cu_2CO_3(OH)_2$, malachite) become quickly saturated in Aquil when copper exceeds the EDTA concentration. Although the hydroxide is not the thermodynamically stable form, it probably is the one which forms initially in the medium for kinetic reasons. Regardless of the precise nature of the solid, good agreement has been obtained between calculated and measured copper concentration in the solid phase in Aquil' medium with a high EDTA concentration, 2 hours after addition of excess copper (29). It is worth noting that the precipitate was very finely dispersed, and that centrifugation was necessary to separate it from the aqueous phase. Ignorance of the formation of a precipitate can obscure completely the meaning of otherwise well controlled experiments. In terms of metal ion activity, the situation is complicated by the change in the nature of the precipitate which might evolve from an active form to a more stable one. In copper saturated media, the cupric ion activity has been measured potentiometrically to decrease markedly over 24 hours, the rate of decrease becoming very small thereafter (22). Such conditions can create large uncertainties in toxicity experiments.

ADSORPTION

The common notion that chelating agents make iron available to algae, seems to be supported by experiments where addition of iron or EDTA salts provide similar growth and carbon uptake enhancement in a variety of algal cultures (3). However, aluminium salts have also been observed to enhance carbon uptake (24). Following Stumm and Barber (41), it is now a prevalent interpretation of such experiments to attribute part, or all of the beneficial effect of the metal additions to a scavenging of other toxic metals by adsorption on precipitating iron or aluminium hydrous oxides. Figure 4-7 illustrates the beneficial effect of iron additions to a *Pyramimonas* culture, and demonstrates how iron and copper behave antagonistically under controlled conditions (28). The growth rate of *Pyramimonas* is reduced at a total copper concentration of 1.2 10^{-7}M, and completely stopped at 4.4 10^{-7}M when the iron concentration is low (1.2 10^{-6}M). Increasing the iron concentration by a factor of 10 completely blocks the toxic effect of the same copper concentrations. The question to be resolved is how much of this "detoxification" of copper by iron is due to adsorption processes, effectively removing the copper from solution and decreasing the cupric ion activity, and how much is due to a genuine physiological antagonistic effect at the cellular level. In a recent study of the adsorption of copper on hydrous iron oxide in seawater (48), it has been observed that under conditions similar to the experiments of Figure 4-7, iron adsorbs copper up to a Fe/Cu molar ratio of 1/3. Adsorption can then certainly account for all of the antagonistic effects in the *Pyramimonas* experiment. What becomes more difficult to explain is the lack of antagonistic effect at the low iron concentration (1.2 x 10^{-6}M) since even then the highest copper concentration (4.4 x 10^{-7}M) should be entirely adsorbed. Note, however, that this is a domain of concentrations where copper starts saturating the colloidal iron surface, and there must be a titration effect



Cu \ Fe	1.2×10^{-5} M		1.2×10^{-6} M
Control 4×10^{-8} M	□	○	△
1.2×10^{-7} M		●	▲
4.4×10^{-7} M	■		▼
	Expt. #1	Expt. #2	Expt. #3

- Experiments #1,2,3 done with inocula from different cultures
- No EDTA

Figure 4-7. Antagonistic effects of iron and copper on the growth rate of Pyramimonas 1 in artificial seawater medium with the usual supplements of F/2 medium except for EDTA which is not added.

Note: Experiments 1, 2, and 3 represent inocula from different cultures. "Fresh" iron is $FeCl_3$ solution prepared the day of the experiment and sterilized by filtration.

(like that of copper on EDTA in Figure 4-3) where the cupric ion activity increases rapidly with increasing copper concentration. Exact quantitification of this phenomenon awaits a better mathematical description of adsorption processes on hydrous iron oxides in seawater. Despite great recent advances in the modeling of adsorption in aqueous systems (50, 42, 15), it is still the least quantifiable chemical process in thermodynamic calculations. The presence of precipitates in a culture medium modifies its global trace metal chemistry to an unpredictable degree. This creates the most common difficulty in interpreting experiments on toxicity of metals to a variety of organisms. Note that adsorption on the walls of a glass culture vessel is equally hard to predict. Choice of container material which minimizes adsorption of solutes is critical to the design of trace metal toxicity experiments.

Adsorption on the surface of algal cells can also be important for the trace metal chemistry of the medium in dense cultures. There is, however, no practical way to distinguish it from intracellular uptake. The effects of cellular uptake processes including adsorption on the cell surface, have been discussed earlier.

