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THE INFLUENCE OF COMPLEXATION AND pH ON INDIVIDUAL AND COMBINED HEAVY METAL TOXICITY TO A FRESHWATER GREEN ALGA

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• Environment Canada

EXECUTIVE SUMMARY

The Influence of Complexation and pH on Individual and Combined Heavy Metal Toxicity to a Freshwater Green Alga

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The effect of metal complexation and pH on heavy metal (Cu, Zn, Pb) toxicity to a freshwater alga, Scenedesmus quadricauda was investigated. It was observed that extracellular excretion produced by S. quadricauda was capable of binding heavy metals and reducing their single and combined toxicities. The effect of apparent complexing capacity of the medium and the ability of the sediment humics and artificial complexing agents such as ethylene-diaminetetraacetic acid (EDTA), citric acid and glycolic acid, to ameliorate Cu, Zn or Pb toxicity were also assessed. EDTA had the strongest power to protect the toxicity of Cu, followed by citric and glycolic acids. This order is not the same for other metals, for instance, for Zn, the citric acid complex is less toxic than the EDTA and glycolic acid complexes. The toxicity of metals to algal growth was generally enhanced at acidic pH. Combined toxicity of these metals was significantly greater at pH 4.5 than at pH 8.5 or pH 6.5. Synergistic effects (between Cu, Zn and Pb) towards algal growth increased at low pH. Specific heavy metals, their respective concentrtations, the presence of complexing ligands and pH, influence both individual and combined heavy metal toxicities.

SOMMAIRE ADMINISTRATIF

Effets de la formation de composés complexes et du pH sur la toxicité individuelle et combinée de métaux lourds pour une algue verte d'eau douce

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On a étudié les effets de la formation de composés métalliques complexes et du pH sur la toxicité de métaux lourds (Cu, Zn, Pb) pour une algue d'eau douce, Scenedesmus quadricauda. On a observé que des excrétions extracellulaires de S. quadricauda pouvaient lier des métaux entre eux et réduire ainsi leur toxicité individuelle et combinée. On a également évalué les effets de l'action complexante apparente du milieu ainsi que la capacité de l'humus sédimentaire et d'agents complexants artificiels comme l'acide éthylènediaminetétracétique (EDTA), l'acide citrique et l'acide glycolique, à réduire la toxicité du Cu, du Zn ou du Pb. On a découvert que l'EDTA était l'agent le plus efficace pour maintenir la toxicité du Cu, suivi de l'acide citrique et de l'acide glycolique. Il n'en allait pas de même pour les autres métaux : par exemple, le complexe acide citrique-Zn était moins toxique que les complexes EDTA-Zn et acide glycolique-Zn. L'effet toxique des métaux sur la croissance de l'algue augmentait généralement en milieu acide. La toxicité combinée des métaux était notablement plus élevée à un pH de 4,5 qu'à un pH de 8,5 ou 6,5. Les effets synergiques (du Cu, Zn et Pb) sur la croissance de l'algue augmentaient à faible pH. Les caractéristiques des métaux lourds, leur concentration respective, la présence de coordinats et le pH influençaient à la fois la toxicité individuelle et combinée des métaux lourds.

Influence de la formation de complexes et du pH sur la toxicité des métaux lourds individuellement ou en groupe pour une algue verte d'eau douce

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RÉSUMÉ

On a étudié l'effet de la formation de complexes et du pH sur la toxicité des métaux lourds (Cu, Zn, Pb) pour une algue verte d'eau douce, Scenedesmus quadricauda. Les ligands extracellulaires fabriqués par S. quadricauda ont la capacité de lier les métaux lourds et de diminuer leur toxicité individuelle ou combinée. On a également étudié l'effet de la formation apparente de complexes et la capacité des acides humiques dans les sédiments et celle d'agents artificiels aptes à former des complexes comme l'acide éthylènediaminetétraacétique (EDTA), l'acide critrique et l'acide glycolique, de diminuer la toxicité du cuivre, du zinc et du plomb. Les métaux inhibent davantage la croissance de l'algue à pH acide. La toxicité combinée de ces métaux est significativement plus élevée à pH 4.5 qu'à pH 8.5 ou 6.5. Les effets synergiques toxiques du cuivre, du zinc et du plomb augmentent à pH acide et diminuent ainsi la croissance des algues. La nature des métaux lourds, leur concentration respective, la présence des ligands pouvant former des complexes et le pH influencent la toxicité individuelle et combinée des métaux lourds.

The Influence of Complexation and pH on Individual and Combined Heavy Metal Toxicity to a Freshwater Green Alga

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ABSTRACT

The effect of complexation and pH on heavy metal (Cu, Zn, Pb) toxicity to a freshwater green alga, Scenedesmus quadricauda was investigated. Extracellular ligands produced by S. quadricauda were capable of binding heavy metals and reducing their single and combined toxicities. The effect of Apparent complexing capacities and the ability of the sediment humics and artificial complexing agents such as ethylenediaminetetraacetic acid (EDTA), citric acid and glycolic acid, to ameliorate Cu, Zn or Pb toxicity were also assessed. The toxicity of metals to algal growth was enhanced at acidic pH. Combined toxicity of these metals was significantly greater at pH 4.5 than at pH 8.5 or

pH 6.5. Synergistic effects (between Cu, Zn and Pb) towards algal growth increased at low pH. Specific heavy metals, their respective concentrations, the presence of complexing ligands and pH, influence both individual and combined heavy metal toxicities.

