Chapter 9

PHYSICO-CHEMICAL PROCESSES AFFECTING COPPER, TIN AND ZINC TOXICITY TO ALGAE: A REVIEW

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9.1 INTRODUCTION

Algal biofouling results from uncontrolled and undesirable algal growth on a surface that has usually been created by and for humans. A comprehensive understanding of this phenomenon requires the ability to quantify nutrient and toxicant interactions among that surface, the algae and the surrounding solution. This goal is complicated by the fact that highly interdependent chemical, biological and physical processes are involved. The discussion presented herein represents a brief review of how various chemical processes, listed as topic headings, affect and are affected by biological processes. These processes, which may interactively affect the availability and hence toxicity of solutes, include: precipitation-dissolution, adsorption-desorption, complexation-dissociation, oxidation-reduction and photoactivation. Each will be discussed with an emphasis on quantifying process effects on algal response. Examples from the literature involving copper (Cu) and zinc (Zn) toxicity have been chosen, because of the well-documented inhibitory effects and common algicidal applications of these two elements. Toxicity of tin (Sn), an antifouling agent used in the form of organic complexes, is also briefly discussed. Some background information is initially given on the relationship between chemical speciation and algal toxicological response in order to establish the importance of discussing the topics in this chapter. Temporal and light effects are mentioned in terms of adaptation mechanisms, reaction kinetics and photochemistry. However, physical processes, which may be important, are not addressed (e.g., effects of temperature and flow regime.) The reader is directed to reviews by Mauersberger (1983) and Bowie et al. (1985) on this topic.

Both Cu and Zn are essential algal micronutrients (Wiessner, 1962; Brand et al., 1983), cofactors in numerous biochemical processes (Lehninger, 1975). Yet, submicromolar activities of these essential elements may inhibit algal growth (Petersen, 1982; Kuwabara, 1985; Brand et al., 1986). Gavis et al. (1981) presented results of an extensive Cu toxicity study using 24 clones of 11 marine phytoplankton species. They reported pCu (negative logarithm of free Cu^{2+} activity) threshold values of typically 8.7 and 11.5. For both Cu and Zn the uncomplexed metal ion seems most readily available to algae (Manahan and Smith, 1973; Sunda and Guillard, 1976; Anderson et al., 1978; Petersen, 1982).

Rueter et al. (1981) suggested a toxicity mechanism for diatoms in which Cu^{2+} inactivates the silicic acid ($Si(OH)_4$) transport site. Intracellular Cu also inhibits diatom growth, resulting in accumulation of intracelluar Si even though Si uptake is inhibited. Cu also adversely affects growth of algae in general by affecting cell membrane permeability as demonstrated by large increases in cell volume and accelerated cell potassium release (McBrien and Hassall, 1965; Riisgard et al., 1980; Rai et al., 1981; Kuwabara and Leland, 1986). Evidence from Zn toxicity experiments indicates that uncomplexed Zn interferes with efficient intracellular phosphate utilization (Bates et al., 1982; Kuwabara, 1985). Bates et al. (1982) hypothesized that Zn accumulates in intracellular polyphosphate bodies until reaching some threshold level, at which point phosphate metabolism ceases. Zn generally appears to be a less effective algicide than Cu, although sensitivity to these elements varies considerably among species (Gavis et al., 1981; Petersen, 1982; Kuwabara, 1985).

Under pH conditions typical of most natural waters (pH 6.5-8.5), chemical speciation of Cu(II) is usually dominated by hydrolysis products or carbonate complexes. Although total dissolved Cu concentrations reported for unpolluted marine and freshwaters are in the nanomolar range (Brewer, 1975; Leland and Kuwabara, 1985), free ion activites are three or more orders of magnitude lower (10^{-11}-10^{-13} $\underline{M}$). When high concentrations of dissolved organic carbon (DOC) are present (> 10 mg/L), Cu free ion activities and hence bioavailability may be further decreased due to chelation (Mantoura et al., 1978; Kremling et al., 1981; Wood, 1983; Wood et al., 1983). Like Cu, nanomolar dissolved concentrations are typical for background Zn in natural waters. However, uncomplexed Zn(II) can be the predominant species in water with low DOC. Other important inorganic complexes include $ZnOH^+$, $ZnCO_3^0$, $ZnCl^+$, $ZnCl_2^0$ and $ZnSO_4^0$. A comprehensive review of Zn cycling in the coastal environment has been presented by Young et al. (1980).