INDIRECT CHEMICAL EFFECTS

The general principles of coordination, precipitation, and adsorption which have been discussed heretofore, are readily understood and their importance in toxicity studies is usually recognized. What is less often perceived is the global interdependency of the chemistry of culture media, the indirect interactions (43, 27). For example, upon variations in the total copper concentrations, it is natural to relate the observed effects to changes in the cupric ion activity. However, as illustrated in Figure 4-8, activities of the zinc and ferric ions are also increased when the total copper is augmented in Aquil. Conceivably, any or all of these increased activities could be responsible for the observed effects. It is then a difficult choice to either maintain all metal activities constant by *adhoc* modification of all analytical concentrations -- a method which multiplies the work for medium preparation and can create other interpretative ambiguities -- or to perform the multitude of necesary controls on an already arduous series of experiments. Table 4-3 shows how the total metal concentrations have to be varied concomitantly with that of copper, to vary exclusively the cupric ion activity in Aquil with two EDTA concentrations (35).

The indirect interactions illustrated in Figure 4-8 are almost exclusively mediated by EDTA, which chelates all the interdependent metals. In principle, a convenient way to avoid the complications created by these interactions is to reduce them to a minimum. This can be achieved by uncoupling the system using more specific complexing agents. Figure 4-8 shows how the metal

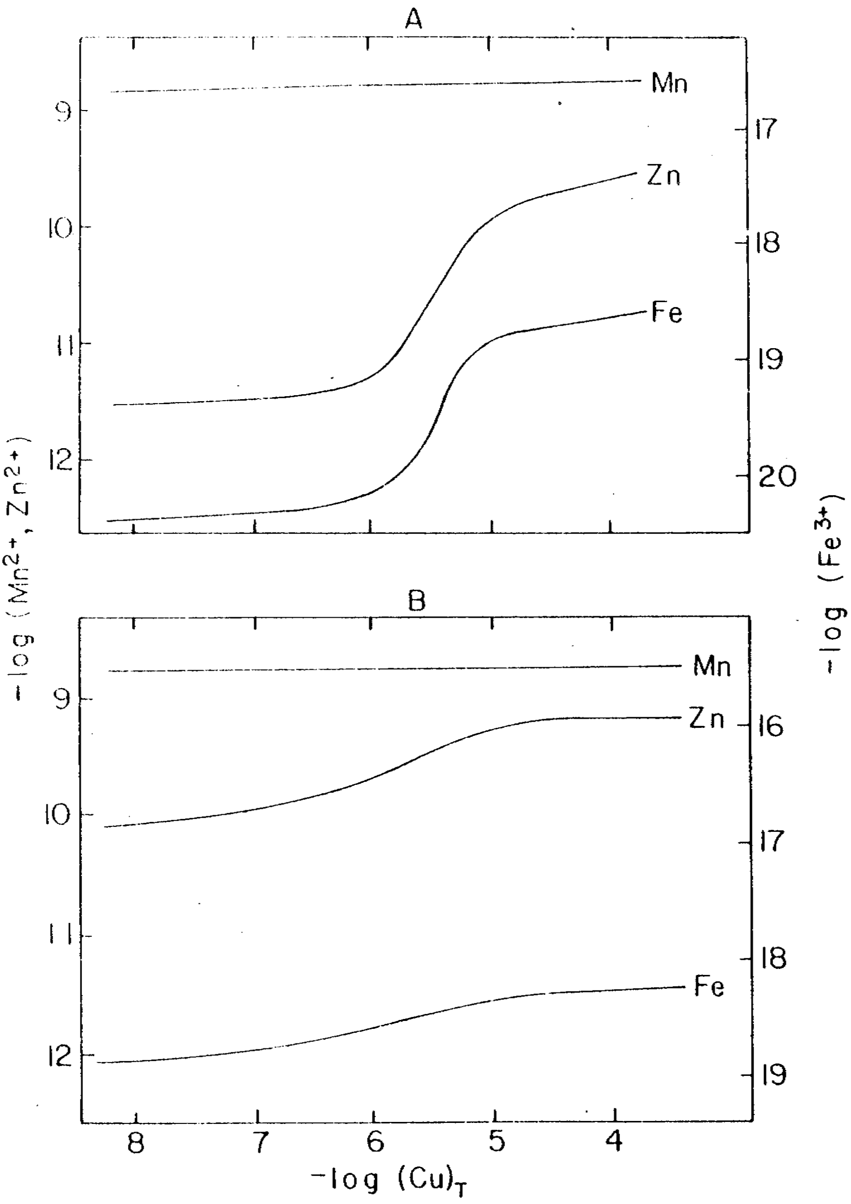


Figure 4-8. Variations in metal activities (M) of manganese, zinc and iron with total copper concentration.