INTRODUCTION

Mobility, bioavailability and toxicity of metals are dependent on their physico-chemical forms in the aquatic environment (Alberts and Giesy, 1983; Giesy, 1983). Metal toxicity is influenced by binding to organics, precipitation, complexation and ionic interactions. For most metals, it is accepted that the free ionic form imparts toxicity. Unfortunately, most of the data reported in the literature quote toxicity with respect to total metal concentrations rather than individual species. In 1979 the United States Environmental Protection Agency noted that the "toxicity of certain compounds (including heavy metals) may be less in some waters because of differences in acidity, temperature, water hardness and other factors. Conversely some natural water characteristics may increase the impact of certain pollutants." The degree to which these abiotic factors influence toxicity is unclear (U.S. EPA, 1979).

Organic complexing agents in natural waters, although generally not as abundant as inorganic ligands, possess a strong affinity for heavy metals, expecially Cu (Borgmann, 1983). Stevenson and Ardakani (1972) and Stiff (1971) found that most metals are transported in natural waters as organic complexes. Hodson et al. (1979) suggest that chelation is the single most

important abiotic factor in the reduction of Cu toxicity in aquatic ecosystems. Many studies have revealed that both natural and synthetic chelators or metal-organic complexes alter the toxicity of metal ions to aquatic organisms (Bitten and Freihofer, 1978; Gadd and Griffiths, 1978; Gnassia-Barelli et al., 1978; Khobot'yer et al., 1975; Van den Berg et al., 1979). Metal organic complexes reduce the bioavailability and toxicity of heavy metals probably by decreasing the attraction between the chelated metals and the negative surface of the cell (Babich and Stotzky, 1983). Although binding of metals to organics or solids usually reduces metal toxicity, toxicity has been known to persist even when there are no free metallic ions present (Gadd and Griffiths, 1978).

Increased toxicity of metals to algae have been observed at alkaline pH (Steemann Nielsen and Kamp-Nielsen, 1970; Gachter, 1976; Hargreaves and Whitton, 1976 a,b; Peterson et al., 1984). Other authors report a general decrease in metal toxicity at high pH (Gachter, 1976; Monahan, 1976; Steemann Nielsen and Bruun Laursen, 1976; Harding and Whitton, 1977; Rai et al., 1981).

Species of metal in aqueous solution influences their chemical behaviour. Studies of the influence of abiotic factors on toxicities of multiple toxicants, including heavy metals, are virtually non-existent. Measurements of the complexing capacities of natural (algal exudates, sediment humics) and artificial (EDTA, citric acid, glycolic acid) complexing agents, and an assessment of the effect of these complexing agents on individual and combined heavy metal toxicity are presented in this paper. Literature reporting different effects of pH prompted our studies of both individual and combined metal toxicity to growth of S. quadricauda over a range of pH.

Materials and Methods

The green freshwater alga S. quadricauda was grown in a modified CHU-10 medium (Wong et al., 1982) containing no complexing agents, pH 8.0, at 20°C on a rotary shaker (100 rpm) under conditions of 18 h light (108 $\mu E/m^2/sec$) and 6 h darkness until cells reached logarithmic phase of growth. This took approximately 1 week. Cell counts were determined by direct microscopic counts with a Zeiss microscope, 200 X magnification, in a Fuchs-Rosenthal counting chamber.

Batch cultures of S. quadricauda were prepared by inoculating glass carboys containing 8 L of CHU-10, pH 8.0 with logarithmic cells to yield an initial population density of approximately 8.0×10^3 cells/mL. Algae were grown phototrophically at 20°C with agitation, aeration and constant illumination (130 $\mu E/m^2/sec$) until senescence. Total incubation period was 4-5 weeks. Algal exudates from each 8 L carboy were collected by pressure filtration through a 0.45 µm membrane filter (Millipore HA filter, 140 mm dia.) and were then concentrated on a rotary evaporator at 50°C to a final volume of 1L. Concentrated exudates were frozen until the contents of 10 8L carboys had been filtered and concentrated. The concentrated exudates were thawed and pooled. Since chloride analysis of pooled concentrated algal exudates with a modified mercuric thiocyanate method (Environment Canada, 1974) revealed an excessive ion concentration they were dialysed in cellulose dialysis tubing (molecular weight 1000). Desalting of the exudates via gel filtration, Sephadex G-25 or anion exchange resins was attempted, but proved unsatisfactory due to separation of various carbohydrates and simultaneous elution of the carbohydrate fractions with the chloride indicator ion. Active

complexing algal exudates were estimated as µg/mL dextrose equivalent by the phenol/sulphuric acid method of Dubois, et al. (1956) and protein as µg/mL bovine serum albumin equivalent by the Lowry method (Lowry et al., 1951).

