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The effect of copper and manganese on phytoplankton in the Grand River (southern Ontario), Lake Erie and Pacific Ocean ecosystems

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The Effect of Copper and Manganese on Phytoplankton in the Grand River (Southern Ontario), Lake Erie and Pacific Ocean Ecosystems

by

Rachel Helen Welbourn

B.Sc. McGill University, 2000.

A thesis

presented to Ryerson University

in partial fulfillment of the

requirement for the degree of

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in the Program of

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ABSTRACT

The Effect of Copper and Manganese on Phytoplankton in the Grand River, Lake Erie and Pacific Ocean Ecosystems

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Master of Applied Science
in the Program of
Applied Environmental Science and Management

With the increased use and loading of metals into the environment, the accumulation of toxic metals by phytoplankton has become a concern. Trace metal interactions with phytoplankton are of particular interest due to the influence of phytoplankton on the biogeochemical cycling of metals in aquatic systems. The study of the accumulation of metals and their toxicity in phytoplantkon is also of interest since phytoplankton lie at the base of many aquatic food webs. Toxic metals therefore have the potential to disrupt food webs and may have important implications on aquatic ecosystems.

This study has chosen to focus on the response of phytoplankton to two trace metals in particular: copper (Cu) and manganese (Mn). Although both Cu and Mn are essential elements for phytoplankton, Cu is of particular interest as a toxicant. A number of laboratory studies have suggested that there exists a physiological interaction between Cu and Mn, and that Cu toxicity can be decreased in the presence of high concentrations of Mn. However, few studies have examined the effects of these metals on phytoplankton in their natural environments. The significance of this study is that it is one of the first to examine whether the importance of Cu toxicity and the interaction between Cu and Mn observed in the laboratory is also observable under natural conditions.

Short-term bioassays were conducted in order to observe the response of phytoplankton from the Grand River (Southern Ontario) and Lake Erie to additions of various concentrations of Cu and Mn under natural conditions. Similar long-term bioassay experiments were also conducted in the Pacific Ocean. Experiments in the Grand River and the Pacific Ocean revealed no significant decrease in phytoplankton biomass or in photosynthetic efficiency with the addition of various concentrations of Cu and Mn. In Lake Erie, phytoplankton biomass was only adversely affected following relatively high additions of Cu of 60 nM, and only under certain conditions. These results seem to indicate that under the tested conditions, Cu toxicity may not be of particular concern to the phytoplankton of the Grand River, Lake Erie and Pacific Ocean ecosystems.

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CHAPTER 1: INTRODUCTION

1.1 Phytoplankton

An examination of the effects of copper (Cu) and manganese (Mn) on phytoplankton in their natural environments requires an understanding of the biota themselves. Planktonic algae (or phytoplankton) are free floating photosynthetic organisms found in both freshwater and oceanic environments. In lakes and oceans, the phytoplankton community usually consists of a relatively large number of different species. Phytoplankton are often divided into three separate size categories: the microplankton which range in size from 20-200 μm; the nanoplankton, which range in size from 2-20 μm; and the picoplankton which are less than 2 μm in diameter.

In the open ocean, the nanoplankton and picoplankton, especially prokaryotes such as *Synechococcus* (cyanobacterium) and *Prochlorococcues* (prochlorophyte), are often more abundant than in the coastal regions, where larger diatoms and dinoflagellates are more widespread (Sze, 1998). In freshwater, the major groups of phytoplankton present are cyanobacteria, green algae, diatoms, synurophytes, chrysophytes, cryptomonads, and occasionally dinoflagellates (Sze, 1998).

Phytoplantkon growth is often dependent on the availability of nutrients, and the availability of nutrients is often dependent on the season. Temperate oceans and most lakes show vertical mixing in the spring and in the fall, which results in resuspension of nutrients and peaks in phytoplankton abundance. In eutrophic lakes however, growths of green algae and cyanobacteria can occur in the summer due to the large availability of nutrients (Sze, 1998).

The most common method for assessing phytoplankton abundance (or total phytoplankton biomass) is the measurement of chlorophyll fluorescence (Fogg and Thake, 1987). One of the most widely used and most sensitive techniques for measuring phytoplankton primary production uses a radioactive isotope of carbon: ^{14}C. Phytoplankton primary production can be estimated by adding a known amount of inorganic carbon containing ^{14}C to a water sample containing phytoplankton and later

measuring the amount of ^{14}C incorporated into the organic material (Welschmeyer and Lorenzen, 1984; Geider and Osborne, 1992).

The species composition of the phytoplankton community as well as the abundance of phytoplankton varies between seasons, between water bodies, and even within the water bodies themselves. Despite these variations, one constant remains: phytoplankton are the primary producers of organic matter upon which most other aquatic organisms depend (Fogg and Thake, 1987). Phytoplankton also influence the biogeochemical cycling of trace metals in aquatic systems (Bruland *et al.*, 1991; Twiss *et al.*, 1996), which in turn can influence community structure. In addition, phytoplankton have the ability to concentrate certain metals out of the water. Since they are the main source of food for many aquatic herbivores, phytoplankton may play an important part in introducing metals into food webs (Fisher and Reinfelder, 1995). Since phytoplankton play such a crucial role in many aquatic environments, toxic metals that affect phytoplankton thereby have the potential to affect entire ecosystems.

1.2 Sources of trace metals in the environment

The anthropogenic release of trace metals into the environment dates back to the discovery of fire in ancient times. However, although humans have been responsible for the release of trace metals into the environment for thousands of years, it is only with the great technological advances of the past century that trace metal pollution has reached never-before-seen levels and has become an important concern. Although trace metals can be emitted from natural sources such as volcanoes, forest fires, and wind-borne particles, the substantial increase in emissions during the past century can be explained by the increased anthropogenic sources of these metals from such activities as mining and smelting (Nriagu, 1990). In fact, the anthropogenic release of trace metals has increased so dramatically that according to some estimates, worldwide atmospheric copper emissions increased 500-fold from 1900 to the mid 1980s (Nriagu, 1996).

Once released into the environment, trace metals can enter a number of different environmental compartments; air, soil or water. Aquatic systems are particularly vulnerable to trace metal pollution since in addition to direct waste inputs

of metals into the system, trace metals can also enter rivers and lakes via runoff and atmospheric deposition. In fact, the atmosphere is one of the main pathways for the transportation of trace metals, and it is often overlooked by the general population. For example, few people realize that atmospheric deposits are responsible for more than 50% of all trace metals entering the Great Lakes (Nriagu, 1990; Sweet *et al.*, 1998). Since the concentrations of trace metals in continental waters are partially dependent upon atmospheric precipitation and weathering of soils and bedrock, the increased atmospheric discharges of trace metals have resulted in increased concentrations of trace metals in water systems across the world (Nriagu, 1990). In addition to atmospheric deposition, trace metals can also enter aquatic systems via sewage discharges, urban and agricultural runoff, domestic and industrial wastewaters, industrial and municipal discharge, surface runoff and waterways, as well as groundwater and diagenic processes in sediments (Kolak *et al.*, 1998; Nriagu, 1990).

1.3 Trace metals in aquatic environments

Once present in aquatic environments, metals can exist in two general forms: dissolved and particulate. Dissolved metal is operationally defined as metal that can pass through a 0.2-μm to 0.45-μm filter. It is important to note that dissolved metal includes not only free metal ions, but also metal ions that have formed complexes with a variety of organic and inorganic ligands. The formation of such complexes can influence the bioavailability, toxicity and mobility of the metal (Bruland *et al.*, 1991; Sunda and Huntsman, 1998a).

The speciation of a metal is of importance when studying the effect of such a metal on aquatic organisms. Several different species of metal can exist in aquatic systems (Mota and Correia Dos Santos, 1995):

-hydrated free metal ions,

-dissolved inorganic complexes,

-dissolved organic complexes,

-metal species in the form of dispersed colloids, and

-metal adsorbed on colloids or suspended matter.

Most cationic trace metals are complexed by inorganic or organic ligands or are adsorbed on the surface of, or bound within particles (Sunda and Huntsman, 1998a). Chloro-, carbonato-, sulfato-, oxo- and hydroxo complexes are the main inorganic dissolved complexes that are present in natural waters (Turner *et al.,* 1981). In the ocean, there is little variation in the inorganic speciation of trace metals since the major ion composition and pH of seawater are relatively constant (Sunda and Huntsman, 1998a). However, in freshwater and estuarine environments, large variations in chloride concentration, pH and alkalinity can lead to variations in inorganic complexation (Sunda and Huntsman, 1998a). Organic complexes found in natural waters are mainly stable complexes between free metal ions and organic matter of biological origin. The amount of organic matter in the open sea (0.3 to 3 $mgC \cdot L^{-1}$) is less than in rivers, lakes and estuaries (1 to 10 $mgC \cdot L^{-1}$) (Morel and Hering, 1993). The percentage of total dissolved metal that exists in complexed form varies depending on a number of factors, including the chemical characteristics of the metal itself, such as its electronic configuration. For example, in seawater, about 20 % of dissolved iron is complexed with natural organic ligands (Nimmo *et al.,* 1989), whereas in both freshwater and seawater environments, more than 90% of copper is bound by organic matter (Nimmo *et al.,* 1989; Achterberg *et al.,* 1997; Coale and Bruland, 1990; Witter *et al.*, 1998; Xue *et al.*, 1996).

1.4 Free Ion Activity Model (FIAM)

Although metals can exist in a variety of different species in aquatic systems, not all of these species have been found to be equally available for uptake by organisms. A variety of laboratory experiments published from 1976 to 1983 determined that the total aqueous concentrations of metals is not a reliable indicator of metal bioavailability to organisms. Instead, bioavailability is in fact a function of the concentration of the free aquo-ion of the metal (M), $M^{z+}(H_2O)$; which is in turn dependent upon the concentration and nature of ligands present (Campbell, 1995). In order to rationalize this, the Free-ion Activity Model (FIAM) was developed. The FIAM suggests that for cationic forms of a given metal, the free-ion species is the form that bioavailability can be most closely related to. The interaction of a free metal

ion at the surface of a cell can be described by the following equations (Campbell 1995):

$$M^{z+} + {}^{-}X\text{-cell} \overset{K}{\longleftrightarrow} M\text{-X-cell} \qquad (1)$$

where $\mathbf{M^{z+}}$ is the free-metal ion, **$^{-}$X-cell** is a cellular ligand at the cell surface, **M-X-cell** represents the surface complex formed by the interaction of the metal with the cell surface, and **K** is the equilibrium constant for the binding of a metal to a reactive site.

From equation 2, it is clear that since X-cell and K are both constant, the binding of a given metal to the surface of a cell is proportional to the free ion concentration of this metal.

$$\{M\text{-X-cell}\} = K\{{}^{-}X\text{-cell}\}\ [M^{z+}] \qquad (2)$$

The FIAM proposes that the nutritional and toxicological effects of a metal on a given organism are therefore proportional to the free ion concentration of the metal rather than to the total metal concentration (Campbell, 1995).

1.5 Role of Cu and Mn in phytoplankton

With the increased use and loading of metals into the environment, the accumulation of toxic metals by phytoplankton has become a concern. Trace metal interactions with phytoplankton are of particular interest due to the influence of phytoplankton on the biogeochemical cycling of metals in aquatic systems (Bruland *et al.,* 1991; Twiss and Campbell, 1998). The study of the accumulation of metals and their toxicity in phytoplankton is also of interest since phytoplankton lie at the base of many aquatic food webs. Toxic metals therefore have the potential to disrupt food webs and may have important implications for marine organisms and for human health (Fisher and Reinfelder, 1995). This thesis focuses on two trace metals in particular: copper (Cu) and manganese (Mn). Both Cu and Mn are essential elements for phytoplankton, and both are involved in photosynthesis.

The most notable role of Cu in phytoplankton is as a component of plastocyanin (PC), which is involved in the electron transport chain. PC is responsible for shuttling electrons from the cytochrome *b6/f* complex to photosystem I (PSI), where they are used to reduce NADP to NADPH (Whitfield, 2001).

Although Cu is an essential micronutrient, the potential toxic effects of Cu at sub-nanomolar concentrations have been well documented (Van den Berg *et al.*, 1979; Sunda and Guillard, 1976; Brand *et al.*, 1986; Gerringa *et al.*, 1995; Sunda and Huntsman, 1998b, 1998c). Also, different species of phytoplankton exhibit varying sensitivities to Cu toxicity. Concentrations of free Cu greater than 10^{-12} M are toxic to eukaryotic phytoplankton (Sunda and Huntsman, 1995; Moffett *et al.*, 1997), and prokaryotes are considered to be even more sensitive. The following sequence shows increasing sensitivity to Cu toxicity: diatom-dinoflagellate-coccolithophore-*Synechococcus* (Whitfield, 2001)

The potential toxicity of Cu may be due to its ability to bind to sulphur sites, and to interfere with the acid-base and redox chemistry of the cell (Whitfield, 2001). It is however thought that the toxic effect of Cu on phytoplankton may often involve the competition by Cu for sites normally occupied by other essential elements, such as Mn (Sunda *et al.*, 1981; Sunda and Huntsman, 1983).