Note: A) Aquil medium, $10^{-5.3}$M EDTA; B) Aquil with $10^{-6.3}$M EDTA, 10^{-3}M Tris.

Table 4-3. Calculation of Total Metal Concentrations Needed to Change the Cupric Ion Activity in Aquil While Maintaining the Other Metal Activities Constant
(-log (concentration) or [activity], M)

$(EDTA)_T$	$[Cu^{2+}]$	$(Copper)_T$	$(Iron)_T$	$(Mang)_T$	$(Zinc)_T$	$(Cobalt)_T$
3.3	8.5	3.30	7.00	7.63	8.7	8.6
	10.9	3.70	4.72	6.40	6.49	6.7
	11.3	4.0	4.6	6.30	6.40	6.6
4.3	9.8	4.35	6.45	7.20	8.30	8.49
	10.9	4.70	5.72	7.20	7.49	7.7
	11.3	5.00	5.58	7.15	7.4	7.6

activities vary with total copper in modified Aquil medium containing "Tris", a ligand known to chelate mostly copper (44). Upon variations in copper concentration, the other metals are seen to have a much more constant activity in Aquil with Tris than in Aquil with only EDTA.

One of the principal ways by which indirect chemical effects can be initiated is through pH variations. For example, pH has an indirect effect on metal complexation due to the acid-base properties of the coordinating ligands. Figure 4-9 illustrates this effect for Mn, Cu and Zn in Aquil, with EDTA and Aquil with Tris. In this case, Tris mediates a much greater indirect effect than EDTA: Zinc and especially cupric ion activities are markedly depressed by increasing pH in the Tris medium, while the activities of all three ions remain essentially constant in the EDTA medium. Increases in pH, which can be brought about by photosynthetic carbon uptake if the aeration of the culture is insufficient, can also result in precipitation as illustrated in Figure 4-6. Adsorption on the fresh precipitate will follow, resulting in an unquantified decrease in the soluble concentration of trace metals. It is apparent that pH is a major factor in determining directly and indirectly the activity and toxicity of trace metals, and should be monitored regularly in metal toxicity experiments.

CONCLUSION

The chemistry of metals in the external milieu of algal cells is only one of the determinants of their toxicity. The literature on bacteria and higher cells abounds with examples of how the sensitivity of a particular strain or clone to a particular toxicant, depends markedly on the physiological status of the cells (9, 4). Although it is often recognized that the same situation should apply to phytoplankton, this concept has received scant attention in recent algal literature. It stands to reason that the previous history of an algal cell, its