Preparation of Hamilton Harbour sediment elutriate was based on the method developed by the U.S. EPA in conjuction with the U.S. Army Corps of Engineers (U.S. EPA, 1973). One part Hamilton Harbour sediment (160 g) was combined with four parts double distilled water (640 ml) in a 1L Erlenmeyer flask. Air bubbling through a deionized water trap was used to aerate the sediment/water mixture for 24 h. During this period the reaction flask was periodically shaken to facilitate a homogeneous suspension. After the 24 h elutriation period aeration was stopped and the sediment suspension allowed to settle for no more than 1 h. The elutriate was decanted off and centrifuged at 6000 x g for 20 min. Filtration of the supernatant through a Whatman No. 4 qualitative paper, followed by a further filtration through a 0.45 µm Millipore HA filter, concluded the collection of the Hamilton Harbour sediment elutriate. Carbohydrate contents were determined with the phenol/sulphuric acid method (Dubois et al., 1956).

Prior to the determination of complexing capacity, non-pooled concentrated algal exudates, non-dialysed pooled concentrated algal exudates, dialysed pooled algal exudates, Hamilton Harbour sediment elutriate, 1 µM EDTA, 1 µM citric acid, and 1 µM glycolic acid were filtered under mild vacuum through 0.45 µm Millipore HA filter, in order to remove particulates. Complexing capacities of the natural and artificial chelators were determined by Differential Pulse Anodic Stripping Voltammetry (D.P.A.S.V.) according to the method of Chau et al., 1974. Aliquots of these chelators were titrated

with various amounts of Cu^{2+} and allowed to equilibrate for at least 1 h at constant room temperature. An acetate buffer, pH 4.6, prepared from glacial acetic acid (suprapure grade, Merck-BDH) and anhydrous sodium acetate (suprapurgrade, Merck-BDH) was used to buffer titrated samples and the free Cu^{2+} concentration in each was measured by D.P.A.S.V. with a total plating period of 2 min. From a plot of peak current (μA) vs. Cu (μg/L) spike, the complexing capacity (x-intercept) of the respective chelator was determined. Complexing capacities were recorded as μmoles/L Cu^{2+} equivalent. The complexation of Zn and Pb individually by non-dialysed pooled algal exudates was also determined by titration of non-dialysed pooled algal exudates with known amounts of the respective metal. Non-dialysed and dialysed pooled algal exudates were titrated with known amounts of Cu, Zn, and Pb in combination, equilibrated and the respective free metal ion concentrations measured by D.P.A.S.V.

To determine the effects of complexing agents on metal toxicity, EDTA, citric acid or glycolic acid was added to 25 ml Erlenmeyer flasks containing CHU-10, pH 8.0 to give a final concentration of 2 μM for each complexing agent. Copper from 0-1000 μg/L was added to sets of flasks containing either 2 μM EDTA, citric acid or glycolic acid which were then autoclaved. Each flask received 1.0 ml of inoculum and was incubated under described conditions for 20 h. After 20 h, 0.1 ml of ^{14}C-sodium bicarbonate (2.0 μCi/ml) was added to each flask and incubated a further 4 h at which time photosynthetic activity was arrested and ^{14}C-uptake per flask was determined according to the method of Wong et al. (1982). This procedure was repeated twice, replacing copper with lead at concentrations from 0-5000 μg/L and finally with

zinc at concentrations of 0-1000 µg/L. the concentration of each metal in the presence of the respective complexing agent that caused a 50% reduction in photosynthesis (EC_{50}) was estimated by probit analysis (Finney, 1971).

The ability of various volumes (0-10 ml) of dialysed pooled concentrated algal exudates, non-dialysed pooled concentrated algal exudates, and Hamilton Harbour sediment elutriate to ameliorate Cu toxicity of 0, 100 and 200 µg/L was investigated. Flasks containing CHU-10, pH 8.0, and metals were autoclaved prior to the addition of the natural complexing agents to avoid denaturing the organic matter in the complexing agents at high temperature. Chelators were added to cooled flasks and held at room temperature for no less than 30 min. to allow the chelators and metals to equilibrate and form complexes before being inoculated with algal cells. Final volume per flask was 15 ml. As described previously, S. quadricauda was exposed to metals in the presence of the chelators for 20 h after which ^{14}C-uptake over an additional 4 h was determined. The influence of dialysed pooled algal exudates on the toxicity of Cu, Zn and Pb (singly and in combination) to photosynthetic activity of S. quadricauda was also examined. The bioassays were carried out with three replicate samples. The metal solutions ($CuSO_4$, $ZnSO_4$ or $PbCl_2$) were prepared by dissolving reagent grade metal compounds in doubly distilled water.

Long term exposure (15 days) of S. quadricauda to Cu, Zn and Pb (singly and in combination) at pH 8.5, 6.5 and 4.5 was investigated. Duplicate tubes (final volume 15 ml) containing metal ions in CHU-10 at each pH were autoclaved and inoculated with 1.0 ml of cells in logarithmic growth phase. The tubes were incubated at 20°C under continuous illumination

(108 $\mu E/m^2/sec$) for a 15 day period; at the end of which the pH of each tube was measured. Growth was monitored daily by optical density at 665 nm. These readings were converted into average growth rates according to the equation: $\mu_{ave} = (\ln X(T) - \ln X_0)/T$ (Nyholm, 1985), where μ_{ave} = average algal growth rate per day; X(T) = biomass concentration at time T; T = time in days; and X_0 = inoculated biomass concentration (at time zero).