It is clear from the toxicological mechanisms discussed above that an ability to provide an accurate description of Cu and Zn speciation and distribution in natural waters enables improved predictions for responses to algal inhibitory treatments. The discussion presented below of physico-chemical processes that regulate solute speciation and distribution is sectionalized by process to enhance readability. It is hoped, however, that this format does not cause one to lose sight of the fact that these processes operate interactively in nature and should be modeled accordingly.

9.2 PHYSICO-CHEMICAL PROCESSES

9.2.1 Precipitation-dissolution

The availability of a nutrient or toxic substance can be significantly affected by precipitation (Anderson and Morel, 1982; De Haan et al., 1985). Furthermore, formation of precipitates can then affect dissolved concentrations of other solutes by adsorption, a subject which will be discussed in a later section of this paper. Precipitation-dissolution reactions are generally quantified by a solubility product (K_{so}) as shown below:

$$\mathrm{Me}_i\mathrm{L}_j(s) \rightarrow i(\mathrm{Me}) + j(\mathrm{L}) \qquad \text{(dissolution reaction)} \quad (1)$$

$$K_{so} = (\gamma_{\mathrm{Me}}[\mathrm{Me}])^i(\gamma_L[\mathrm{L}])^j \qquad \text{(solubility product)} \quad (2)$$

Equation 1 represents the dissolution of a solid composed of i moles of metal Me and j moles of ligand L. Equation 2 defines the activities of Me and L ions when controlled by the dissolution of the solid. Activity coefficients for the metal (γ_{Me}) and the ligand (γ_L) may be determined by the Davies Equation for ionic strengths less than 0.5 $\underline{\mathrm{M}}$ (Stumm and Morgan, 1981) or by Pitzer Equations for ionic strengths equivalent to seawater and higher (Whitman, 1975). The simple case described above may be extended for a solid composed of more than one metal or ligand. For example, the dissolution of malachite ($Cu_2(OH)_2CO_3(s)$) under acidic conditions, is defined by:

$$K_{so} = 10^{14.16} = (\gamma_{Cu}[Cu^{2+}])^2 P_{CO_2}(\gamma_H[H^+])^{-4} \qquad \text{(Schindler,1967)} \quad (3)$$

It has been noted that inorganic tin (Sn) is not an effective algicide relative to organotin complexes that are commonly used in antifouling paints (Hallas and Cooney, 1981; Wong et al., 1982). This result may be due to the low solubility of Sn(IV) in water. A pSn(IV) of approximately 20 is predicted from solubility data (Hogfeldt, 1982) for a system controlled by Sn oxide at pH 7. Chelation, photochemical and redox processes, to be discussed in later sections, appear to be more important in controlling Sn toxicity. Numerous computer programs (Westall et al., 1976; Felmy et al., 1983) are available to determine whether a solubility product is exceeded (i.e., whether formation of a precipitate is thermodynamically favorable.) The applicability of such equilibrium computations is dependent on the accuracy of the thermodynamic data base, the validity of the analytical measurements, and the importance of reaction kinetics over the time scales of interest (Pankow and Morgan, 1981).

9.2.2 Adsorption-desorption

Like precipitation-dissolution reactions, adsorption-desorption reactions affect solute bioavailability through interactions of solid and aqueous phases. Kuwabara et al. (1986) demonstrated a dramatic difference between availability of dissolved and particulate-bound forms of both Zn and orthophosphate to a freshwater chlorophyte. Partitioning coefficients are often used in field studies to quantify solute distribution between dissolved and particulate phases (Ambrose et al., 1983). Yet recent research has pointed out limitations to the applicability of a partitioning coefficient approach for determining solute availability to algae. Many factors affect solute adsorption and desorption, and hence tend to make partitioning coefficient data site specific. These factors include:

1. Surface minerology - Oxide and clay surfaces react very differently with different solutes (Hart, 1982; Wauchope and McDowell, 1984),
2. Sediment particle size - Smaller particles generally have a higher adsorption site density (i.e., sites per unit mass) (Onishi et al., 1982),
3. Reactions involving particulate and dissolved organic matter - Dissolved organic material may form strong chelates with the solute or form reactive organic films around inorganic particles (Toledo et al., 1982; Luoma and Davis, 1983),
4. Chemical speciation - Factors like pH and ionic strength affect chemical speciation and the reactivity of an adsorbent. If adsorption sites are limited, competition between adsorbates can occur (Benjamin and Leckie, 1980),
5. Reaction kinetics - Rapid desorption as a result of changing chemical environment can effectively buffer dissolved solute concentrations and thus enhance bioavailability (Kuwabara et al., 1986). Kinetics may also be dependent on physical processes like diffusive mass transfer rates (i.e. solute velocity to surface reaction sites).
6. Interdependence with biological processes - Some photosynthesizing algae can create a high pH microenvironment (> 2 pH units above the bulk solution) that can decrease availability of metals like Cu and Zn while increasing anion (e.g., phosphate) availability (Revsbech et al., 1984; Kuwabara et al., 1986).