Unlike Cu, Mn is not usually considered as a potential toxicant to phytoplankton. However, like Cu, Mn is an essential element for phytoplankton. Mn is involved with the splitting of water in photosystem II and thus is essential to the evolution of oxygen (Figure 1). Four Mn atoms sequentially lose electrons, resulting in the release of one O_2 molecule from the oxidation of 2 H_2O (Falkowski and Raven, 1997).

It should also be noted that both Mn and Cu are also present in superoxide dismutases, enzymes that aid in the intracellular removal of active oxygen species (Raven *et al.*, 1999; Whitfield, 2001).

1.6 Role of Cu and Mn in society

In addition to their essential roles in phytoplankton, Cu and Mn are also widely used in today's society. Cu is one of the main metals used by humans due to its

malleability, ductility, durability and high electrical and thermal conductivity (ATSDR 2000; Mansilla-Rivera and Nriagu, 1999). The uses for Cu are varied, ranging from telecommunications and electrical wiring, to fungicides for plants, algicides for water treatment, piping for potable water, and even as a preservative for wood, fabric and leather products (ATSDR 2000).

Manganese is the second most abundant metal found in nature, surpassed only by iron. (Forstner and Wittman, 1983; ATSDR, 2000). Mn is mainly used industrially in the production of steel to improve hardness, stiffness and strength (ATSDR 2000). However, Mn compounds are also used in the production of dry-cell batteries, matches and fireworks, in addition to glazes, varnishes and ceramics. Mn compounds can also be found in animal feed, fertilizer and water purification systems (ATSDR 2000). In Canada, organic Mn is also a component of MMT, which has replaced tetraethyl lead as a fuel additive in gasoline (ATSDR 2000).

1.7 Previous studies of antagonistic effects of Cu and Mn

1.71 Laboratory studies (with oceanic and estuarine species of phytoplankton)

Sunda and Huntsman have published most of the data regarding the study of the effects of Cu and Mn interactions on phytoplankton. It is suspected that one of first publications on this subject dates back to 1981, when Sunda, Barber and Huntsman assessed the role of Fe, Mn, and Cu in the growth of phytoplankton in recently upwelled seawater. They collected water from a depth of 800 m (the nitrate maximum) off the coast of North Carolina and found that additions of iron, manganese and chelators increased the growth rate of unialgal cultures of *Chaetoceros socialis* or of a natural phytoplankton community inoculated into the seawater. Additions of Cu, on the other hand caused a decrease in growth and a shift in the dominant species present in the natural community. What was of particular interest however was that they found that the toxic effects of Cu could be reversed by additions of Fe, Mn or chelator. Based on the results of this experiment, the authors suggested that there exists a physiological interaction between Cu and Mn. They hypothesized that Cu can bind to Mn cellular sites thereby competing with Mn and interfering with Mn metabolism (Figure 1).

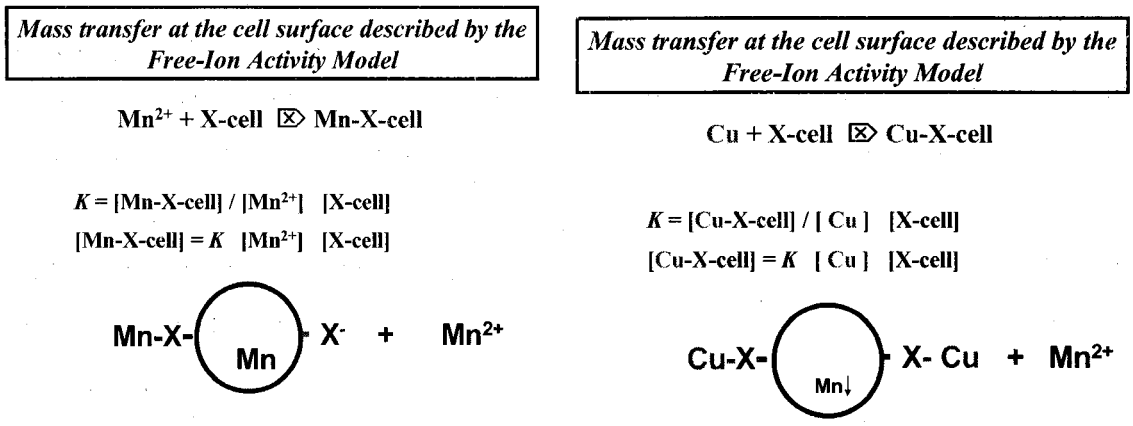

Figure 1: Mass Transfer at the cell surface described by the Free-Ion Activity Model. Where X-cell is the binding site, which is constant, and K is a constant. Therefore the rate of uptake of a given metal by the cell is dependent upon the free ion concentration of the metal. (Modified from Campbell, 1995)

The reversal of the negative effects of Cu, observed by Sunda *et al.* (1981), upon the addition of the chetalors and the Fe, can be explained by the ability of the chelators and presumably the hydrous iron oxides to complex more readily with Cu than with Mn, resulting in an increased Mn to Cu ion ratio. Sunda *et al.* (1981) therefore concluded that high concentrations of Cu ion combined with low Mn concentrations may limit the growth of phytoplankton due to the inhibition of Mn uptake, which results in the Mn starvation of the cells (Sunda *et al.*, 1981). Such conditions of high Cu:Mn ratios could very well occur naturally in upwelled seawater that is usually rich in Cu and low in Mn. These experiments may therefore have important implications for marine phytoplankton in their natural environments.

In a subsequent study, Sunda and Huntsman (1983) further investigated the competitive interactions between Cu and Mn in two related species of diatoms, the estuarine species *Thalassiosira pseudonana* (clone 3H) and a related oceanic species *Thalassiosira oceanica* (clone 13-1). Both species of diatom were exposed to various manganese and cupric ion activities. It was determined that the growth rate of the cultures was related to the concentration of Mn within the cell, which was in turn related to the manganese ion activity and inversely related to cupric ion activity. These results supported their earlier hypothesis (Sunda *et al.*, 1981) that phytoplankton growth can be inhibited by low manganese ion concentrations combined with high cupric ion concentrations, conditions that are typical of recently upwelled seawater.

In 1998, Sunda and Huntsman published the results of two series of laboratory experiments that focused on Cu and Mn antagonisms, in addition to the effect of varying concentrations of Zn, and the effect of light in regulating trace metal uptake, chlorophyll *a* and specific growth rate of phytoplankton (Sunda and Huntsman, 1998b, 1998c). Culture experiments were carried out with the green alga *Chlamydomonas* sp. and the estuarine diatom *T. pseudonana* in metal ion-buffered media. In the *Chlamydomonas* experiment, antagonistic interactions were found between toxic metals (Cu and Zn) and nutrient metals (Zn and Mn). Low levels of Mn combined with high levels of Cu and Zn resulted in inhibition of cellular Mn uptake by competitively blocking Mn binding to the high-affinity Mn uptake system. It was also found that high levels of Cu combined with low levels of Zn inhibited zinc uptake by a high Zn uptake system (Figure 3). In the *T. pseudonana* experiment, it was found that independent of light intensity, Cu and Zn inhibited growth rate at low Mn concentrations; this was completely accounted for by the fact that both Cu and Zn were inhibiting Mn uptake (Sunda and Huntsman, 1998b). These results once again support the earlier of findings of Sunda and Huntsman (1981, 1983) which indicated that high concentrations of Cu can competitively block the Mn binding site, resulting in decreased uptake of Mn by the cell. This study also strongly suggests that metals do not act in isolation, and that it is not the individual metals themselves, but rather the interactions between various metals, that regulate cellular metal accumulation, nutrition and toxicity (Sunda and Huntsman, 1998b).

1.72 Laboratory studies (with Lake Erie phytoplankton isolates)

Recently, another team of scientists decided to further investigate the effects of Cu and Mn on phytoplankton using Lake Erie isolates. Twiss *et al.* (2003) conducted a laboratory experiment which examined the specific growth rate per day of *Chlorella* and *Microsystis* under the following conditions: free cupric ion concentrations ranging from 10^{-13} M to 10^{-10} M, low and high light levels and low (0.08 nM) and high (4.8 nM) concentrations of Mn. Their findings contrasted somewhat with the results of Sunda and Huntsman's study described previously, which found that Cu toxicity was independent of light. Twiss *et al.* (2003) observed that Cu levels had no effect on the growth rate of *Chlorella* at high light levels, however, under low light levels, Cu had a

detrimental effect under both low and high concentrations of Mn. *Microsystis* on the other hand showed sensitivity to 10^{-10} M Cu at both high and low levels of light and at both high and low levels of Mn (Twiss *et al.*, 2003). The difference in the response of the two species was attributed to the fact that cyanobacteria such as *Microsystis* are considered to be more susceptible than eukaryotic algae to toxic metals since they lack an additional protective cell membrane (Twiss *et al.*, 2003). The marked difference between the results of the study conducted by Twiss *et al.*, and Sunda and Huntsman's study, indicate that Cu toxicity may not only be a function of Mn concentrations and of light intensity, but that the conditions which induce Cu toxicity may vary depending on the species.

1.73 Field studies in the Pacific

Although trace metal interactions with phytoplankton cultures have gained more interest recently, few experiments with natural assemblages of plankton have been performed and those that have, have chosen to examine the effects of single metals on phytoplankton. For example, in 1991, Coale conducted metal enrichment experiments on natural plankton populations from the surface waters of the subarctic Pacific. Small concentrations of Fe (0.89 nM), Mn (1.8 nM), Cu (3.9 nM) and Zn (0.75 nM) were added in order to simulate natural changes in metal concentrations that marine phytoplankton are likely to be exposed to. Treated samples were incubated for 7 days. Incubations took place on deck in running surface seawater and under PVC screens in order to simulate natural light and temperature conditions. The biomass and productivity of the samples were assessed over time in order to monitor the effects of the metal additions on the phytoplankton. Mn treatments showed an increase in productivity and biomass (especially for diatoms), indicating Mn limitation of productivity in the subarctic. Substantial increases in phytoplankton productivity, chlorophyll *a* and cell densities were observed in treatments with added Fe and Cu. The increase in the Fe treatments is attributed to an increase in photosynthetic efficiency due to the fact that Fe may be limiting to marine phytoplankton. The increase in the Cu treatments however, is surprising since it is well known that even small concentrations of Cu can produce toxic effects. Coale (1991) attributes the

increase in the Cu treatments to a decrease in grazing by the microzooplankton thus resulting in an increased rate of phytoplankton production.

1.74 Field studies in the Great Lakes

1.741 Lake Superior, September 2000

In September 2000, Twiss *et al.* (2003) conducted an experiment on Lake Superior in order to assess the effects of increasing concentrations of Cu on the phytoplankton community. Water was sampled from a 20 m depth from the pelagic western arm of Lake Superior. Samples were submitted to a 2 day exposure of various concentrations of added Cu ranging from 0 nM to 20 nM. Total chlorophyll *a* and ^{14}C uptake over a 9 hour incubation period were measured. It was found that both phytoplankton biomass and total photosynthetic activity decreased with increasing concentrations of copper (Figure 2)

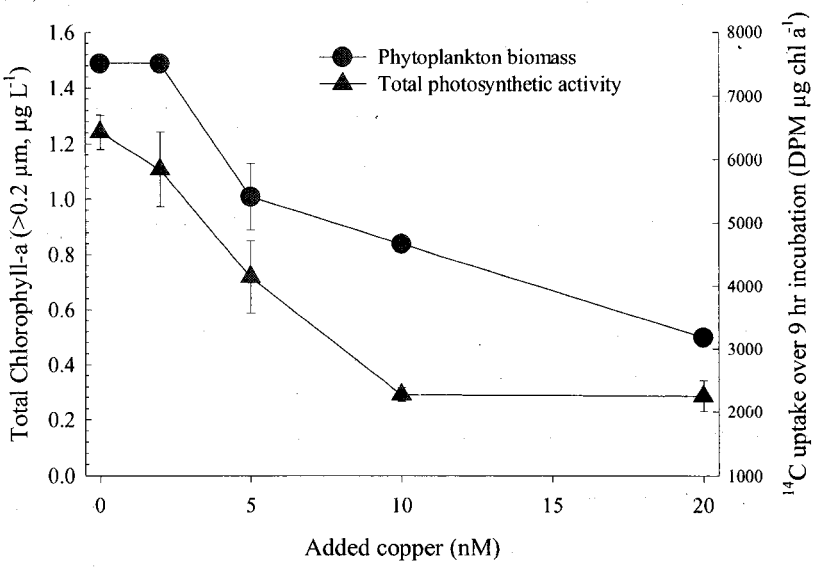

Figure 2: The response of phytoplankton sampled from Lake Superior in September 2000 to additions of Cu. (Twiss *et al.*, 2003)

This experiment supported previous studies which suggested that high concentrations of Cu can be detrimental to organisms (Van den Berg *et al.*, 1979; Sunda and Guillard, 1976; Brand *et al.*, 1986; Gerringa *et al.*, 1995; Sunda and Huntsman, 1998b, 1998b).