Results and Discussion

Complexation: The complexing capacities of the artificial complexing agents (EDTA, citric acid and glycolic acid) and natural complexing agents (non-pooled and non-dialysed concentrated algal exudates, dialysed pooled concentrated algal exudates, non-dialysed pooled concentrated algal exudates and Hamilton Harbour sediment elutriate) are listed in Table 1. For each natural complexing agent the carbohydrate (CHO) content as µg/ml dextrose equivalent, pH, and Cl^- concentration as µg/ml are listed in Table 1. Protein contents of algal exudates (unconcentrated, concentrated, and pooled concentrated) were below the detection limits of the Lowry method (Lowry et al., 1951).

The toxic effects of Cu, Zn and Pb on the photosynthesis of S. quadricauda in the presence of three artificial complexing agents are recorded in Table 2. In the absence of a complexing agent, Cu was the most toxic to algal photosynthesis with an EC_{50} value of 100 µg/L followed by Zn at 250 µg/L while Pb was relatively non-toxic with an EC_{50} value of 2700 µg/L. In the presence of 2 µM EDTA the order of heavy metal toxicity to S. quadricauda was Zn > Cu >>> Pb. Citric acid (2 µM) had a slight protective

effect on heavy metal toxicity. Glycolic acid (2 μM) reduced Pb toxicity and slightly enhanced Cu and Zn toxicity.

Algal cells by releasing extracellular products may control the bioavailability of nutrients and metals (i.e. enhance uptake of nutrients, or prevent uptake of metals) in their immediate environment (Van den Berg et al., 1979).

Citric and glycolic acids form weak and labile complexes with Cu which had no significant ameliorative effect on Cu toxicity to S. quadricauda (Tables 1 and 2). However, each acid did offer minimal protection against Pb toxicity to algal photosynthesis. Zinc toxicity was also reduced by citric acid (2 μM), although glycolic acid (2 μM) slightly enhanced Zn toxicity (Table 2). Complexes formed between glycolic acid and Zn may have enhanced algal uptake of this metal, allowing higher concentrations of Zn to penetrate the cell. In contrast, EDTA, a synthetic complexing agent and contaminant of natural waters, demonstrated a strong complexing capacity for Cu (Table 1).

The ability of dialysed pooled concentrated algal exudates with complexing capacity of 0.34 μmoles Cu^{2+}/L to ameliorate single (Cu, Zn and Pb) and combined heavy metal toxicity to photosynthesis of S. quadricauda is presented in Table 3. Addition of Cu (100 and 200 μg/L), Zn (250 and 500 μg/L) or Pb (3000 and 6000μg/L) inhibited the photosynthesis about 42-50%. The algal exudates ameliorated the single metal and the metal mixture toxic effects.

Previous work of Chau and Wong (1975) revealed that natural waters low in organic carbon have little to no complexing capacity. Complexing capacities of the natural chelators, algal exudates and Hamilton Harbour

sediment elutriate increased with increased carbohydrate content (Table 1). The observed increase in complexing capacity, however, was not proportional to the increased carbohydrate content, which suggested that something other than carbohydrates was involved in the observed complexation. Protein contents of the algal exudates were well below the detection limits of the Lowry method and therefore probably did not contribute to this observation. Amide groups present in low amounts could have contributed to the complexing capacity of the algal exudates. According to the respective complexing capacities of the algal exudates for Cu, Zn and Pb, Cu was the most strongly complexed metal. However, when Cu, Zn and Pb were allowed to equilibrate simultaneously with these exudates, Zn was preferentially bound, then Cu and lastly Pb. The apparent selective binding of Zn may be related to its requirement as an essential nutrient and could have significantly affected the observed multiple heavy metal toxicity to S. quadricauda.

An elutriate prepared from Hamilton Harbour sediments had a high carbohydrate content and demonstrated a strong complexing capacity (Table 1). The observed effect of 0.10 mL of Hamilton Harbour sediment elutriate (with complexing capacity of 1.5 μmoles Cu^{2+}/L) on single and multiple heavy metal toxicity to S. quadricauda is presented in Table 4. Unlike the results in Table 3, the addition of sediment elutriate had little protective effect against metal toxicity. While the presence of 0.10 mL of elutriate significantly reduced individual toxicities of Cu (100 μg/L) and Zn (500 μg/L), the individual toxicities of Pb (3000 and 6000 μg/L), Cu (200 μg/L) and Zn (250 μg/L) were only slightly reduced (Table 4). No significant reduction in toxicity was observed for any combinations of Cu, Zn and Pb examined. The

sediment elutriate in the absence of metals was slightly inhibitory to photosynthesis. The elutriate may include inorganic and organic pollutants from the heavy industrial use of the water that may counteract the protective effect of the elutriate.