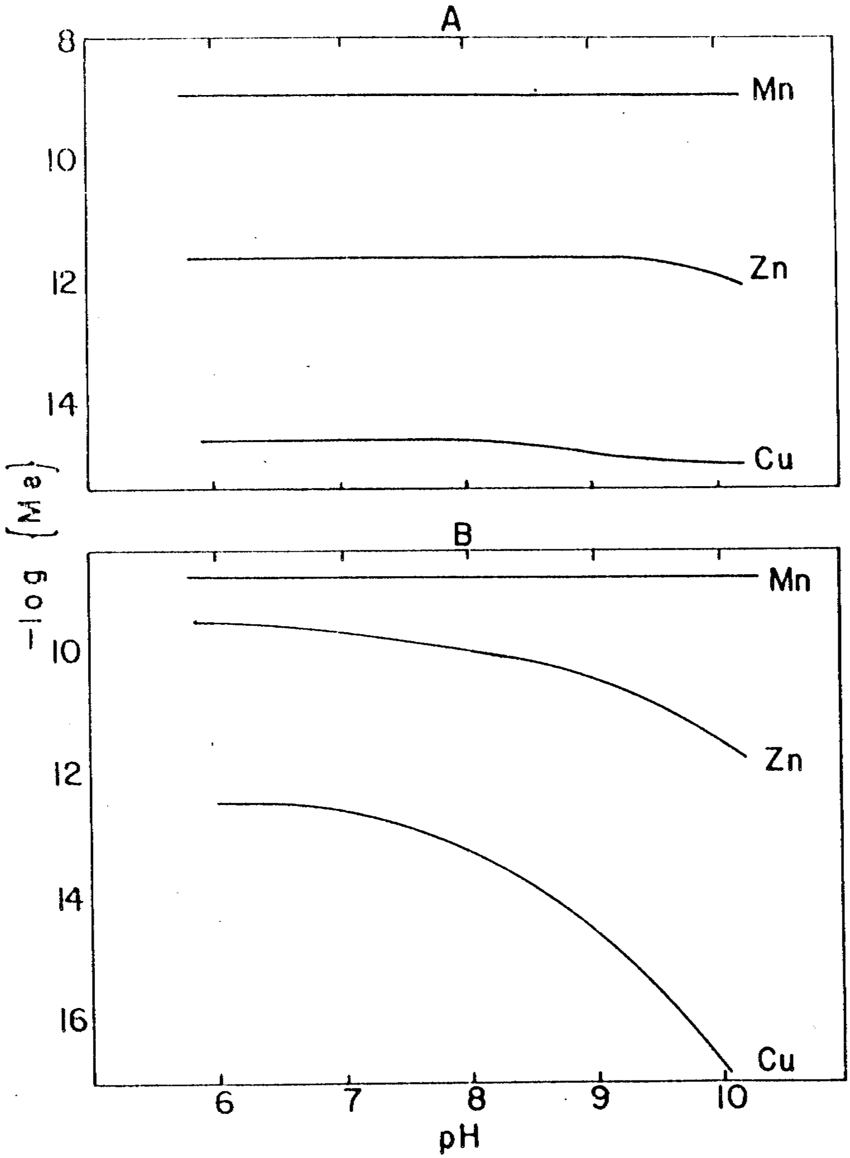


Figure 4-9. Variations in metal activities with pH.

Note: A) Aquil medium, $10^{-5.3}$M EDTA; B) Aquil with $10^{-6.3}$M EDTA, 10^{-3}M Tris.

nutritional status, and the particular phase of the cell cycle during which the experiment is conducted to name but a few obvious determinants of physiological status must affect its sensitivity to trace metals. Batch culture experiments which, so far, have been used principally for metal toxicity studies, have inherent restrictions to resolve the importance of these physiological factors. Toxicity studies in continuous phytoplankton cultures promise to be enlightening in this respect; they also promise to accentuate the difficulties in controlling precisely the chemistry of the system.

It is hoped that the conceptual framework presented here will help in designing and interpreting experiments where physiological responses to trace metal toxicity are clearly assessed, distinctly from purely chemical effects in the growth medium. It is also hoped that this study will help to increase phytoplankton physiologists' awareness of the important chemical processes which can affect their studies: It is, for example, surprising that so little attention has been paid to the possible importance of phosphate speciation in nutrient uptake experiments. Understanding the ecology of phytoplankton requires detailed resolution of the cells' physiological responses to the total aquatic chemistry of their environment.

ACKNOWLEDGMENTS

We thank S.W. Chisholm for her critical review of the manuscript and R.C. Selman for her excellent job in typing the manuscript. This work was funded by National Science Foundation grant no. DES75-15023, Environmental Protection Agency grant no. R-803738 and the office of Sea Grant in the National Oceanic and Atmospheric Administration grant no. 04-6-158-4407.

REFERENCES

1. Anderegg, Von G., F.L'Eplattenier, and G. Schwarzenbach. 1963. Hydroxamatkomplexe (III^1). Eisen (III)-Austausch zwischen Sideraminen und Komplexonen, Diskussion der Bildungskonstanten der Hydroxamatkomplexe. Helv. Chim. Acta. 46:1409-1422.

2. Anderson, D.M. and F.M.M. Morel 1978. Copper Sensitivity of *Gonyaulax tamarensis*, Limnol. Oceanogr. (in press).

3. Andrew, R. W., K.E. Biesinger, and G.E. Glass. 1976. Effects of Inorganic Complexing on the Toxicity of Copper to *Daphnia Magna*, Water Res. 11:309-315.

4. Baird-Parker, A.C., and R. Holbrook 1971. In: Inhibition and Destruction of the Microbial Cell (ed.), W.B. Hugo, Acad. Press.

5. Barber. R.T., R.C. Dugdale, J.J. MacIsaac, and R.L. Smith. 1971. Variations in Phytoplankton Growth Associated with the Source and Conditioning of Upwelling Water, Inv. Peso. 35(1):171.

6. Barber, R.T. 1973. In: Trace Metals and Metal-Organic Interactions in Natural Waters, P. C. Singer (ed.), Ann Arbor Science, Mich.