It should be noted that this experiment was repeated in May 2001 at the same station on Lake Superior as well as at an additional station, with a few minor adjustments: ^{14}C uptake was not measured and the incubation period was extended to 3 days. The results of the May 2001 experiments however showed that Cu had no significant effect on phytoplankton biomass. This was in sharp contrast with the September 2000 experiment, where just 5 nM of Cu adversely affected the phytoplankton population. The lack of response in May 2001 is explained by the fact that phytoplankton productivity was constrained by the cool water temperature of 3-4 degrees Celsius, as compared to 15 degrees Celsius in September 2000 (Twiss *et al.*, 2003).

1.742 Lake Erie, July 2001

In July 2001, another field experiment was carried out on the Great Lakes, and on this occasion, water was sampled from Station 23 in the Eastern Basin of Lake Erie. The experimental design used was similar to that employed on Lake Superior in September 2000. Water samples were submitted to additions of Cu ranging from 0 nM to 20 nM. It was found that chlorophyll *a* biomass and photosynthetic efficiency did not vary amongst the treatments. However, when 5 nM of Mn was added to the 5 nM Cu treatment, a significant increase was observed in chlorophyll *a* biomass (Twiss, unpublished) (Figure 3)

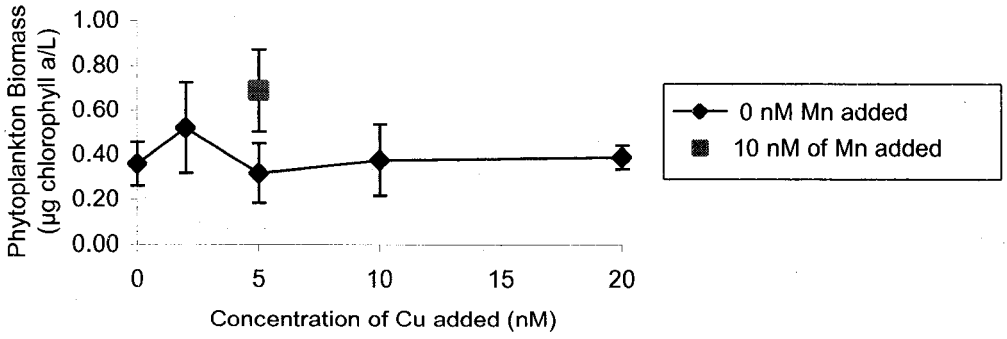

Figure 3: Response of phytoplankton sampled from Lake Erie in July 2002 to additions of Cu and Mn (Twiss, unpublished)

1.8 Review of current state of knowledge

The field and laboratory experiments described previously indicate that under certain conditions, high concentrations of Cu can indeed have a toxic effect on phytoplankton by inhibiting the uptake of Mn, thus causing Mn starvation of the cells. These experiments also draw attention to the importance of the antagonistic and synergistic relationships amongst trace metals. Since trace metals are not present in isolation in natural environments, it is important to consider the combined influences of a variety of metals that may have synergistic or antagonistic effects on each other rather than focusing on one metal in particular (Bruland *et al.*, 1991). However, as Bruland *et al.* (1991) point out, experiments regarding the antagonistic interactions among trace metals are almost all from laboratory studies. The field studies that have been conducted on trace metals have mostly examined the effect of a single metal or a number of metals individually (see Coale, 1991). It is therefore necessary that more field studies be conducted concerning metal antagonisms in order to test the hypotheses that have been largely based on the results of laboratory studies.

1.9 Description of study areas

As stated in the previous section, although many laboratory studies have examined the antagonistic relationship between Cu and Mn, few studies have examined this relationship in natural environments. We therefore decided to study the response of phytoplankton to additions of Cu and Mn in three separate aquatic environments: a river, a lake and an ocean, all of which are briefly described below.

1.91 The Grand River

The Grand River Watershed in Southern Ontario occupies an area of 6,734 km^2 and is one of the most important rivers in the province (Grand River Conservation Authority, 1979). The Grand River has its origins in springs and marshes in Dufferin County and flows south into the Eastern Basin of Lake Erie (Figure 4).

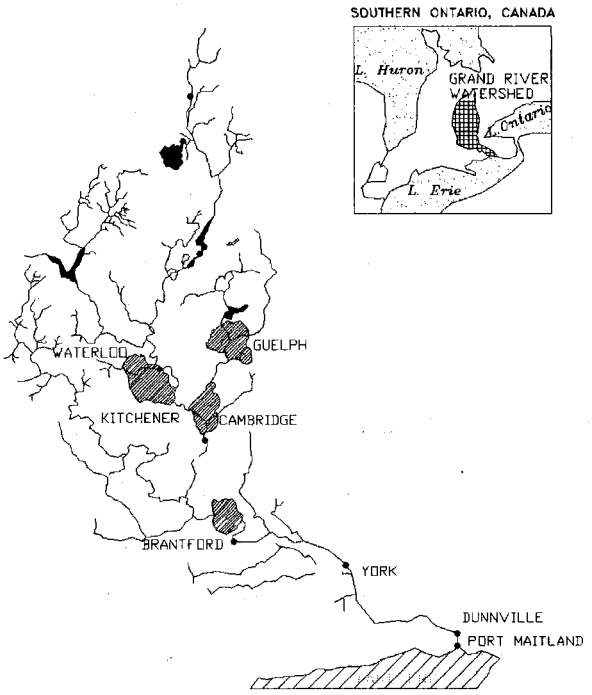

Figure 4: Map of the Grand River, Southern Ontario (adapted from Rott *et al.*, 1998)

The dominant land use in the watershed is agriculture, although the central portion of the river runs through some large urban centers such as Guelph, Kitchener-Waterloo, Cambridge and Brantford (Grand River Conservation Authority, 1979). The river has therefore been subjected to pollutants from a variety of sources, the main one being agricultural. In the 1970s, the Grand River was even known as one of the most polluted rivers in Southern Ontario and was estimated to contribute up to 25% of the total phosphorus loadings into Lake Erie (Nicholls *et al.,* 1983). Today, the water quality of the Grand River has improved substantially, in part due to the efforts of the Grand River Conservation Authority, which now monitors water quality on a regular basis. It should however be noted that a recent study suggests that although phosphorus loadings into the Grand River have decreased since the 1960s, certain areas of the Grand River are showing signs of increased eutrophication probably as a result of an increase in nitrates (Rott *et al.*, 1998).

Despite the polluted nature of the Grand River, previous studies of Grand River phytoplankton and their influence on Lake Erie have focused mainly on distribution and taxonomy (Nicholls *et al.*, 1983; Rott, 1995). There is a lack of

studies examining the presence of trace metals in the river as well as the potential effect of these metals on phytoplankton in the mixing zone as the Grand River flows into Lake Erie.

1.92 Lake Erie and the North American Great Lakes

The Great Lakes contain approximately 18% of the world's supply of fresh water (Fuller and Shear, 1995). They span a total of 244,000 km^2 and contain 23,000 km^3 of water. Despite their large size, the Great Lakes remain vulnerable to a number of pollutants. The large regions surrounding the Great Lakes are intensely industrialized in some areas; one tenth of the population of the United States and one quarter of the population of Canada presently live in the Great Lakes Basin (Fuller and Shear, 1995). Pollution can enter the lakes from a variety of sources including runoff from soils and agricultural lands, wastewater from cities, discharges from industrial areas and through leaching of waste disposal sites. Given the large surface area of the lakes, they are also vulnerable to direct atmospheric inputs. It should also be noted that since the hydraulic residence time of the Great Lakes is high, pollutants that enter the lakes remain in the system for some time. (Fuller and Shear, 1995).

Lake Erie is the shallowest and the smallest of the Great Lakes in volume. It is divided into three distinct basins: the Western Basin, the shallowest with an average depth of 7 m, the Central Basin, with an average depth of 18 m and the Eastern Basin, with an average depth of 25 m. Among the Great Lakes, Lake Erie is the most exposed to agricultural and urbanization effects (Fuller and Shear, 1995). There are also a number of industrialized areas located on the shores of Lake Erie, including Buffalo, Cleveland, Toledo, Sandusky, Detroit and Windsor in addition to those within the watershed including Windsor, Guelph, Kitchner-Waterloo, Cambridge and Brantford. All of these factors make Lake Erie particularly vulnerable to pollution from a variety of sources. In fact, about three decades ago, Lake Erie was known as a "dead lake". Lake Erie was so christened because excessive loading of phosphorus from sewage and agricultural drainage caused an explosion of growth in the phytoplankton community, which, upon their decomposition, dramatically reduced the oxygen content of the lake, hence the term "dead lake" (Nicholls, 1997). The water quality of Lake Erie has greatly improved since then, in part due to the initiatives of the Great

Lakes Water Quality Agreements of 1972 and 1978, which set reduced phosphorus loading targets for both Canada and the United States (Nicholls, 1997). Although phosphorus loadings have been successfully reduced, large amounts of toxic metals continue to enter the Great Lakes (Nriagu *et al.*, 1996)

Despite continued large inputs of trace metals into the Great Lakes, very little is known about their distribution, their chemical behaviour and their potential effects on organisms (Nriagu *et al.*, 1996). Nriagu *et al.* (1996) therefore decided to determine the baseline levels of trace metals in Lakes Superior, Ontario and Erie. Their results indicate that levels of Cu are fairly constant throughout Lake Superior, Lake Ontario, and Lake Erie whereas concentrations of Mn vary greatly throughout Lake Superior and Lake Ontario (Table 1).

Table 1: Total dissolved concentrations (nM) of Cu and Mn in Lake Ontario (Data from Nriagu *et al.*, 1996)

Metal	Total dissolved (<0.4μm) concentration, nmol/L		
	Mean ± SD, n=47	Minimum	Maximum
Cu	13.1 ± 1.5	9.8	15.9
Mn	1.4 ± 1.6	0.1	8.2

Although the data in Table 1 are for Lake Ontario, concentrations of Cu in Lake Erie fall within approximately the same range: 8 -13 nM (Leslie and Lum, 1983; Rossman and Barres, 1988; Coale and Flegal, 1989; Nriagu, 1996). As for Mn, it should be noted that there are no published studies detailing the range of dissolved Mn concentrations throughout Lake Erie using ultra-clean techniques. However, in 1978, prior to the widespread use of ultra-clean techniques, it was found that concentrations of Mn in Lake Erie ranged from 5 nM to 1275 nM (Leslie and Lum, 1983). It is possible that these concentrations may be overestimated, since ultra-clean techniques were not employed during the collection, sampling and analysis of the samples. In fact, during the course of an experiment carried out using ultra-clean techniques at a

discrete location in the Central Basin of Lake Erie (Station 84), the total dissolved concentration of Mn was determined to be 2 nM (Twiss *et al.*, 1994), lower than the range reported by Leslie and Lum (1983). Although there is a lack of published trace metal clean data regarding the range of total dissolved Mn concentrations throughout Lake Erie, it is expected that Mn concentrations in Lake Erie would be comparable to those in Lake Ontario, and that they would also exhibit a wide range of variation throughout the lake. It is therefore relevant to examine the effects of Cu and Mn on phytoplankton in Lake Erie since phytoplankton may naturally be exposed to conditions of high concentrations of Cu and low concentrations of Mn, conditions which have the potential for reducing phytoplankton growth by inhibiting Mn uptake.

1.93 Pacific Ocean

Our final area of study is the Pacific Ocean. The oceans are less affected by anthropogenic inputs than the Grand River and the Great Lakes due mostly to their large surface area; they account for more than 70% of the Earth's surface. In fact, the Pacific Ocean is even less affected than the Atlantic, which receives more atmospheric contaminants and windborne dust (Whitfield, 2001). Even though the oceans may be less affected by contaminants, in contrast to freshwater environments, trace metals in the oceans have been extensively studied. Consequently, much is known regarding the distribution and speciation of trace metals in the Pacific.

The vertical distribution of total Cu concentration in the Pacific shows a trend of increasing concentration with depth (Figure 4), from 0.5 nM at the surface to 1.5 nM at 500 m and up to 5 nM at 4000 m (Coale and Bruland, 1990). This type of distribution seems to be intermediate between a nutrient type element, which is depleted on the surface and enriched in deep water, and an element that is scavenged throughout the water column (Coale and Bruland, 1990). Although anthropogenic inputs of Cu in the open ocean are presently not a great concern, given the vertical profile of Cu concentrations in the Pacific, Cu toxicity to phytoplankton is still thought to be possible following upwelling events (Brand *et al.*, 1996).

Although the total concentrations of Cu in sea water are very close to, or above the toxic levels for cyanobacteria and eukaryotic phytoplankton, the biologically available concentration of copper is much less, due to the formation of strong organic complexes that bind >99% of the total Cu (Coale and Bruland, 1988). The source of some of these organic complexes may be the phytoplankton themselves. It has been demonstrated that, in response to increasing ambient concentrations of Cu, both prokaryotes and eukaryotes have the ability to produce Cu-complexing ligands (Croot *et al.,* 2000). In fact, the speciation of Cu(II) in the upper ocean is dominated by a very selective strong ligand. This strong ligand, denoted L_1, is found only near the surface, suggesting that it is of biological origin (Coale and Bruland, 1988). The concentration of L_1 averages 1.8 nM in the upper 100 m of the ocean and exceeds the total concentration of Cu(II) in the upper 200 m of the ocean (Bruland *et al.*, 1991). The presence of L_1 contributes to a free Cu concentration of less than 0.1 $pmolL^{-1}$, that is 2 orders of magnitude lower than the free Cu concentration in deeper parts of the water column (Figure 5).