Competition for complexing ligands may result in either antagonism or synergism between metals. Competition between metals for the same functional groups in the exudates may cause a net decrease in the free ion concentration of both metals and result in a decrease in toxicity; in other words, an antagonistic response. Conversely, a synergistic response may arise from the same competition between metals for binding sites. However, in this case one metal may successfully outcompete the other, consequently increasing the free metal ion concentration of the other metal and rendering it more toxic in the presence of the second metal than in its absence.

Many models are available for describing the complex interactions between toxic chemicals and organisms (Borgmann, 1980; Marking, 1985). In the present study, three different models (Colby, 1967; Borgmann, 1980 and Voyer and Heltshe, 1984) were used to analyze our results (Tables 5 and 6). In general, most Cu, Zn and Pb interactions were antagonistic. Several additive metal interactions were also found. Zinc (500 µg/L) combined with Pb (6000 µg/L) interacted antagonistically with no complexing agents (Table 5). In the presence of dialysed algal exudates synergism between the two metals was found with the Colby method. The same difference between metal interactions in the absence and presence of dialysed algal exudates was observed for mixtures of Zn (250 µg/L) and Pb (6000 µg/L). Synergism was also determined according to the Voyer and Heltshe (1984) model for the following mixtures of all three

metals in the presence of algal exudates: Cu (100 µg/L), Zn (250 µg/L) and Pb (6000 µg/L), and Cu (100 µg/L), Zn (500 µg/L) and Pb (6000 µg/L). This observed synergism may be related to the preferential binding of algal exudates for Zn and Cu, rather than Pb when exposed to all three metals simultaneously. Based on their measured complexing capacities, one would expect that Zn and Cu would successfully outcompete Pb for the functional ligands. Hence the relative concentrations of Zn and Cu would be reduced leaving the free toxic Pb ion available in solution in a relatively higher concentration than in the absence of the algal complexing agents. This would be interpreted as a synergistic interaction between the three metals. In the absence of the algal exudates all three metals would be present in solution as the toxic free metal ion and would compete equally to exert a toxic effect on the test organism.

Metal interactions in the presence or absence of Hamilton Harbour sediment elutriate followed the same pattern (Table 6). All interactions appeared to be antagonistic, except for those of: Cu (100 µg/L) and Pb (3000 µg/L); Cu (100 µg/L) and Zn (500 µg/L); and Cu (100 µg/L), Zn (500 µg/L) and Pb (3000 µg/L) which were additive. These observed differences in metal interactions seemed to be related to the significant protective effect against Cu (100 µg/L) and Zn (500 µg/L) individual toxicities afforded by the elutriate. Antagonism in the presence and absence of Hamilton Harbour elutriate was probably due to competition between these divalent cations for the same binding sites on the cell surface, which reduced the overall cellular uptake of the metals.

pH: The influence of pH on long term heavy metal toxicity to growth of S. quadricauda was also investigated. Heavy metal toxicity to growth at initial pH values of 8.5, 6.5 and 4.5 is presented in Table 7. In general the toxicity of individual metals and metal mixtures increased with a corresponding increase in acidity. For example, even though the highest growth rate for control cells was observed at pH 4.5, no growth was observed for algal cells exposed to either Cu (200 µg/L) or Zn (500 µg/L). Algal metabolism may have been stimulated in the acidic environment and resulted in an increased metabolic uptake of metals, thus heightening their toxicity under acidic conditions. On the other hand, species difference and bioavailability of metals are influenced by pH. Increasing acidity increases the free metal ion concentration in solution, due to competition between bound metal ions and free H^+ ions for negatively charged exchange sites on organics and inorganics. Under acidic conditions metals tend to exist in the free ionic form which is believed to impart metal toxicity. However, reports found in the literature of the effect of pH on metal toxicity are inconclusive. Several authors noticed an increase in Cu toxicity to phytoplankton at acidic pH (Monahan, 1976; Harding and Whitton, 1977; Rai et al., 1981), while other authors reported an increase in metal toxicity at alkaline pH (Gachter, 1976; Hargreaves and Whitton, 1976 a,b; Steemann Nielsen and Kamp-Nielsen, 1970).

Algal cells subjected to combinations of either 2 or 3 metals exhibited a pronounced lag and overall reduction in growth. The short initial lag in growth of algae exposed to individual metals indicates an immediate toxic effect that was followed by a period of adaptation or tolerance which resulted in their recovery. Due to the longer exposure period (15 days) used to assess

the effect of pH on metal toxicity, algal by-products capable of complexing metals accumulated in the growth medium. Binding of Cu, Zn or Pb by these algal exudates would have reduced their bioavailablility and toxicity which could account for the noticed recovery.

It appeared that upon exposure to multiple metal mixtures competition between the metal ions for the active ligands of algal exudates was not effective in reducing their individual toxic free ion concentration. Thus, a severe toxic effect on growth was observed for which there was no recovery. All toxic effects exerted by Cu, Zn and Pb on growth of S. quadricauda were enhanced at acidic pH. An increase in complex stability has been observed at high pH (Cheng et al., 1975), which may also contribute to reduce toxicity at alkaline pH. Under acidic conditions, competition may occur not only between H^+ and metal ions for active sites on the cells' surface, but also for weakly acidic ligands (Gould and Genetelli, 1978). Therefore competition between the added metals and H^+ ions for complexing ligands present in accumulated algal exudates may influence metal toxicity to algal growth.