7. Davey, E.W., M.J. Morgan, and S.J. Erickson. 1973. A Biological Measurement of Copper Complexation Capacity of Seawater, Limnol. Oceanogr. 18:993-997.

8. Droop, M.R. 1961. Some Chemical Considerations in the Design of Synthetic Culture Media for Marine Algae. Botanica Marina. 2:231.

9. Farwell, J.A., and M.R. W. Brown 1971. In: Inhibition and Destruction of the Microbial Cell (ed.), W.B. Hugo, Acad. Press.

10. Gächter, R., K. Lum-Shue-Chan, and Y.K. Chau. 1973. Complexing Capacity of the Nutrient Medium and its Relation to Inhibition of Algal Photosynthesis by Copper, Schweizerische Zeitschrift für Hydrologie. 35:252-260.

11. Guillard, R.R.L., and J. Lorenzen. 1972. Yellow-Green Algae with Chlorophyllide-C, J. Phycol. 8:10-14.

12. Guillard, R.R.L., and J.H. Ryther. 1962. Studies on Marine Planktonic Diatoms I. *Cyclotella nana* Hustedt and *Detonula Confervacea* (Cleve) Gran, Can. J. Microbiol. 8:229-239.

13. Hansen, E.H., C.G. Lamm, and J. Rizicka 1972. Anal. Chim. Acta. 59:403.

14. Jackson, G.A., and J.J. Morgan 1977. Trace Metal-Chelator Interactions and Phytoplankton Growth in Seawater Media, Limnol. Oceanogr. (in press).

15. James, R.O., and T.W. Healey. 1972. Adsorption of Hydrolyzable Metal at the Oxide-Water Interface, J. Colloid and Interface Sci. 40:42.

16. Jensen, A., B. Rystad, and S. Melsom. 1976. Heavymetal Tolerance of Marine Phytoplankton II. Copper Tolerance of Three Species in Dialysis and Batch Cultures, J. Exp. Mar. Biol. Ecol. 22:249-256.

17. Johnston, R. 1964. Seawater, the Natural Medium of Phytoplankton. II. Trace Metals and Chelation, and General Discussion, J. Mar. Biol. Assoc. U.K. 44:87-109.

18. Jones, G.E. 1967. Precipitate from Autoclaved Seawater, Limnol. Oceanogr. 12:165-167.

19. Lange, Willy. 1974. Chelating Agents and Blue-Green Algae, Can.J. Microbiol. 20:1311-1321.

20. Lockhart, H.B., Jr., and R.V. Blakeley. 1975. Aerobic Photodegradation of Fe(III)-(Ethylenedinitrilo) tetraacetate (Ferric EDTA), Environ. Sci. and Technol. 9:1035-1038.

21. McDuff, R.E., and R.M. Morel. 1972. T.R. #EA-73-02, Keck Laboratories, California Institute of Technology, Pasadena, Calif.

22. McKnight, D.M. Unpublished results.

23. McNeel, T. Unpublished results.

24. Menzel, D.W., E.M. Hulbert, and J.H. Ryther. 1963. Effects of Enriching Sargasso Sea Water on the Production and Species Composition of the Phytoplankton, Deep-Sea Res. 10:209-219.

25. Morel, F.M., and Morgan, J.J. 1972. A Numerical Method for Coupling Equilibrium in Aqueous Chemical Systems, Envir. Sci. and Technol. 6:58.

26. Morel, F.M., J.C. Westall, J.G. Rueter, and J.P. Chaplick. 1975. Description of the Algal Growth Media "Aquil" and "Fraquil", Technical Note No. 16, Water Quality Lab., Ralph M. Parsons Laboratory for Water Resources and Hydrodynamics, Dept. of Civil Engineering, Mass. Instit. of Technol., Cambridge, Mass.

27. Morel, F.M., R.E. McDuff, and J.J. Morgan. 1973. In: Trace Metals and Metal-Organic Interactions in Natural Waters, Interactions and Chemostatis in Aquatic Chemical Systems, P.C. Singer (ed.), Ann Arbor Sci.

28. Morel, N.M.L., and F.M. Morel. 1976. Lag Phase Promotion in the Growth of *Pyramimonas* 1 by Manipulation of the Trace Metal Chemistry of the Medium, Technical Note No. 17, Water Quality Lab., Ralph M. Parsons Laboratory for Water Resources and Hydrodynamics, Dept. of Civil Engineering, Mass. Instit. of Technol., Cambridge, Mass.