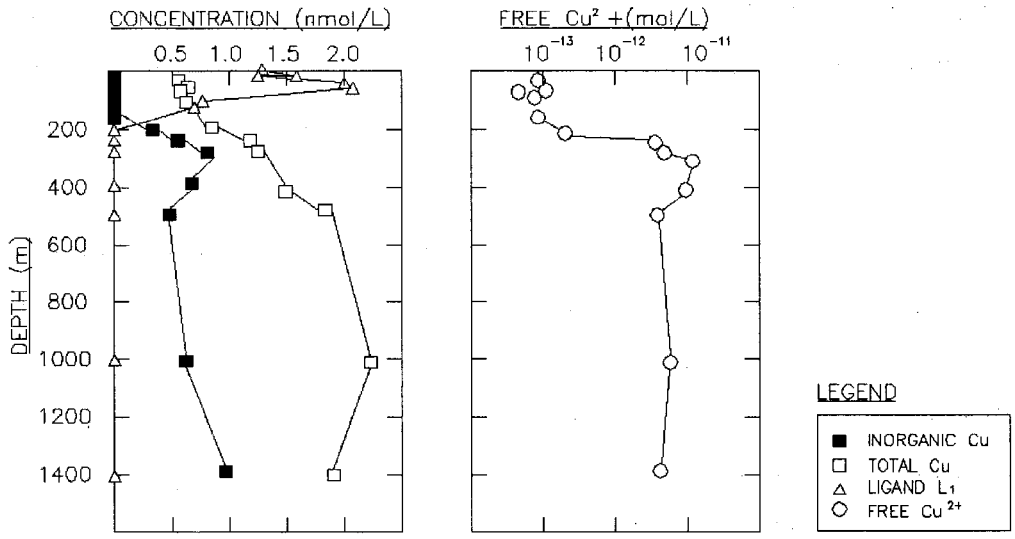

Figure 5: The vertical distribution of inorganic Cu, total Cu and the strong ligand L1 from a station in the Northeast Pacific is shown in the left panel. The right panel shows the vertical distribution of free Cu^{2+} (Modified from Coale and Bruland, 1988).

A number of studies have found that the cyanobacterium *Synechococcus* sp. produces a Cu-binding ligand whose conditional formation constant is identical to L_1 (Moffett *et al.* 1990; Coale and Bruland, 1988, 1990). Considering that

Synechococcus sp. is widespread throughout the ocean, it could have an impact on the speciation of Cu(II) in many oceanic regions (Hunter and Boyd, 1999). Biological processes therefore play an important role in the distribution and speciation of Cu in marine environments.

The presence of another, weaker Cu-complexing, ligand denoted L_2, has also been detected in seawater (Coale and Bruland, 1998). In contrast with L_1 however, L_2 is found throughout the water column. L_2 is thought to be a component of the dissolved organic matter in seawater that results from the degradation of plant and animal tissues (Hunter and Boyd, 1999). This weaker ligand is also reportedly similar in nature to humic acids, which are present in freshwater systems (Hunter and Boyd, 1999).

As for Mn, the vertical profile shows clear decreasing concentration with depth: from 1 nM at the surface to 0.2 nM at 400 m in the North Pacific central gyre (Bruland *et al.*, 1991). It is therefore relevant to examine the effects of Cu and Mn on phytoplankton in the Pacific since an upwelling or deep vertical mixing of water could expose phytoplankton to high concentrations of Cu and low concentrations of Mn, conditions which have the potential for inhibiting phytoplankton growth by inhibiting Mn uptake.

1.10 Thesis Objective

Given the current state of knowledge regarding the antagonistic interactions between the trace metal Cu and Mn and their effect on phytoplankton, and given the dearth of field studies that have been conducted to date, the objective of my thesis is as follows:

To investigate the response of phytoplankton to various concentrations of Cu and Mn under natural conditions in three different aquatic environments:

-The mouth of the Grand River (a river)

- The Eastern and Central Basins of Lake Erie (a lake)

-The Northern Pacific Ocean (an ocean)

Following enrichment of samples with varying concentrations of Cu and Mn, the response of phytoplankton will be determined by assessing ^{14}C uptake (a measure of photosynthetic activity) and/or chlorophyll *a* production (a measure of phytoplankton biomass).

1.11 Hypothesis

I hypothesize that given the known antagonisms between Cu and Mn (Sunda *et al.*, 1981; Sunda and Huntsman, 1983, 1998a, 1998b), high concentrations of Cu are more likely to have a negative effect on phytoplankton biomass and chlorophyll *a* production in waters with low concentrations of Mn than in waters with high concentrations of Mn.

1.11 Outline of Thesis

Chapter 2 provides a description of the standard methods used in all experiments from Chapters 3 through 5.

Chapter 3 examines the response of phytoplankton from the Eastern Basin of Lake Erie and the Grand River to additions of Cu and Mn in November 2001 and again in July 2002. The response of the phytoplankton to the various treatments was assessed by measuring chlorophyll *a* (a measure of phytoplankton biomass) and ^{14}C uptake (a measure of photosynthetic efficiency).

Chapter 4 examines the response of phytoplankton from the Central Basin of Lake Erie to additions of Cu and Mn in September 2002. Although the experiments described in Chapter 4 examine phytoplankton from Lake Erie, as in Chapter 3, it was decided for a number of reasons to divide these experiments into two separate chapters. The main reason was that the methods used in the experiments in the Central Basin was based upon the results obtained from the experiments in the Eastern Basin of Lake Erie. Finally, experiments in the Central Basin assessed the response of phytoplankton by measuring only phytoplankton biomass rather than phytoplankton biomass and photosynthetic efficiency as was the case in the Eastern Basin.

Chapter 5 examines the response of phytoplankton to additions of Cu and Mn at three stations in the North Pacific in June/July 2002. The response of phytoplankton

to the various treatments was assessed by measuring chlorophyll *a* (a measure of phytoplankton biomass).

Chapter 6 summarizes the results and conclusions from Chapters 3 through 5.

CHAPTER 2: STANDARD METHODS

The following methods are common to all experiments and are therefore described here for the sake of brevity.

2.1 Trace Metal Clean Technique

2.11 Background

The study of trace metals in the natural environment differs from most other disciplines in that special precautions must be taken in order to minimize contamination of samples. The concentrations of trace metals in most aquatic systems are so low that samples can be easily contaminated by materials that are commonly found in the average chemistry laboratory (dust, dander, paint flakes) (Hunter and Boyd, 1999). Therefore, without careful precautions, water samples may be easily compromised, resulting in an overestimation of trace metal concentrations. In fact, with the recent development of sophisticated technology and techniques for the sample collection and analysis of trace metals, today, we are able to state with certainty that the trace metal concentrations of many aquatic systems are generally orders of magnitude lower than they were believed to be in the mid-1970s (Hunter and Boyd, 1999).

In order to obtain reliable results when conducting experiments involving trace metals, it is imperative that special attention be paid to all steps of the process from sample collection to analysis. It is therefore necessary to ensure that whenever possible: all containers used be cleaned appropriately in order that they be free of trace metals, the collection and handling of samples be undertaken with the utmost care and that possible external sources of contamination be controlled by using specialized clothing, as well as working in clean-room conditions (Hunter and Boyd, 1999). According to Hunter and Boyd (1999), experience has shown that in order to minimize contamination, it is the attention to detail and the working methods that are the most important; with the clean room itself merely providing the right kind of environment for these methods.

It is important to note that in the past, many Great Lakes researchers assumed that such stringent methods were not necessary when studying trace metals on the Great Lakes. Given the "polluted" nature of these waters, the concentrations of trace metals was assumed to be high relative to seawater, whereby a few researchers adopted the theory that "an extra nmol or two" from contamination would not noticeably influence the results (Nriagu, *et al.,* 1993). Recent studies however, have shown that most of the available data regarding trace metal concentrations in the Great Lakes are overestimates, resulting from artifact contamination during the collection, handling or analysis of samples (Nriagu *et al.*, 1993). It is therefore important that meticulous measures be taken when working with trace metals, in order to minimize contamination from a variety of sources including labware, equipment, reagents and even the surrounding air.

As much as was possible, such meticulous measures were employed in the experiments described in Chapters 3 through 5. Collection and analysis of samples was done using ultra-clean techniques (Nriagu *et al.,* 1993). All preparatory and analytical work was conducted in the Ryerson University Clean Room (Class 100), in a portable clean room aboard the C.C.G.S. *Limnos*, and under a HEPA laminar flow hood onboard the R/V *Lake Guardian* and the R/V *Melville*. In order to minimize contamination, Class 1.5 compatible clean room gloves (Oak Technical; Stow, Ohio) were worn during the sampling, handling and analysis of samples. Also, ultrapure Milli-Q water (Millipore Corp., Bedford, Mass.) or ultrapure Nanopure water was used for rinsing labware and in the preparation of solutions.

2.12 Washing procedure for labware

Prior to each research cruise, all labware was washed meticulously in the Ryerson University Trace Metal Clean Laboratory. All labware was rinsed twice with Milli-Q water, then left to soak for at least 24 hours in a solution of 3-10% Trace Metal Grade HCl (Fisher Scientific; Ottawa, ON). Following the acid soaking, labware was rinsed seven times with copious amounts of Milli-Q water, dried in a laminar flow hood and then put into polyethylene bags (Zip-Loc) until use, in order to minimize contamination.

2.13 Washing procedure for new polycarbonate bottles

Prior to the research cruise, new polycarbonate bottles (or those that had only been previously used in the field) were rinsed twice with Milli-Q water. Trace Metal Grade HCl solution (100 mL of 3-10%) was then poured into each bottle. The bottles were capped, shaken, and left to sit for a minimum of 24 hours. The bottles were then rinsed seven times with copious amounts of Milli-Q water, dried in a laminar flow hood, and sealed in polyethylene bags.

2.14 Washing procedure for polycarbonate bottles previously used in culture experiments

Additional precautions were taken polycarbonate bottles that had been previously used in culture experiments in order to remove any residual organic compounds left adhered to the glass from the algal cultures. Bottles were rinsed two times with Milli-Q water. They were then soaked for at least 24 hours with a 10 % industrial soap (Citranox), 90 % Milli-Q water solution. This was followed by five rinses with Milli-Q water. Bottles were then rinsed with a 10 % solution of ethanol, followed by five rinses with Milli-Q water, another rinsing with 10 % ethanol solution, followed by five more rinses with Milli-Q water. Approximately 100 mL of 5-10 % dilute trace metal clean HCl solution was added to each bottle and allowed to soak for a minimum of 24 hours. Bottles were then rinsed seven times with copious amounts of Milli-Q water, dried in a laminar flow hood, and then placed in polyethylene bags.

2.2 Preparation of stock solutions of Cu and Mn

A solution of 10 μM of Cu or Mn was prepared in a 100 mL flask from a dilution of atomic absorption spectroscopy standards (VWR). Concentrated trace metal grade HCl (5 μL of 12 M) was also added to the solution to ensure solubility. For experiments in the Central Basin of Lake Erie, this methodology was somewhat altered. Instead of making the metal solutions by diluting atomic absorption spectroscopy standards, $CuSO_4$ and MnCl salts were diluted in Milli-Q water. By using metal salts, we were erring on the side of caution, since the atomic adsorption spectroscopy standards are diluted in HCl (0.5%), which may alter the pH, and therefore the metal speciation of the water samples. It should, however, be noted that

in all experiments, the pH of samples was checked occasionaly before and after metal additions using pH paper and no change in pH was ever observed.

2.3 Spiking of samples with metals

Once the polycarbonate bottles were filled with water, they were gently shaken and then spiked with various concentrations of Cu and Mn. When both Cu and Mn were added to a sample, the Mn was added prior to the Cu. Following the spiking of samples with metals, bottles were capped and gently shaken in order to evenly distribute the added metal throughout the sample. A wax film (Parafilm) was wrapped around the necks of the bottles in order to prevent contamination of samples. Bottles were then transported to the incubator in black garbage bags, so as not to damage the phytoplankton by exposing them to direct sunlight.

2.4 Filtration of samples for total dissolved concentrations of metals

At each station, water for trace metal analysis was filtered using a Teflon filtering apparatus attached to a vacuum pump (Figure 6).

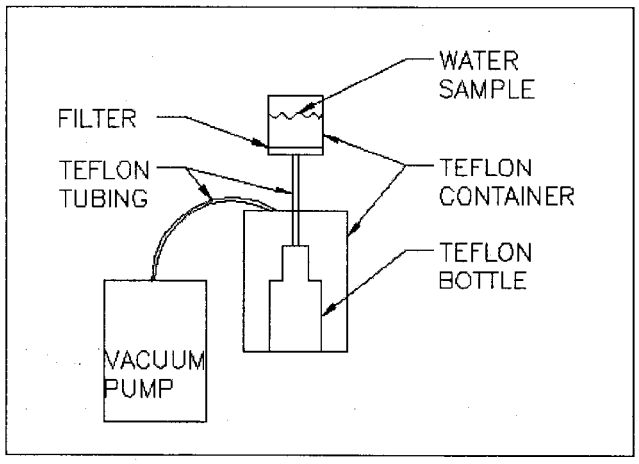

Figure 6: Schematic diagram of trace metal clean filtration set-up used for filtering water samples for total dissolved metal analysis.