The effect of pH on heavy metal interactions determined by the methods of Colby (1967), Borgmann (1980) and Voyer and Heltshe (1984) are listed in Table 8. Acidic conditions appeared to enhance the occurrence of synergisms or additive effects between the heavy metals investigated. A great deal of variation in metal interactions occurred with changes in pH. Hydrogen ion mediated displacement of complexed metals from algal waste products at low pH resulted in a greater toxic free metal ion concentration in solution which probably caused the observed increase in toxicity and synergism at pH 4.5. In addition to the increased bioavailability of metal ions, acidic conditions

may have altered algal cell membrane solubility, so that damage to the membrane and penetration of the algal cell by metals was enhanced. Metabolic uptake (active or passive) may have been stimulated by the high ion level at acidic pH, which would result in a greater toxic effect. Data collected from exposure of S. quadricauda to heavy metals indicate that both single and multiple metal toxicities are influenced by their respective concentrations and pH.

The data presented emphasize that the physico-chemical properties of an aquatic ecosystem determine the speciation and hence toxicity of metals in the aquatic environment. Competition between metals can result in either synergistic, antagonistic or additive interactions, depending on the concentration and speciation of the respective metals. The study, therefore, of metal interactions and combined toxicity to the aquatic biota is a complex subject that requires further research.

Table 1: Measured Complexing Capacities for both Natural and Artificial Complexing Agents.

Complexing Agent	Complexing Capacity µmoles Cu^{2+}/l equiv.	CHO µg/ml Dextrose	pH	Cl µg/ml
EDTA 1 µmole/l	0.90	-	-	-
Citric Acid 1 µmole/l	N.D.[1]	-	-	-
Glycolic Acid 1 µmole/l	N.D.[1]	-	-	-
CHU-10	N.D.[1]	N.D.[2]	8.0	20
Non-dialysed non-pooled concentrated Algal exudates	0.12	8.2	-	-
Dialysed pooled concentrated Algal exudates	0.34	4.60	7.00	14
Non-dialysed pooled concetrated Algal exudates	0.71	12.45	7.90	144
Hamilton Harbour Sediment Elutriate	1.50	60.00	-	-

1. Not detectable by D.P.A.S.V. method of Chau et al. 1974.

2. Not detectable by the method of Dubois et al. 1956.

Table 2: Effect of various complexing agents on the toxicity of copper, zinc and lead to the photosynthesis of S. quadricauda.

Metal	EC_{50} [1] (µg/L)			
	No complexing agent	EDTA (2 µM)	citric acid (2 µM)	glycolic acid (2 µM)
Copper	100	300	135	90
Zinc	250	250	290	210
Lead	2700	N.T.[2]	3200	N.T.[2]

1. EC_{50} = concentration causing a 50% reduction in photosynthesis after a 4-hr. incubation.

2. No toxic effect observed.

Table 3: Amelioration of single and combined heavy metal toxicity to photosynthesis by dialysed pooled concentrated algal exudates (1.0 mL).

Metals Cu	Zn	Pb	No Complexing Agents % control[a] ± S.D. (N = 3)	Dialysed Algal Exudates % control[b] ± S.D. (N = 3)
	µg/L			
control				
0	0	0	100 ± 10	100 ± 20
100	0	0	* 50 ± 1	77 ± 7
200	0	0	* 42 ± 2	* 53 ± 4
0	250	0	* 44 ± 7	72 ± 1
0	500	0	* 49 ± 5	95 ± 4
0	0	3000	47 ± 2	112 ± 4
0	0	6000	51 ± 5	139 ± 28
100	0	3000	* 66 ± 6	87 ± 15
200	0	6000	82 ± 3	87 ± 11
100	0	6000	86 ± 1	*151 ± 17
200	0	3000	88 ± 1	97 ± 4
100	250	0	* 59 ± 3	81 ± 5
200	500	0	* 49 ± 3	92 ± 0
100	500	0	* 57 ± 4	86 ± 9
200	250	0	* 52 ± 5	81 ± 8
0	250	3000	* 42 ± 5	78 ± 7
0	500	6000	* 71 ± 1	112 ± 3
0	500	3000	* 55 ± 6	98 ± 10
0	250	6000	* 56 ± 2	86 ± 13
100	250	3000	* 59 ± 7	78 ± 10
200	250	3000	* 53 ± 3	77 ± 1
100	250	6000	* 49 ± 4	90 ± 6
200	250	6000	* 48 ± 5	76 ± 4
100	500	3000	* 55 ± 5	80 ± 5
200	500	3000	* 48 ± 4	74 ± 1
100	500	6000	* 61 ± 5	96 ± 5
200	500	6000	* 52 ± 2	77 ± 3

a. ^{14}C-uptake is presented as % control without complexing agents; 100% = 29428 dpm.

b. ^{14}C-uptake is presented as % control with dialysed algal exudates; 100% = 20894 dpm.