The algal cell surface has been recently studied as a trace metal adsorbent (Crist, 1981). Fisher et al. (1984) found that uptake of trace metals by marine phytoplankton was initially a passive sorption process. Our recent studies (unpublished) indicate that active uptake of Zn and orthophosphate by the chlorophyte _Selenastrum capricornutum_ is not significant over the first

24 h. of exposure. While uptake of orthophosphate (10 uM total P) by heat-killed Selenastrum cells after 24 h. was nearly 100% efficient over a pH range of 6.5 to 8.5, Zn uptake (1 uM total Zn) increased from 30 to 70% over the same pH range (Fig. 1). These results suggest that the algal cell surface can successfully compete with inorganic surfaces for dissolved nutrients and toxic substances within this environmentally significant pH range.

Methods for modeling the effects of adsorption-desorption reactions have been previously reviewed (Onishi et al., 1982; Kuwabara and Helliker, 1986). Operational methods for determining biological availability of particulate-bound metals have also been presented and reviewed (Luoma and Jenne, 1976; Tessier et al., 1984).

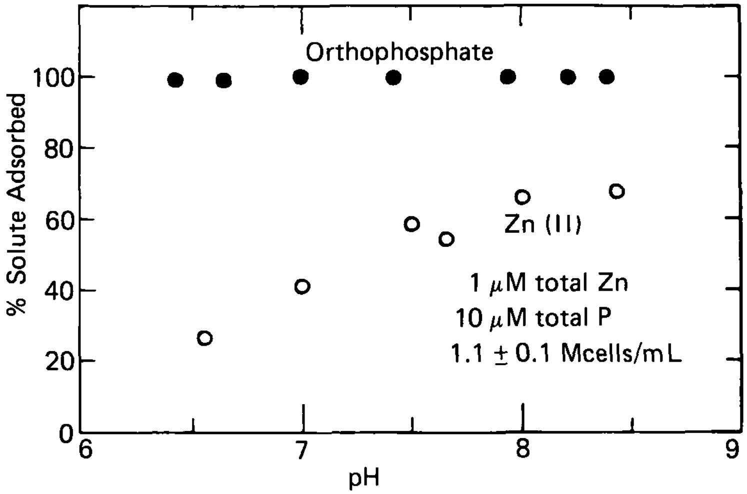

Fig. 9.1. Effects of culturing media pH on Zn(II) and orthophosphate adsorption by heat-killed Selenastrum cells. A $1.1 \pm 0.1 \times 10^6$ cells/mL concentration was used for both experiments with 1 uM and 10 uM total Zn or orthophosphate, respectively.

9.2.3 Complexation-dissociation

It was previously noted that the free ion forms of Cu and Zn appear to be most available to algae. Complexation and dissociation reactions therefore represent an important process regulating availability of these solutes. An equilibrium constant (K), analogous to the precipitation-dissolution K_{so}, describes the association of i moles of metal (Me) and j moles of ligand (L) that form complex Me_iL_j.

$$i(Me) + j(L) \rightarrow Me_iL_j \tag{4}$$

$$K = (\gamma_{complex}[Me_iL_j])/(\gamma_{Me}[Me])^i(\gamma_L[L])^j \tag{5}$$

The activity coefficients (γ_{Me}, γ_L, $\gamma_{complex}$) may be estimated from the same equations cited previously for precipitation reactions. Though chemical equilibrium programs can be helpful in modeling speciation of solutes (Westall et al., 1976; Felmy et al., 1983), field applicability of such computations is highly dependent on: (1) the accuracy of equilibrium constants used to model the particular environment, (2) the extent to which the sample represents the in-situ conditions, (3) and the degree to which kinetics of these reactions affects the departure from assumed equilibrium conditions. Consider for example, the discharge of inorganic Cu into an environment with dissolved organic matter. Though formation of strong Cu chelates and subsequent reduction of Cu availability to algae is thermodynamically favorable, Cu chelation may be suppressed by slow dissociation of existing metal-organic chelates. This kinetic effect, which enhances Cu availability, has been observed in several laboratory experiments (Anderson and Morel, 1978; Kuwabara and Leland, 1986).