Filters (0.2-μm pore–size; polycarbonate membrane) were soaked in a 3 % Trace Metal Grade HCl solution for 12 hours prior to use. Immediately prior to

filtering, filters were removed from the acid solution and rinsed with copious amounts of Milli-Q water. Following filtration, the filtrate (collected in a Teflon® bottle) was frozen until analysis by GFAAS in the Ryerson University Clean Room. Plastic tweezers were used in the handling of the filters in order to minimize metal contamination.

2.5 Graphite furnace atomic absorption spectroscopy (GFAAS)

2.51 Lake Erie and Grand River Samples

Once the samples were thawed, 1mL of concentrated HNO_3 (Seastar) was added to every 1L of sample in order to dislodge any metal that had adsorbed onto the walls of the Teflon® bottles. Analysis of metals was carried on a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS: Perkin Elmer) in the Ryerson University Clean Room. Hollow cathode lamps were used to analyze Cu and Mn concentrations. Whenever a sample was run, standard solutions of 0 nM, 5 nM, 20 nM, 30 nM of either Cu or Mn were prepared by diluting atomic absorption spectroscopy standards (VWR). Concentrated HNO_3 (Seastar) was added to the standard solutions in order to ensure matrix consistency. Standard addition techniques (0 nM and 10 nM) were used to compensate for matrix effects. The calibration curve obtained from the standard solutions was then compared to the standard addition curve of each sample. We ensured that the slopes of both equations were similar, thus reducing potential matrix interference. Prior to each analysis, three replicates of SLRS-4 (National Research Council Canada) standard riverine water were also analyzed in order to assure the accuracy of the GFAAS. Once the slopes of the calibration curve and the standard addition curve were found to be similar and the SLRS-4 samples had determined the accuracy of the GFAAS, three replicates of water from each station were analyzed for total dissolved concentrations of Cu or Mn.

2.52 Pacific Ocean Samples

The methodology used to analyze the Pacific Ocean samples was slightly altered from above. Since the Pacific Ocean samples had a relatively high salt content, this could cause interference with the metal signals. Prior to analysis by GFAAS, these samples were therefore UV oxidized, in order to remove organics, then

chelexed in order to remove any salts. UV oxidation and chelexing of samples was carried out by Clarkson University, Potsdam, NY. The samples were then analyzed at Ryerson University by GFAAS using the same methodology described previously, with the exception that the Pacific samples were not acidified with HNO_3 (Seastar), since they had already been acidified during the chelexing process. The analyses of the water samples from the Pacific are pending.

2.6 Photosynthesis measurements

Following incubation, bottles were removed from the incubator, covered with a black garbage bag (to avoid damaging the phytoplankton by exposing them to direct sunlight) and transported into the lab for filtering. Prior to filtering, each bottle was gently shaken in order to homogenize its contents. For total radioactivity analysis, 1 mL was taken out of each bottle and added to 100 µl of phenethylamine (Aldrich), this was performed in duplicate. A given volume of water was then filtered onto a 0.2, 2.0 or 20 µm-membrane filter. Following the filtration of each sample, the filtering apparatus was rinsed with 10 mL of 0.2 µm-filtered lake water, to ensure that minimal amounts of phytoplankton remained adhered to the apparatus. Filters were removed from the apparatus and immediately frozen for future ^{14}C analysis. Analysis of ^{14}C in was carried out by liquid scintillation at the University of Western Ontario.

2.7 Chlorophyll *a* analysis

Following incubation, bottles were removed from the incubator, covered with a black garbage bag (to avoid damaging the phytoplankton by exposing them to direct sunlight) and transported into the lab for filtering for chlorophyll *a* analysis. Prior to filtering, each bottle was gently shaken in order to homogenize its contents. A given volume of water was then filtered onto a 0.2, 2.0 or 20 µm-membrane filter. Following the filtration of each sample, the filtering apparatus was rinsed with 10 mL of 0.2 µm-filtered lake or seawater, to ensure that minimal amounts of phytoplankton remained adhered to the apparatus. Filters were removed from the apparatus, added to a 90% acetone solution and refrigerated in the dark for approximately 24 hours (refer

to Appendix A) before being analyzed for chlorophyll *a* content using a fluorometer (Turner Designs, model TD-700) using the method of Welschemeyer (1994).

2.8 Statistical analysis

Statistical analysis was performed on the results from all experiments using the software program Statistica (StatSoft, Inc.). Analysis of variance (ANOVA) tests were performed for each experiment in order to determine whether any treatments were statistically different from the others. If the ANOVA test was statistically significant, Neuman-Keuls post hoc tests were run to determine which specific treatments varied from the others. All statistical differences are based on $p<0.05$. The results of the statistical analysis for all experiments can be found in Appendices B though M.

CHAPTER 3: CU AND MN ENRICHMENT EXPERIMENTS: EASTERN BASIN OF LAKE ERIE AND GRAND RIVER

3.1 Cu and Mn enrichment experiments: Eastern Basin of Lake Erie and Grand River, November 2001

3.11 Introduction

A research cruise on Lake Erie was conducted from November 5th to November 9th, 2001 aboard the CCGS *Limnos*. Experiments were conducted in order to examine the response of phytoplankton from the Eastern Basin of Lake Erie and the Grand River to additions of various concentrations of Cu and Mn. All experiments were carried out using trace metal clean techniques (Nriagu *et al.*, 1993) in a Class 100 portable clean room fixed to the deck of the CCGS *Limnos* (refer to Nriagu *et al.*, 1993).

Lake water was collected at depth and delivered directly into the clean laboratory via a trace metal clean pumping system (polyethylene-coated Teflon® tubing suspended at depth, using a Kevlar line, which was weighted with a Teflon® – coated stainless steel weight), employing a Teflon® double-diaphragm pneumatic pump (Husky model no 307).

Incubations were carried out in a ship-deck incubator through which surface lake water was continuously pumped. The transparent walls of the incubator were covered with two layers of neutral density screen in order to simulate the light at 10 m depth.

Three stations were sampled during the course of this cruise, Station 23 (November 5th 2001) and Station 938 (November 6th 2001) in the Eastern basin of Lake Erie, as well as at the mouth of the Grand River (November 8th 2001) between Dunnville and Port Maitland, Ontario (Figure 7).

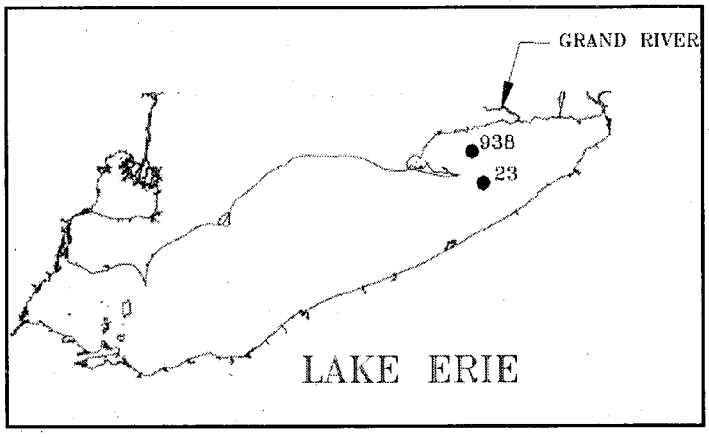

Figure 7: Map of Lake Erie showing the position of the study sites in the Eastern Basin of the lake.

At each station, water was sampled and then filtered through a 0.2 μm-filter (using the methodology described in Section 2.1). At Stations 23 and 938, 200 mL were filtered in this manner. A smaller volume of water (100 mL) was filtered from the Grand River due to the higher concentration of suspended solids in the river as compared with the lake. Filtrations were performed in duplicate and samples were frozen for analysis of total dissolved concentrations of Cu and Mn by GFAAS upon return to Ryerson University (refer to Chapter 2)

3.12 Methods

3.121 Station 23 and Station 938

A similar experimental design was employed at both Station 23 and Station 938. Lake water was collected from a 10 m depth, filtered through a 210 μm-filter (Spectrapor), and placed into polycarbonate bottles (18 × 1300 mL), that had been previously rinsed with 210 μm-filtered lakewater. The samples were then submitted to 5 different treatments in addition to a control: 5 nM Cu, 20 nM Cu, 10 nM Mn, 5 nM Cu + 10 nM Mn and 20 nM Cu + 10 nM Mn (Table 2). Each treatment was prepared in triplicate.

Table 2: Metal concentrations (nM) added to each treatment at Stations 23 and 938 in Lake Erie in November 2001.

Treatment number	Metal additions
1	Control
2	10 nM Mn
3	5 nM Cu
4	20 nM Cu
5	5 nM Cu + 10 nM Mn
6	20 nM Cu + 10 nM Mn

Following the metal additions, treatments from Stations 23 and 938 were incubated for 12.5 hours and 19.5 hours respectively, after which 5 μCi of radioactive [^{14}C]-HCO_3^- were added to each treatment and the bottles were returned to the incubator for a further 5 hours and 5.75 hours respectively. Following a total incubation time of 17.5 hours at Station 23 and 24.25 hours at Station 938, the samples were transported to the clean room for filtering. From the experiment at Station 23, 250 mL from each bottle were filtered onto a 20 μm-filter, 100 mL onto a 2 μm-filter and 50 mL onto a 0.2 μm-filter. Filtrations from Station 23 were performed in duplicate, one for ^{14}C analysis and one for chlorophyll *a* analysis.

In order to increase the number of replicates, size-fractionated filtering was not performed at Station 938. From Station 938, 100 mL from each bottle were filtered onto a 0.2 μm-filter and filtrations were performed in quadruplicate two for ^{14}C analysis and two for chlorophyll *a* analysis.

3.123 Grand River

On November 8th 2001, 30 L of water was collected from the Grand River between Dunnville and Port Maitland. As the CCGS *Limnos* was too large to enter the Grand River, a smaller motor boat was used. A polycarbonate bottle (2 L) was dipped into the water, and brought back to the surface at a slight angle. A total of fifteen bottles were filled in this manner.

Upon return to the CCGS *Limnos,* a similar experimental design as was used at Station 938 was employed. Polycarbonate bottles (18 × 1300 mL) were filled with

unfiltered Grand River water and spiked with varying concentrations of Mn and Cu (Table 3). Each treatment was prepared in triplicate.

Table 3: Metal concentrations added to water sampled from the Grand River in November 2001

Treatment number	Metal Additions
1	Control
2	10 nM Mn
3	20 nM Cu
4	200 nM Cu
5	20 nM Cu + 10 nM Mn
6	200 nM Cu + 10 nM Mn

Higher concentrations of Cu (20 nM Cu and 200 nM Cu) were used in the Grand River experiments as opposed to the Lake Erie ones since the Grand River is a lot more particle-rich than Lake Erie. It was therefore expected that most of the added Cu would form surface complexes and would therefore not be available for uptake by the organisms.

Following 18 hours of incubation, 5 μCi of radioactive $[^{14}C]$-HCO_3^- were added to each treatment. The bottles were then returned to the incubator for a further 5.25 hours. Following a total of 23.25 hours incubation, 25 mL from each bottle were filtered onto a 0.2 μm-filter. Filtrations were performed six times per bottle, three for ^{14}C analysis and three for chlorophyll *a* analysis.

3.13 Results

3.131 Station 23

Total phytoplankton assemblages were not affected by the metal additions; no significant effect ($p<0.05$) was detected for phytoplankton biomass or for photosynthetic efficiency (Figure 8) (Appendix B1).