29. Morel, N.M.L., J.G. Rueter, and F.M. Morel. 1977. Copper Toxicity to *Skeletonema Costatum*, J. Phycol. (in press).

30. Murphy, T.P., D.R.S. Lean, and C. Nalewajko. 1976. Blue-Green Algae: Their Excretion of Iron Selective Chelators Enables Them to Dominate Other Algae, Science 192:900-902.

31. Pagenkopf, G.K., R.C. Russo, and R.V. Thurston. 1974. Effect of Complexation on Toxicity of Copper to Fishes, J. Fish. Res. Bd. Can. 31:462-465.

32. Provasoli, L., J.J.A. McLaughlin, and M.R. Droop. 1957. The Development of Artificial Media for Marine Algae, Arch. Mikrobiol. 25:392-428.

33. Ringbom, A. 1963. Complexation in Analytical Chemistry, Interscience.

34. Ross, J.W. 1969. In: Ion Selective Electrodes, R. Durst (ed.), Nat. Bur. Standards (USA) Spec. Pub. 314.

35. Rueter, J.G. 1977. The Response of *Skeletonema costatum* to Copper Relating to the Question of Medium Conditioning, Master's Thesis, Mass. Instit. of Technol., Cambridge, Mass.

36. Sillen, L.G., and A.E. Martell. 1964, 1971. Stability Constants, Special Publication, No. 17 and No. 25, The Chem. Soc. of London.

37. Smayda, T.J. 1974. Bioassay of the Growth Potential of the Surface Water of Lower Narragansett Bay over an Annual Cycle Using the Diatom *Thalassiosira pseudonana* (Oceanic Clone, 13-1), Limnol. Oceanogr. 19:889-901.

38. Smith, R.M., and A.E. Martell. 1976. Critical Stability Constants, Vol. 4, Inorganic Complexes, Plenum, N.Y.

39. Steemann Nielsen, E., And S. Wium-Anderson 1971. The Influence of Cu on Photosynthesis and Growth in Diatoms, Physiol. Plant. 24:480-484.

40. Stolzberg, R.J., and D.N. Hume 1975. Rapid Formation of Iminodiacetate from Photochemical Degradation of Fe(III) Nitrilotriacetate Solutions, Environ. Sci. and Technol. 9:654.

41. Stumm, W. 1969. Personal Communication to Barber, Referenced in (1973) Barber, Trace Metals and Metal-Organic Interactions in Natural Waters, P.C. Singer (ed.), Ann Arbor Science, Ann Arbor, Mich.

42. Stumm, W., Herbert Hohl, and Felix Dalang 1976. Interaction of Metal Ions with Hydrous Oxide Surfaces, Croatica Chemica Acta. 48:491-501.

43. Stumm, W., and J.J. Morgan 1970. Aquatic Chemistry, Wiley-Interscience, New York.

44. Sunda, W.G. 1975. The Relationship Between Cupric Ion Activity and the Toxicity of Copper to Phytoplankton, Doctoral Thesis, Woods Hole Oceanographic Instit., Woods Hole, Mass.

45. Sunda, W.G., D.W. Engel, and R.M. Thuotte 1978. Cadmium Toxicity to the Grass Shrimp, *Palaemonetes pugio*, as a Function of Free Cadmium Ion Concentration (to be published in Envir. Sci. and Technol.).

46. Sunda, W.G., and R.R.L. Guillard 1976. Relationship Between Cupric Ion Activity and the Toxicity of Copper to Phytoplankton, J. Mar. Res. 34:511-529.

47. Swallow, K.C. 1977. Adsorption of Trace Metals by Hydrous Ferric Oxide in Seawater, Ph.D. Thesis, Dept. of Chemistry, Mass. Instit. of Technol., Cambridge, Mass.

48. Swallow, K.C., J.C. Westall, D.M. McKnight, N.M.L. Morel, and F.M. Morel 1977. Potentiometric Determination of Copper Complexation by Phytoplankton Exudates, Limnol. Oceanogr. (in press).

49. Westall, J.C., J.L. Zachary, and F.M. Morel 1976. MINEQL, a Computer Program for the Calculation of Chemical Equilibrium Composition of Aqueous Systems, Technical Note No. 18, Water Quality Lab., Ralph M. Parsons Laboratory for Water Resources and Environmental Engineering, Dept. of Civil Engineering, Mass. Instit. of Technol., Cambridge, Mass.

50. Yates, D.E., S. Levine, and T.W. Healey 1974. Site Binding Model of the Electrical Double Layer at the Oxide/Water Interface, Far Soc. I., J. Chem. Soc. 70:1802.