* Significantly different from the control; P = 95%; DF = 5.

Table 4: Amelioration of single and combined heavy metal toxicity to photosynthesis by Hamilton Harbour sediment elutriate (0.1 mL).

Metals Cu	Zn	Pb	No Complexing Agents % control[a] ± S.D. (N = 3)	Sediment Elutriate % control[b] ± S.D. (N = 3)
	µg/L			
control				
0	0	0	100 ± 2	100 ± 15
100	0	0	* 61 ± 9	83 ± 20
200	0	0	* 53 ± 4	* 58 ± 11
0	250	0	* 42 ± 2	* 56 ± 10
0	500	0	* 55 ± 6	75 ± 5
0	0	3000	* 49 ± 6	* 67 ± 8
0	0	6000	* 58 ± 5	* 68 ± 5
100	0	3000	* 55 ± 1	* 65 ± 5
200	0	6000	* 51 ± 5	* 59 ± 8
100	0	6000	* 57 ± 6	75 ± 7
200	0	3000	* 47 ± 2	* 53 ± 2
100	250	0	* 50 ± 4	* 61 ± 6
200	500	0	* 50 ± 2	* 63 ± 8
100	500	0	* 51 ± 5	* 61 ± 5
200	250	0	* 52 ± 2	* 59 ± 10
0	250	3000	* 43 ± 6	* 69 ± 2
0	500	6000	* 61 ± 3	* 62 ± 5
0	500	3000	* 49 ± 2	* 71 ± 5
0	250	6000	* 52 ± 5	* 60 ± 6
100	250	3000	* 53 ± 1	* 55 ± 3
200	250	3000	* 47 ± 1	* 57 ± 15
100	250	6000	* 49 ± 1	* 61 ± 3
200	250	6000	* 53 ± 3	* 63 ± 6
100	500	3000	* 63 ± 4	* 65 ± 10
200	500	3000	* 49 ± 5	* 66 ± 2
100	500	6000	* 55 ± 6	* 66 ± 1
200	500	6000	* 54 ± 4	* 61 ± 7

a. ^{14}C-uptake is presented as % control without complexing agents; 100% = 55894 dpm.
b. ^{14}C-uptake is presented as % control with elutriate; 100% = 49063 dpm.
* Significantly different from the control; P = 95%; DF = 5.

Table 5: The effect of algal exudates (dialysed pooled concentrated) on multiple heavy metal interactions towards primary productivity (short term exposure).

Metals			No Complexing Agents			Dialysed Algal Exudates		
Cu	Zn µg/l	Pb	Colby	Borgmann	Voyer and Heltshe	Colby	Borgmann	Voyer and Heltshe
100		3000	+	A	+	+	+	+
200		6000	A	A	A	A	A	+
100		6000	A	A	A	A	A	+
200		3000	A	A	A	A	A	A
100	250		A	A	A	A	A	A
200	500		A	A	A	A	A	A
100	500		A	A	A	A	+	A
200	250		A	A	A	A	A	A
	250	3000	+	+	+	+	+	+
	500	6000	A	A	A	S	A	+
	500	3000	+	+	+	+	+	A
	250	6000	A	A	+	S	+	A
100	250	3000	A	A	A	A	+	A
200	250	3000	A	A	A	A	A	A
100	250	6000	A	A	A	A	A	S
200	250	6000	A	A	A	A	A	A
100	500	3000	A	A	A	+	A	+
200	500	3000	A	A	A	A	A	A
100	500	6000	A	A	A	+	+	S
200	500	6000	A	A	A	A	A	A

A. Antagonistic.
S. Synergistic.
\+ Additive.

Table 6: The effect of Hamilton Harbour elutriate on heavy metal interactions towards primary productivity (short term exposure).

Metals			No Complexing Agents			Hamilton Harbour Elutriate		
Cu	Zn µg/l	Pb	Colby	Borgmann	Voyer and Heltshe	Colby	Borgmann	Voyer and Heltshe
100		3000	A	A	A	+	+	A
200		6000	A	A	A	A	A	A
100		6000	A	A	A	A	+	A
200		3000	A	A	A	A	A	A
100	250		A	A	A	A	+	A
200	500		A	A	A	A	A	+
100	500		A	+	A	+	+	A
200	250		A	A	A	A	+	A
	250	3000	A	A	A	A	A	A
	500	6000	A	A	A	A	+	A
	500	3000	A	A	A	A	A	A
	250	6000	A	A	A	A	A	A
100	250	3000	A	A	A	A	A	A
200	250	3000	A	A	A	A	+	A
100	250	6000	A	A	A	A	A	A
200	250	6000	A	A	A	A	A	A
100	500	3000	A	A	A	+	+	A
200	500	3000	A	A	A	A	A	A
100	500	6000	A	A	A	A	A	A
200	500	6000	A	A	A	A	A	A

A. Antagonistic.
S. Synergistic.
\+ Additive.

Table 7: Heavy metal toxicity to growth of S. quadricauda at pH 4.5, 6.5 and 8.5.