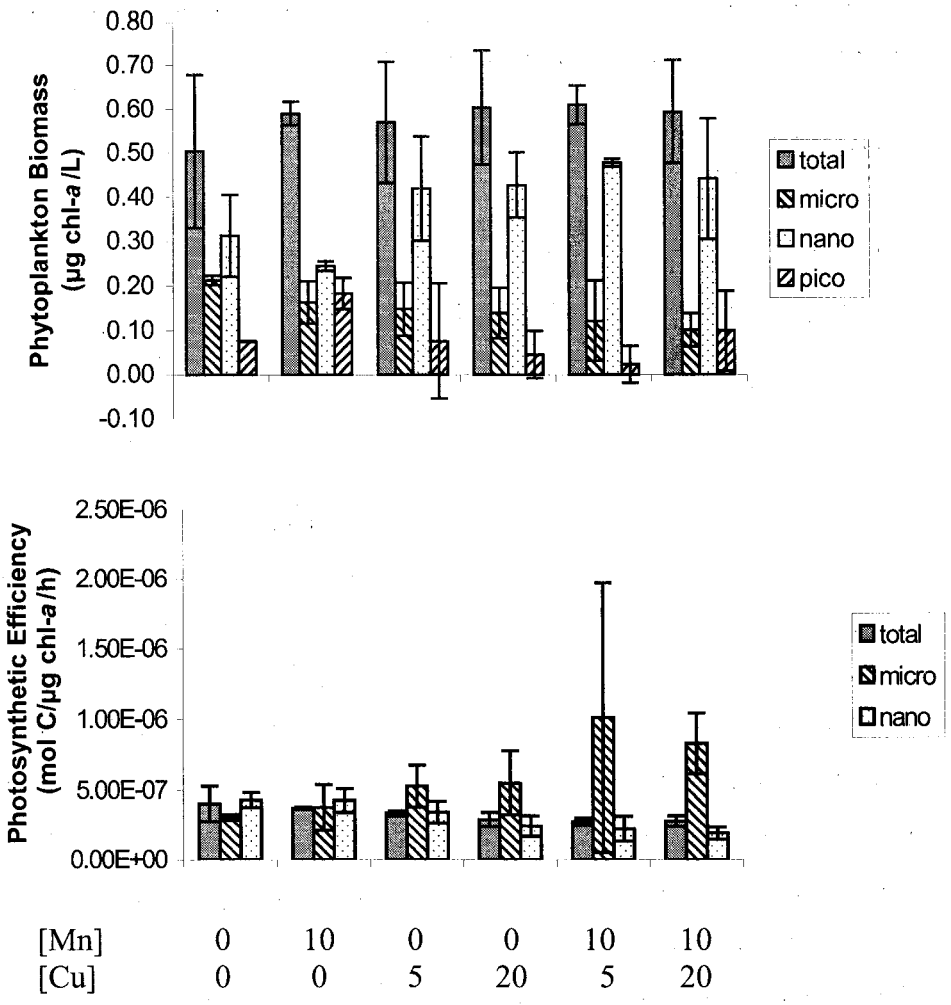

Figure 8: Response of phytoplankton (biomass and photosynthetic efficiency) sampled from Station 23 in November 2001 and treated for 24 hours with various additions of Mn and Cu (nM). All values are mean ± standard deviation.

The microplankton assemblage (>20 µm) was also unaffected by the metal additions; no significant effect ($p<0.05$) was detected on phytoplankton biomass or on photosynthetic efficiency ($p<0.05$) (Appendix B2).

Regarding the nanoplankton size fraction (>0.2 µm), metal additions did not affect the phytoplankton biomass ($p<0.05$). Metal additions did however affect the photosynthetic efficiency of the nanoplankton assemblage ($p<0.05$). Treatments with additions of 10 nM Mn had significantly greater photosynthetic efficiencies than all other treatments with the exception of the control and the treatment with 5 nM Cu added. In addition, the treatment with 5 nM Cu +10 nM Mn added and the treatment

with 20 nM Cu+10 nM Mn added had significantly lower photosynthetic efficiencies than the control ($p<0.05$) (Appendix B3).

The picoplankton (<0.2 μm) biomass was unaffected by metal additions ($p<0.05$). As for the photosynthetic activity of the picoplankton assemblage, statistical analysis could unfortunately not be performed. This was due to the fact that in some cases, the quantity of μg of chlorophyll *a* attributable to picoplankton was equal to 0; thus the photosynthetic activity measured as a ratio of mol C fixed/μg chlorophyll *a*/h would be an unrealistic value (Appendix B4).

The total dissolved background concentrations of Cu and Mn at Station 23 in November 2001 as determined using GFAAS were 9.89 nM Cu and 2.73 nM Mn.

3.132 Station 938

The photosynthetic efficiency of the total phytoplankton assemblage was not affected by the metal additions ($p<0.05$). Phytoplankton biomass was however affected ($p<0.05$). The treatment with 10 nM Mn added had significantly greater phytoplankton biomass ($p<0.05$) than all other treatments, with the exception of the treatment with 20 nM Cu added. Also, the treatment with 20 nM Cu added had significantly greater phytolankton biomass ($p<0.05$) than the treatments with 5 nM Cu added, 5 nM Cu + 10nM Mn added, and 20 nM Cu+10 nM Mn added. In addition, the treatment with 20 nM Cu + 10 nM Mn added had significantly lower phytoplankton biomass ($p<0.05$) than the control (Figure 9) (Appendix C).

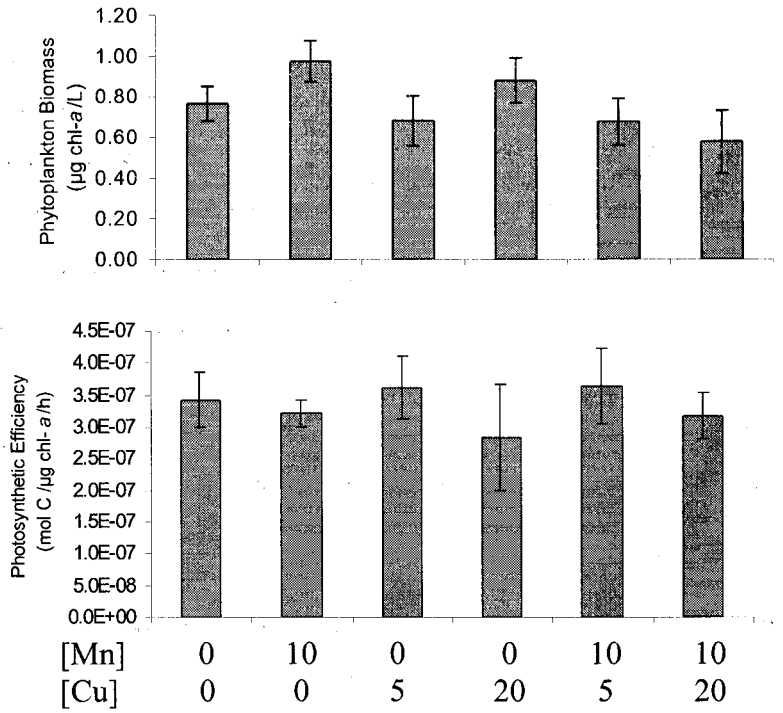

Figure 9: Response of phytoplankton (biomass and photosynthetic efficiency) sampled from Station 938 in November 2001 and treated for 24 hours with various additions of Mn and Cu (nM). All values are mean ± standard deviation.

The total dissolved background concentrations of Cu and Mn at Station 938 in November 2001, as determined using GFAAS, were 11.37 nM Cu and 16.02 nM Mn.

3.133 Grand River

The total phytoplankton assemblage was not affected by the metal additions with regards to photosynthetic efficiency ($p<0.05$). However, significant differences were found with regards to phytoplankton biomass ($p<0.05$). The treatment with 200 nM Cu + 10 nM Mn added, had significantly greater chlorophyll *a* concentrations ($p<0.05$) than all other treatments with the exception of the control (Figure 10) (Appendix D).

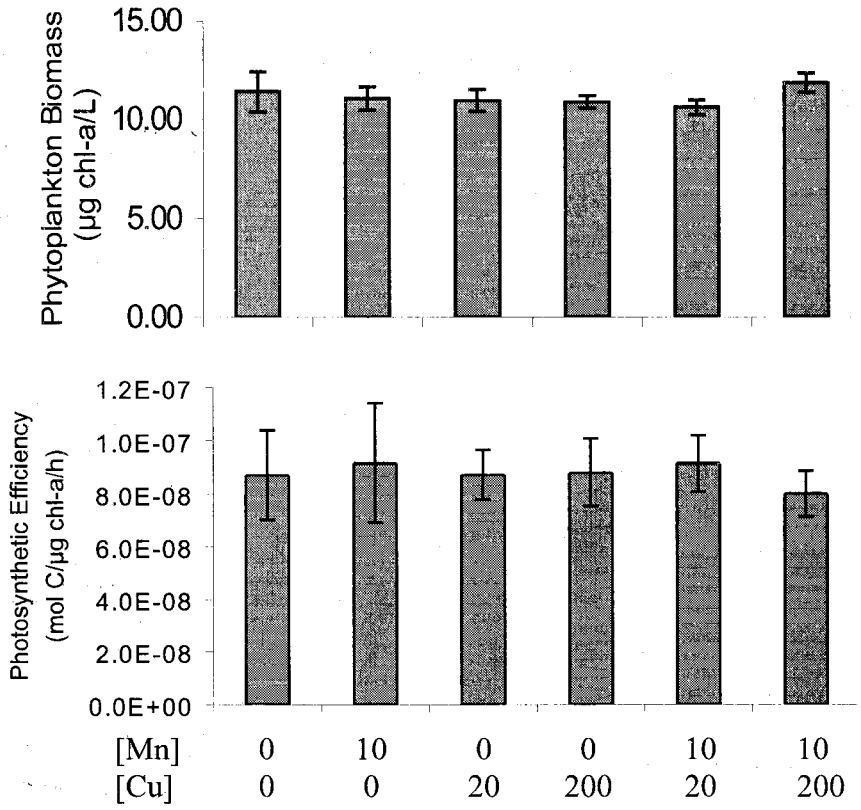

Figure 10: Response of phytoplankton (biomass and photosynthetic efficienty) sampled from the Grand River in November 2001 and treated for 24 hours with various additions of Mn and Cu (nM). All values are mean ± standard deviation.

The total dissolved background concentrations of Cu and Mn in the Grand River in November 2001, as determined using GFAAS, were 15.56 nM Cu and 156.24 nM Mn.

3.134 Summary of Results

The results from Station 23 indicate that Cu additions of up to 20 nM did not produce a significant decrease in phytoplankton biomass for any size group following 17.5 hours incubation. In addition, no significant difference was observed amongst the treatments for the total phytoplankton assemblage and the microplankton size group with regards to photosynthetic efficiency. The only significant difference observed at Station 23 was in the photosynthetic efficiency of the nanoplankton size fraction, where the treatment with 10 nM Mn added had significantly greater photosynthetic efficiency than all treatments with the exception of the control and the treatment with

5 nM Cu added. In addition, the treatments with 5 nM Cu + 10 nM Mn and 20 nM Cu + 10 nM Mn added had significantly lower photosynthetic efficiencies than the control.

The results from Station 938 reveal no significant difference amongst the treatments with regards to photosynthetic efficiency. However, significant differences in total phytoplankton biomass were observed. The treatment with 10 nM Mn added and the treatment with 20 nM Cu added had significantly greater phytoplankton biomass than most of the other treatments. The only treatment that had significantly lower phytoplankton biomass than the control was that with 20 nM Cu +10 nM Mn added.

As for the Grand River experiments, concentrations of added Cu up to 200 nM had no significant effect on photosynthetic activity. However, with regards to phytoplankton biomass, the treatment with 200 nM + 10 nM Mn had significantly greater biomass than all other treatments with the exception of the control.

3.14 Discussion

3.141 Station 23

Analysis of the data from Station 23 in Lake Erie in November 2001 indicate that there is no significant decrease in phytoplankton biomass with additions of up to 20 nM Cu. This is surprising given the previous study in Lake Superior in September 2000 in which a significant decrease in phytoplankton biomass was observed with only 5 nM Cu added (Twiss *et al.*, 2003). Although the study in Lake Superior in May 2001 also revealed little sensitivity to Cu, the authors attributed it to the ambient cold water temperature of 3°C that slowed productivity (Twiss *et al.*, 2003). In the present case, the water temperature at Station 23 in November 2001 was 12°C and is therefore not likely to explain the lack of Cu sensitivity. Given that the background concentration of Cu at Station 23 in Lake Erie in November 2001 is comparable to that in Lake Superior in September 2000, (9.89 nM versus 10.5 nM), but that the Mn concentration in Lake Erie was higher than in Lake Superior (2.73 nM versus 1.19 nM), the differences in the responses observed may be possibly due to the difference in total background concentrations of Mn in each lake. The higher concentration of

Mn in Lake Erie may have lessened the toxic effect of Cu, thus accounting for the lack of a toxic response.

The difference in the responses of phytoplankton to Cu in the Lake Superior experiment versus the Lake Erie experiment may also be due to the fall turnover that occurred just prior to the Lake Erie experiment. Runoff from the fall rains into the Grand River and the mixing of the deeper waters of the lake during fall turnover could have resulted in an increased amount of particles and dissolved organic carbon (DOC) in the surface waters of the Eastern Basin of Lake Erie, particles to which free Cu may bind with relatively high affinity. The fall turnover in Lake Erie in November 2001 may therefore have rendered the sampled water very rich in particles and organic matter, thus decreasing the bioavailability of the Cu to the phytoplankton. Further study of the effects of Cu on phytoplankton in Lake Erie is needed in order to confirm if the results observed in Lake Erie were possibly due to the fall turnover, indicating that Cu toxicity may have a seasonal component.

The only significant differences observed at Station 23 were in the nanoplankton size fraction; the treatment with 5 nM Cu +10 nM Mn added and the treatment with 20 nM Cu + 10 nM Mn added had significantly lower photosynthetic efficiencies than the control and the treatment with 10 nM Mn added. If Cu were having a toxic effect on the phytoplantkon, we would expect the treatments with 5 nM Cu added and 20 nM Cu added to also show significantly lower photosynthetic efficiencies than the control, which is not the present case. The significant differences observed therefore cannot be explained at this time. However, due to the breaking of one of the filters, there were a small number of replicates in the control group (N=2) which may have been a factor.