Availability, and hence potential algal toxicity of Cu and Zn, may also be dependent on the availability of other solutes. The competition of Cu for $Si(OH)_4$ transport sites in marine diatoms (Rueter et al., 1981) has been previously discussed. Button et al. (1973) noted that manganese (Mn) contamination of phosphate stock solutions used in culture media reduced copper sensitivity of the marine yeast Rhodotorula rubra. Sunda et al. (1981) later proposed that for marine phytoplankton Cu interferes with manganese metabolism by competing with Mn for nutritional sites. Another type of interaction was reported by Rana and Kumar (1974) in which Zn toxicity was ameliorated by increasing orthophosphate concentrations. The mechanism of Zn interference with intracellular phosphate utilization has been mentioned earlier (Bates et al., 1982; Kuwabara, 1985). Increased availability of cadmium (Cd) also reduces Zn toxicity, but total Cd concentrations orders of magnitude above typical background concentrations were needed to induce this interaction (Braek et al., 1980).

While algae may be inhibited, they may also show adaptive mechanisms in response to elevated Cu and Zn ion activities. These include intracellular and cell wall exclusion mechanisms (Silverberg et al., 1976; Rai et al., 1981; Reed and Moffat, 1983) that may dramatically increase tolerance to these metals (Wikfors and Ukeles, 1982). It is clear therefore that the environmental history of an algal population is an important consideration in analysis or prediction of algal growth (Bates et al, 1983, Kuwabara and Leland, 1986).

One would also expect that under field conditions, algal community composition would change over the long term to adapt to changes in metal ion activity (Say and Whitton, 1980). Metal resistant algal strains have often been isolated from natural waters known or suspected to be impacted by heavy metal pollution (Gavis et al. 1981; Murphy et al., 1982). It should be noted that in contrast to the above reports of algal adaptation to Cu and Zn, marine diatoms do not appear to adapt to organotin biocides (Walsh et al., 1985).

Although the format of this paper focuses on interacting chemical processes that affect biological response, it should again be mentioned that biological processes in turn affect the chemistry of natural waters. Useful predictions of algal response require an understanding of the relative importance of these interactions (both magnitude and time dependence). For example, it has been shown that certain algal species release organic molecules that may significantly decrease biologically available Cu (McKnight and Morel, 1979; Van den Berg et al. 1979; Lumsden and Florence, 1983). In contrast, Swallow et al. (1978) found only one of nine test phytoplankton species that produced sufficient exudate to significantly complex Cu in a defined medium. However, it is not clear from these experiments of Swallow et al. (1978) if exudate production is stimulated in response to elevated toxicant concentration, because the algae were not directly exposed to Cu.

By solute uptake and release, algae can also affect water chemistry. Algal blooms represent the extreme example of this phenomenon. Button and Hostetter (1977) observed that both Cu uptake and release by algae are rapid, although uptake is considerably more rapid than the rate of Cu release (estimated first order rate coefficents of approximately $10^{-4}\ s^{-1}$ and $10^{-5}\ s^{-1}$, respectively). Similar results have been determined for marine algae by Mandelli (1969) and for periphyton in a mountain stream by Kuwabara et al. (1984).

9.2.4 Oxidation-reduction

Reactions involving electron transfer from an oxidant to a reductant are of significance to phycologists and aquatic toxicologists, because these reactions are often biologically mediated. Because redox reactions can be written in a manner similar to other chemical reactions, chemical speciation programs may be useful in estimating results of redox equilibria (Westall et al., 1976). Full reactions represent the combination of redox couples containing an electron acceptor and an electron donor as shown in the example below for the oxidation of ferrous to ferric Fe (Sillen and Martell, 1971; Hogfeldt, 1982):

$$Fe^{2+} \rightarrow Fe^{3+} + e^- \text{ ; equilibrium constant } K_o = 10^{-13.0} \qquad (6)$$

$(^1/_4)O_2(aq) + H^+ + e^- \rightarrow (^1/_2)H_2O$; equilibrium constant $K_r = 10^{20.8}$ (7)

Full reaction: $Fe^{2+} + (^1/_4)O_2(aq) + H^+ \rightarrow Fe^{3+} + (^1/_2)H_2O$; (8)

equilibrium constant $K = K_o K_r =$

$$(\gamma_{Fe(III)}[Fe^{3+}])(\gamma_{Fe(II)}[Fe^{2+}])^{-1}(\gamma_H[H^+])^{-1}p_{O_2}^{-1/4} = 10^{7.8}. \quad (9)$$