Metals Cu	Zn	Pb	pH 4.5 Growth Rate[a] (S.D.)[b]		pH 6.5 Growth Rate[a] (S.D.)[b]		pH 8.5 Growth Rate[a] (S.D.)[b]	
	µg/l							
0	0	0	0.194	(0.027)	0.171	(0.001)	0.154	(0.017)
50	0	0	0.167	(0.010)	0.181	(0.033)	0.144	(0.013)
100	0	0	0.018	(0.018)*	0.244	(0.001)*	0.168	(0.021)
200	0	0	0.000	(0.000)*	0.047	(0.007)*	0.197	(0.026)
0	100	0	0.100	(0.011)*	0.101	(0.030)	0.122	(0.013)
0	225	0	0.040	(0.009)*	0.042	(0.011)*	0.131	(0.029)
0	500	0	0.000	(0.000)*	0.026	(0.013)*	0.109	(0.004)*
0	0	1000	0.173	(0.004)	0.140	(0.010)*	0.147	(0.001)
0	0	3000	0.141	(0.019)	0.148	(0.000)*	0.077	(0.010)*
0	0	6000	0.028	(0.013)*	0.135	(0.002)*	0.063	(0.007)*
100	100	0	0.009	(0.009)*	0.086	(0.032)*	0.137	(0.003)
100	225	0	0.004	(0.004)*	0.061	(0.010)*	0.107	(0.011)*
100	500	0	0.000	(0.000)*	0.000	(0.000)*	0.127	(0.050)
100	0	1000	0.011	(0.011)*	0.244	(0.005)*	0.183	(0.001)
100	0	3000	0.016	(0.016)*	0.196	(0.020)	0.088	(0.017)*
100	0	6000	0.000	(0.000)*	0.280	(0.012)*	0.093	(0.017)*
50	225	0	0.014	(0.000)*	0.035	(0.032)*	0.105	(0.007)
200	225	0	0.000	(0.000)*	0.114	(0.006)*	0.136	(0.007)
0	225	1000	0.029	(0.003)*	0.043	(0.004)*	0.110	(0.013)*
0	225	3000	0.007	(0.007)*	0.013	(0.005)*	0.134	(0.004)
0	225	6000	0.000	(0.000)*	0.069	(0.017)*	0.070	(0.004)*
50	0	3000	0.156	(0.033)	0.288	(0.001)*	0.069	(0.004)*
200	0	3000	0.035	(0.008)*	0.113	(0.011)*	0.154	(0.033)
0	100	3000	0.038	(0.012)*	0.058	(0.016)*	0.129	(0.022)
0	500	3000	0.016	(0.016)*	0.012	(0.006)*	0.039	(0.017)*
100	225	1000	0.016	(0.016)*	0.049	(0.003)*	0.119	(0.013)
100	225	3000	0.000	(0.000)*	0.083	(0.006)*	0.150	(0.041)
100	225	6000	0.000	(0.000)*	0.086	(0.030)*	0.136	(0.017)
100	100	3000	0.011	(0.011)*	0.090	(0.006)*	0.120	(0.001)*
100	500	3000	0.006	(0.006)*	0.027	(0.001)*	0.125	(0.014)
50	225	3000	0.011	(0.011)*	0.075	(0.001)*	0.109	(0.006)*
200	225	3000	0.000	(0.000)*	0.038	(0.024)*	0.111	(0.035)*

a. $\mu_{ave} = (\ln X(T) - \ln X_0)/T$; (Nyholm 1985).
b. N = 3 samples.
* Significantly different from control at 95% C.I. (Student's test).

Table 8: The effect of pH on heavy metal interactions towards algal growth (long term exposure).

Metals			Colby			Borgmann			Voyer and Heltshe		
Cu	Zn	Pb									
	µg/l		pH 8.5	6.5	4.5	pH 8.5	6.5	4.5	pH 8.5	6.5	4.5
100	100		+	+	+	A	+	+	A	+	+
100	225		S	+	+	S	+	S	S	+	+
100	500		+	S	+	S	S	S	+	S	+
100		1000	+	A	+	+	A	+	A	A	+
100		3000	+	+	+	S	+	+	+	+	+
100		6000	A	A	S	+	A	S	+	A	+
50	225		S	+	S	S	+	S	S	+	+
200	225		S	A	+	S	A	S	+	A	+
	225	1000	+	+	+	+	+	S	+	A	A
	225	3000	A	S	S	A	S	S	A	+	+
	225	6000	A	+	S	A	+	S	A	A	+
50		3000	+	A	+	+	A	+	+	A	S
200		3000	+	A	A	A	A	S	A	A	A
	100	3000	+	+	S	+	S	S	A	+	+
	500	3000	+	+	+	+	+	S	+	+	+
100	225	1000	A	+	+	S	+	+	+	A	+
100	225	3000	A	A	S	+	A	S	A	A	+
100	225	6000	A	+	+	A	+	S	A	+	+
100	100	3000	A	S	+	A	S	+	A	+	+
100	500	3000	A	S	+	A	+	+	A	A	+
50	225	3000	A	A	+	A	A	S	A	A	+
200	225	3000	+	+	+	+	+	S	+	A	+

A. Antagonistic.
S. Synergistic.
\+ Additive.

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