3.142 Station 928

The significant differences observed with regards to phytoplankton biomass at Station 928 are interesting when compared with past research. The treatment with 10 nM Mn added and the treatment with 20 nM Cu added had significantly greater phytoplankton biomass than most of the other treatments. The increase in the treatment with 10 nM added could explained by the fact that the Lake Erie may be Mn limited. However, the increase in biomass in the treatment with 20 nM Cu added is

surprising, since even sub-nanomolar concentrations of Cu are known to be toxic to phytoplankton (Van den Berg *et al.*, 1979; Sunda and Guillard, 1976; Brand *et al.*, 1986; Gerringa *et al.*, 1995; Sunda and Huntsman, 1998b, 1998c). Even if we were to subscribe to Coale's (1991) theory that Cu inhibits grazers, so that the net effect of added Cu is an apparent stimulation, we would also expect an increase in biomass in the treatment with 20 nM Cu + 10 nM Mn added, which is not the present case. The significant differences observed cannot therefore be explained at this time.

3.143 Grand River

The lack of Cu toxicity observed in the Grand River, with additions of up to 200 nM Cu, may be due to the high background levels of Mn or to the fact that the added Cu may not be bioavailable to organisms. We would expect the Grand River to be very rich in particles and organic matter since it is subjected to discharges from agricultural land as well as from a number of cities (refer to Chapter 1). Free Cu could possibly bind to these particles and organic matter with relative ease, rendering the Cu less bioavailable to the phytoplankton. In a study of copper complexation in Swiss rivers, it was suggested that although there was no correlation between Cu complexation and dissolved organic carbon, colloidal particles may play a role as Cu-binding ligands (Xue *et al.*, 1996). The possible role of colloidal particles as ligands could explain the lack of toxicity observed in the present case.

The significantly higher phytoplankton biomass in the treatment with 200 nM Cu + 10 nM Mn added in the Grand River is surprising given the fact that Cu is known to be toxic to phytoplankton at very low concentrations. It is clear from Figure 3 and from the mean concentrations of chlorophyll *a* in Appendix D, that there appears to be no observable difference amongst the treatments. Although statistical analysis indicates a significant difference, this difference seems to only have been revealed since the standard deviations of all the treatments are so low. The significant differences observed cannot therefore be explained at this time.

3.2 Cu and Mn enrichment experiments: Eastern Basin of Lake Erie and Grand River, July 2002

3.21 Introduction

In order to confirm whether the results observed on Lake Erie and the Grand River in November 2001 were in fact due to the fall turnover and runoff from fall rains, experiments were again carried out at Station 23 and in the Grand River, in the summer of 2002, when the lake was stratified. If the lack of response to the metal additions in November 2001 was in fact due to the fall turnover, we would expect to observe a toxic response to additions of 5 nM Cu and 20 nM Cu at Station 23 in July 2002.

3.22 Methods

3.221 Station 23

The methodology employed was similar to that used at Station 23 in November 2001. The same concentrations of Cu (0 nM, 5 nM, 20 nM) and Mn (0 nM, 10 nM) were added on 22nd July 2002 as were added in November 2001 (Table 4). In the event that no toxicity was once again observed with additions of up to 20 nM Cu, we also prepared treatments of 60 nM Cu and 60 nM Cu + 30 nM Mn (Table 4) in the hopes of determining whether this amount of added Cu was toxic to phytoplankton.

Table 4: Metal concentrations (nM) added to water sampled from Station 23 on Lake Erie on July 22nd 2002.

Treatment number	Metal Additions
1	Control
2	10 nM Mn
3	5 nM Cu
4	20 nM Cu
5	5 nM Cu + 10 nM Mn
6	20 nM Cu + 10 nM Mn
7	60 nM Cu
8	60 nM Cu + 30 nM Mn

Following the metal additions, treatments were incubated for 20 hours, spiked with 3 μCi radioactive [^{14}C]-HCO_3^-, and returned to the incubator for a further 5 hours. Following a total of 25 hours incubation, filtrations for chlorophyll *a*, ^{14}C analysis and total metal concentrations were performed in the same manner as at Station 23 in November 2001 with the exception that the microplankton size fraction (>20 μm) was omitted in order to increase the number of replicates (refer to Section 3.)

3.222 Grand River

The Grand River was sampled in the morning of 24th July 2002. Water was collected from three locations on the Grand River using 20 L carboys in the same manner as in November 2001:

-**GR-1** (in the vicinity of Dunnville)
at 42 53'.908 N and 79 36'.875 W (9:20 am)

-**GR-2** (further down the river)
at 42 52'.211 N and 79 34'.522 W (9:48 am)

-**GR-3** (at the mouth of the river, just south of Port Maitland)
at 42 50'.997 N and 79 34'.872 W. (10:02 am)

Upon return to the C.C.G.S. *Limnos*, polycarbonate bottles (24 ×1300 mL) were rinsed and filled with unfiltered Grand River water and then spiked with varying concentrations of Mn and Cu. Samples from all three stations were submitted to 3 different treatments (20 nM Cu, 100 nM Cu and 200 nM Cu) and a control (Table 5). Each treatment was prepared in duplicate.

Table 5: Metal concentrations (nM) added to water sampled from three sites along the Grand River between Dunnville and Lake Erie on 24th July 2002

Treatment number	Metal Additions
1	Control
2	20 nM Cu
3	100 nM Cu
4	200 nM Cu

Following the metal additions, treatments were incubated for 19 hours, spiked with 1 μCi of radioactive [^{14}C]-HCO_3^-, and returned to the incubator for a further 5 hours. Following incubation, 30 mL from each bottle was filtered onto a 0.2 μm-filter and 50 mL onto a 2.0 μm-filter. Filtrations were performed in duplicate, one for ^{14}C analysis and one for chlorophyll *a* analysis.

3.23 Results

3.231 Station 23

The total phytoplankton assemblage was affected by the metal additions; although no significant effect on photosynthetic efficiency was detected ($p<0.05$), a significant effect was observed with regards to phytoplankton biomass ($p<0.05$). The treatments with 60 nM Cu added and 60 nM Cu + 30 nM Mn added had significantly lower phytoplankton biomass ($p<0.05$) than all other treatments (Figure 11) (Appendix E1).

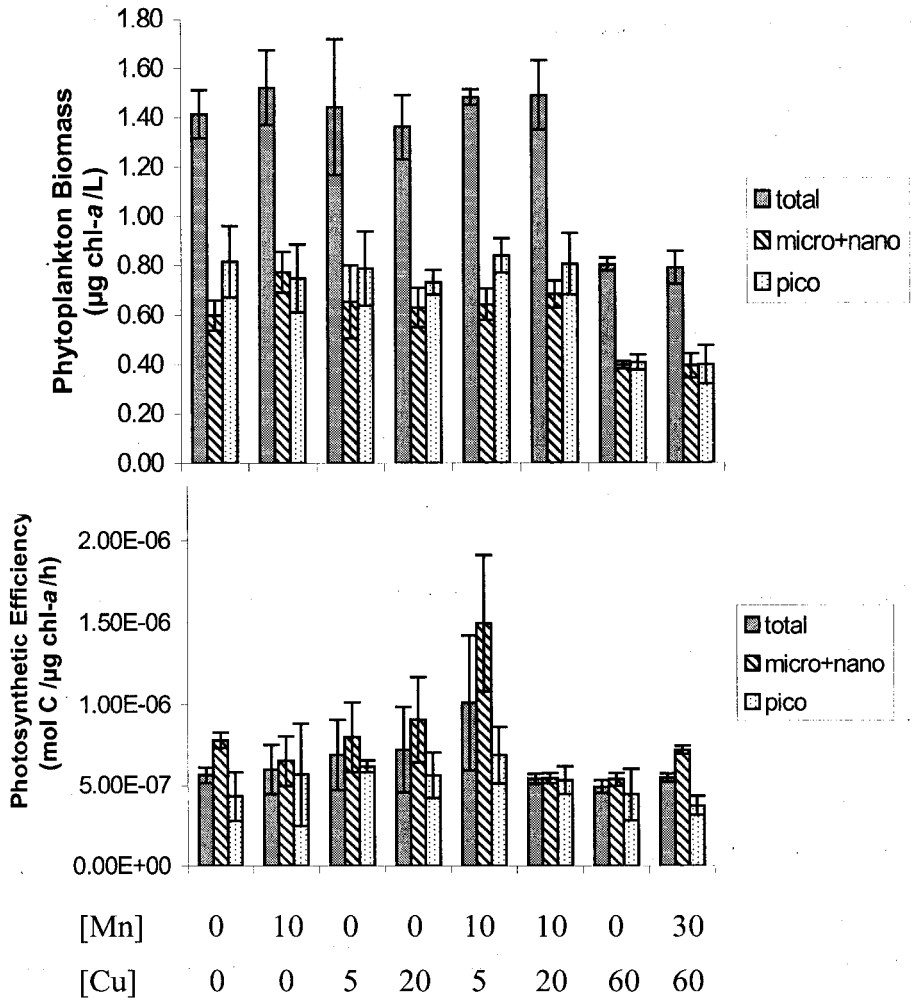

Figure 11: Response of phytoplankton (biomass and photosynthetic efficiency) sampled from the Station 23 in July 2002 and treated for 24 hours with various additions of Mn and Cu (nM). All values are mean ± standard deviation.

The nanoplankton + microplankton assemblage as well as the picoplankton assemblage revealed similar results; the treatments with 60 nM Cu and 60 nM Cu + 30 nM Mn added had significantly lower phytoplankton biomass ($p<0.05$) than all other treatments (Appendix E2 and Appendix E3).

In summary, all size classes showed a significant decrease in phytoplankton biomass in treatments with 60 nM Cu added and 60 nM Cu + 30 nM Mn added. There was no significant difference in photosynthetic activity amongst the treatments throughout all size classes.

The total dissolved background concentrations of Cu and Mn at Station 23 in the Eastern Basin of Lake Erie in July 2002, as determined using GFAAS, were 9.92 nM Cu and 4.80 nM Mn.

3.232 Grand River

GR-1

Total phytoplankton assemblages were not affected by the metal additions; no significant effect ($p<0.05$) on phytoplankton biomass or photosynthetic efficiency was detected (Figure 12) (Appendix F1).

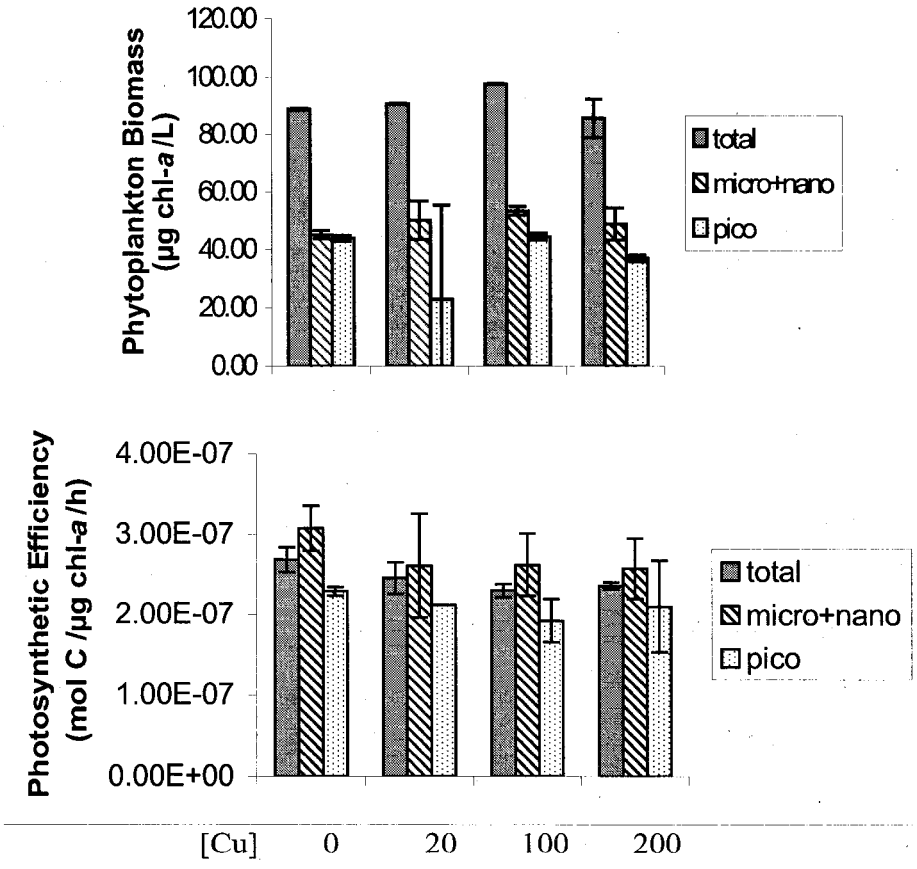

Figure 12: Response of phytoplankton (biomass and photosynthetic efficiency) sampled from the Grand River (GR-1) in July 2002 and treated for 24 hours with various additions of Cu (nM). All values are mean ± standard deviation.

The microplankton + nanoplankton (>0.2 µm) and picoplankton (<0.2 µm) assemblages revealed similar results, with no significant difference amongst the

treatments ($p<0.05$) with regards to phytoplankton biomass or photosynthetic efficiency (Appendix F2 and Appendix F3).

The total dissolved background concentrations of Cu and Mn at GR-1 in July 2002, as determined using GFAAS were 13.09 nM Cu and 19.47 nM Mn.