It is again stressed, however, that an assumption of thermodynamic equilibrium may be a distinct limitation for certain solutes. Reactions like the one above or biologically-mediated redox processes may be kinetically controlled. For example, the fact that the photic zone in most aquatic environments is usually well oxygenated might lead one to assume that reduced states of solutes would not be present at significant concentrations. In the case of Cu, this assumption is generally true; Cu exists primarily in the divalent state in oxidizing environments. Zn does not participate in redox processes under these conditions and always is present as Zn^{2+}. Tin exists primarily as Sn (IV) in aerobic environments in both inorganic and organic forms. Concentrations of these Sn(IV) species are typically in the ng/L (of the order of 10^{-10} $\underline{M}$) range (Braman and Tompkins, 1978). Biological methylation has been proposed as an explanation for the ubiquitous presence of methyltin compounds in the environment (Ridley et al, 1977; Braman and Tompkins, 1978; Maguire et al., 1982). In contrast to Cu, Zn and Sn, there are a number of elements for which the above assumption may be misleading. Through photosynthesis, organic molecules (reduced states) are produced from oxidized nutrients. Algae are involved in the reduction of inorganic arsenic (As) species to methylated As complexes (Andreae, 1978). Immobilization reactions involving the reduction of toxic selenate and selenite species can also be biologically mediated (Doran, 1982). Consequently, deviation from redox equilibrium may partially be related to the rates of these biological processes. Ambrose et al. (1983) modeled the concentrations of reactants and product for a solute oxidation reaction in the following manner:

$$\frac{d[\text{oxidized product}]}{dt} = K_{redox}[\text{oxidant}][\text{reductant}] \quad (10)$$

where K_{redox} = a second order rate constant that is process dependent.

9.2.5 Photoactivation

Photochemical reactions represent a biologically significant subset of redox processes that affect trace metal availability by the conversion of light to chemical energy. Methods for including photochemical reactions in solute transport equations have been reviewed (Jorgensen, 1983). Cu(II) complexes are primarily photosensitive to radiation in the ultraviolet wavelengths. A detailed review of Cu photooxidation-reduction reactions involving various ligands has been presented by Balzani and Carassiti (1970). The effect of photochemical reactions on Cu bioavailability in aquatic systems has not yet been determined. Cu may exist in water in forms other than the divalent state. In moderately reducing environments, Cu_2O can control Cu solubility at pH 7 and in reduced low-sulfur environments, metallic Cu is stable (Garrels and Christ, 1965). The unstable Cu^{+} ion disproportionates into Cu^{2+} and Cu^{0}. Monovalent Cu species could possibly be stabilized by complexation with organic ligands, although photochemical processes would more likely degrade the organic ligands. In their review, Balzani and Carassiti (1970) also pointed out a lack of photochemical information for Zn complexes. In that Zn has a much lower susceptibility toward redox processes, Zn(II) is the only stable oxidation state in the water domain.

Photoreduction and dissolution of Mn oxides by dissolved humic substances has been proposed as an important factor regulating dissolved Mn distribution in seawater (Sunda et al., 1983). The authors suggest that the typically-observed surface maximum in dissolved Mn concentrations in the sea is due to riverine and aeolean inputs and photoactivated reduction of Mn(IV) oxides to Mn^{2+}. Both these processes are worth consideration when assessing Cu toxicity to coastal algal populations because of the proposed Cu-Mn interaction on algal growth mentioned previously (Sunda et al., 1981) and because adsorbed Cu and Zn may be released into solution upon dissolution of Mn oxides. Availability of Cu and Zn may therefore be indirectly affected by photochemical and redox processes.

Photodegradation of iron (Fe) chelates also enhances Fe uptake by algae. Anderson et al. (1982) observed that Fe uptake by Thalassiosira weissflogii in a chemically-defined medium dominated by FeEDTA complexation doubled in the presence of light (cool white spectrum, 95 uE m^{-2} s^{-1}). This photodegradation of the FeEDTA complex is contrasted by the insensitivity of the CuEDTA complex to photochemical reactions (Hill-Cottingham, 1955). Organotin compounds are also photosensitive, but Walsh et al. (1985) noted that the kinetics of the degradation reactions appear to be slow (of the order of months). Degradation products of trialkyl tin compounds have been detected in an area of heavy industrial and antifouling paint use (Hodge et al. 1979; Maguire et al., 1982). In contrast to the enhancement of Fe availability by photodegradation, degradation of organotin compounds would probably decrease Sn toxicity to algae. Recent algal studies (Laughlin and French, 1980; Wong et al., 1982; Walsh et al., 1985) found that toxicity of alkyltin compounds increased with carbon chain length; with butyl compounds generally being the most toxic. Toxicity was also directly related to solubility of the Sn compound in octanol and to its molar surface area (Laughlin et al., 1984). Therefore, unlike the availability of Cu and Zn free ion forms, the mechanism for Sn toxicity appears to reflect the strong partitioning in algal tissue of certain organic compounds.

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