GR-2

Total phytoplankton assemblages were not affected by the metal additions; no significant effect ($p<0.05$) on phytoplankton biomass or photosynthetic efficiency was detected (Figure 13) (Appendix G1).

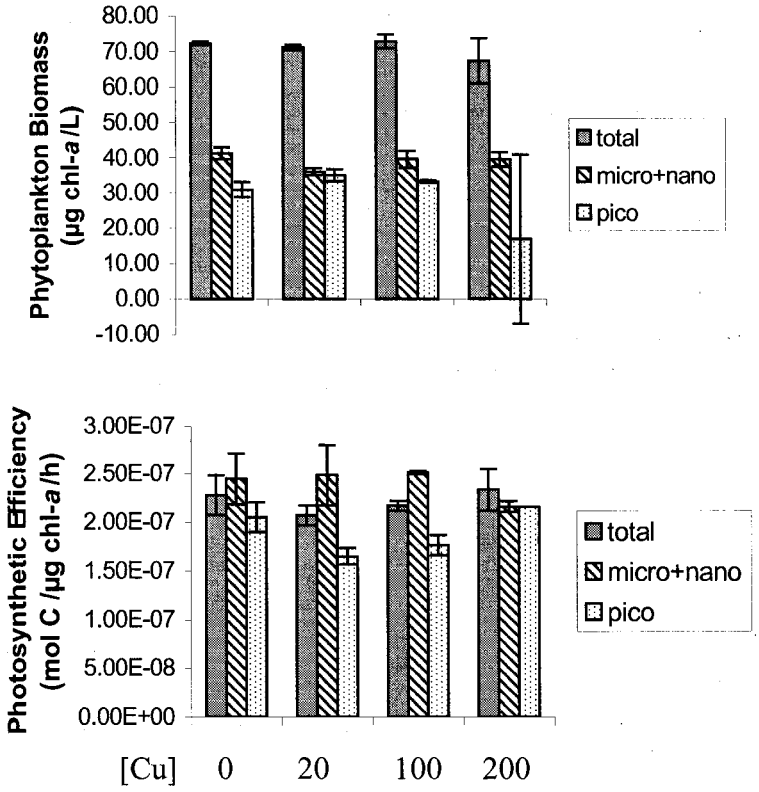

Figure 13: Response of phytoplankton (biomass and photosynthetic efficiency) sampled from the Grand River (GR-2) in July 2002 and treated for 24 hours with various additions of Cu (nM). All values are mean ± standard deviation.

The microplankton + nanoplankton (>0.2 µm) and picoplankton (<0.2 µm) assemblages revealed similar results, with no significant difference amongst the

treatments ($p<0.05$) with regards to phytoplankton biomass or photosynthetic efficiency (Appendix G2 and Appendix G3).

The total dissolved background concentrations of Cu and Mn in the Grand River in November 2001 as determined using GFAAS were 14.6 nM Cu and 15.09 nM Mn.

GR-3

Total phytoplankton assemblages were not affected by the metal additions; no significant effect ($p<0.05$) on phytoplankton biomass or photosynthetic efficiency was detected (Figure 14) (Appendix H1).

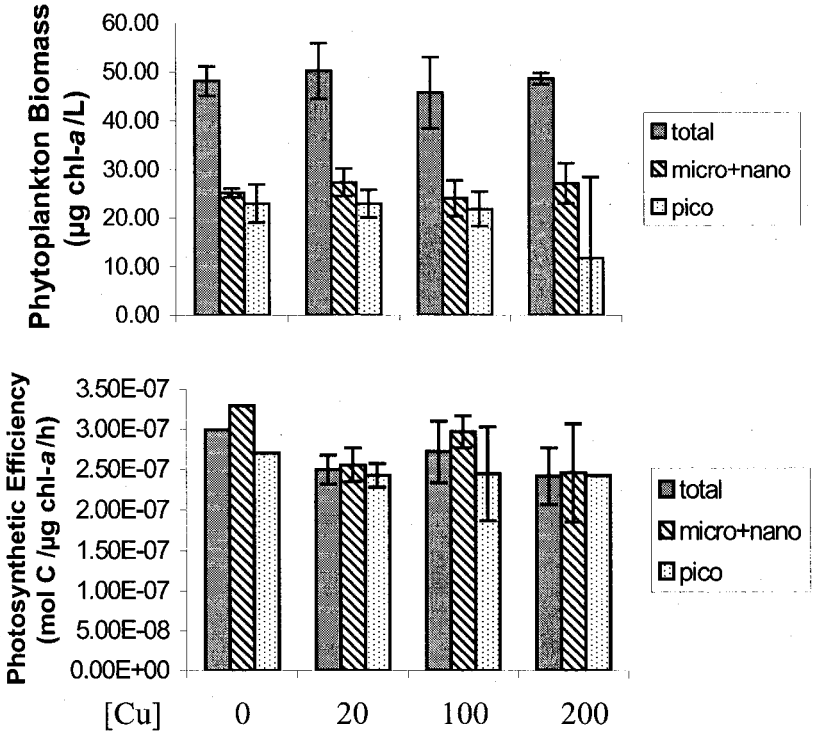

Figure 14: Response of phytoplankton (biomass and photosynthetic efficiency) sampled from the Grand River (GR-3) in July 2002 and treated for 24 hours with various additions of Cu (nM). All values are mean ± standard deviation.

The microplankton + nanoplankton (>0.2 µm) and picoplankton (<0.2 µm) assemblages revealed similar results, with no significant difference amongst the

treatments ($p<0.05$) with regards to phytoplankton biomass or photosynthetic efficiency (Appendix H2 and Appendix H3)

The total dissolved background concentrations of Cu and Mn in the Grand River in November 2001 as determined using GFAAS were 13.06 nM Cu and 20.01 nM Mn.

3.233 Summary of results

Results from Station 23 in Lake Erie in July 2002 indicate a significant decrease in phytoplankton biomass in treatments with 60 nM Cu added and 60 nM Cu + 30 nM Mn added, across all size classes. There was no significant difference in photosynthetic efficiency amongst the treatments throughout all size classes. As for the experiments conducted on the Grand River, no significant decrease in phytoplankton biomass or photosynthetic efficiency was observed with concentrations of added Cu of up to 200 nM.

3.24 Discussion

3.241 Station 23

As seen in November 2001 at Station 23, there was no significant difference in phytoplankton biomass at Station 23 in July 2002 with treatments of up to 20 nM of added Cu. Even though no toxicity was observed in Lake Erie with additions of up to 20 nM Cu, when 60 nM Cu was added to samples from Station 23 in July 2002, a significant decrease in chlorophyll *a* was observed. It is interesting that the treatment with 30 nM Mn and 60 nM Cu did not differ with respect to chlorophyll *a* as compared to the treatment of 60 nM Cu, indicating that the increase in Mn concentration did not seem to lessen the toxic effect of the Cu over the time frame of this experiment

The difference in the responses of phytoplankton to additions of 5 nM Cu and 20 nM Cu in Lake Superior in September 2000 versus Lake Erie is probably therefore due to reasons other than the fall turnover. A possible explanation for the difference in responses between the two lakes is that the higher background concentrations of Mn in Lake Erie may have lessened the toxic effects of the added

Cu. Another possible reason for the difference in the response of phytoplankton between the two lakes may be that the Cu added to the Lake Superior samples was more bioavailable to phytoplankton than the Cu added to the Lake Erie samples. One explanation for a possible difference in the bioavailability of added Cu between the two lakes may be due to the different chemical characteristics of the lakes: Lake Superior is considered oligotrophic whereas Lake Erie is mesotrophic. A study of Swiss lakes found that Cu complexation was the strongest in eutrophic lakes, in comparison to an oligotrophic lake (Xue *et al.*, 1996). This was explained by the presence of low levels of very specific ligands that appear to be linked to high algal productivity (Xue *et al.*, 1996).

Like the studied Swiss Lakes, the chemical speciation of Cu in Lake Superior is probably tightly regulated by organic ligands, and that these ligands are most likely present in concentrations very similar to the concentration of total dissolved Cu as seen in oligotrophic marine systems (Moffett, 1995). It can therefore be suggested that any addition of Cu into this type of environment would saturate the organic Cu-complexing ligands and any excess Cu would be bioavailable to phytoplankton (Twiss *et al.*, 2003). In Lake Erie, the speciation of Cu is also probably dominated by organic complexes. However, since Lake Erie is more eutrophic than Lake Superior, the organic ligand concentration may be higher than the organic ligand concentration of Lake Superior. Given that the total dissolved concentrations of Cu in Lake Superior and Lake Erie are similar (~10 nM), a certain amount of Cu added to Lake Superior water may readily saturate the organic Cu-complexing ligands and readily spill over and be available to organisms. However, it may take the addition of a larger concentration of Cu to produce the same effect in Lake Erie, if Lake Erie did in fact have a higher concentration of organic Cu-complexing ligands than Lake Superior.

One caveat to this explanation is that the total phytoplankton biomass in the Lake Superior experiments was not lower than the total phytoplankton biomass in the Lake Erie experiments (~1.5 μg chlorophyll *a*/L in Lake Superior in September 2001 versus ~0.70 μg chlorophyll *a*/L in November 2001 and ~1.4 μg chlorophyll *a*/L in Lake Erie in July 2002). However, as noted in Chapter 1, not all species of plankton produce Cu-complexing ligands, therefore differences in species composition (and in

turn possible differences in the concentration of Cu-complexing ligands) between the two lakes or different inherent Cu sensitivities of the phytoplankton in each lake could also explain the increased sensitivity to Cu toxicity in Lake Superior in September 2000.

Another explanation for the possible difference in the bioavailability of added Cu in Lake Superior versus Lake Erie may be due to the fact that we would expect there to be more suspended particles in Lake Erie, therefore providing more surfaces to which free Cu could bind.

It is also possible that the difference in the responses of phytoplankton to additions of 5 nM and 20 nM Cu in Lake Superior versus Lake Erie may be due to the phytoplankton sampled in Lake Superior possibly being more Mn limited than the phytoplankton sampled in Lake Erie. The water sampled in Lake Superior was from a depth of 20 m whereas the water sampled in Lake Erie was from a depth of only 7 to 10 m. Since the water sampled in Lake Superior was from a deeper depth, the phytoplankton would be living in darker conditions that those sampled from the shallower depth in Lake Erie. It is known that under darker conditions, phytoplankton requirements for Mn increase. The increased Mn quotas in the Lake Superior phytoplankton compared with the Lake Erie phytoplankton may therefore explain the toxic response in Lake Superior with additions of Cu as low as 5 nM.

3.422 Grand River

These results for the experiments conducted in the Grand River support those from November 2001, in that additions of up to 200 nM Cu do not induce a toxic effect in the Grand River phytoplankton. This may be due to the fact that, as mentioned previously, the Mn concentrations in the Grand River are relatively high, thereby possibly lessening the toxic effects of Cu; alternatively, the lack of toxicity observed may be due to the fact that we know the Grand River to be very rich in particles relative to the Lake Erie, and that these particles would probably have an affinity for binding Cu, rendering the metal unavailable to phytoplankton. These results also indicate that the increase in phytoplankton biomass observed in the

treatment with 200 nM Cu added in November 2001 may not have been typical and representative.

CHAPTER 4: CU AND MN ENRICHMENT EXPERIMENTS: CENTRAL BASIN OF LAKE ERIE, SEPTEMBER 2002

4.1 Introduction

Cu and Mn enrichment experiments were conducted in the Central Basin of Lake Erie during the fall of 2002, aboard the USEPA's R/V *Lake Guardian*. Two studies were conducted during the course of this cruise that followed up on those conducted in the Eastern Basin of Lake Erie and described in the previous chapter. The two stations that were sampled were ER-31M and ER-78M, both located in the Central Basin of Lake Erie (Figure 15).

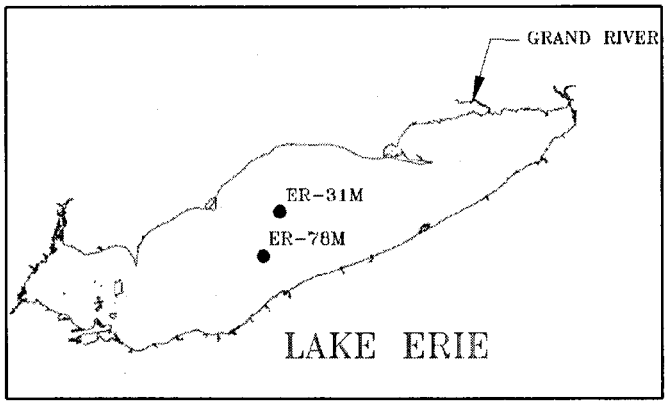

Figure 15: Map of Lake Erie showing the position of the study sites in the Central Basin of the Lake.

The first experiment, at ER-31M, attempted to determine the threshold concentration of added Cu, which evoked a toxic response in Lake Erie phytoplankton. Once the concentration of Cu that produced a toxic effect had been determined, another experiment, at ER-78M, would investigate whether higher concentrations of Mn could lessen the toxic effects of Cu; this would be done by adding various concentrations of Mn to the treatments.

As aboard the CCGS *Limnos*, lake water for all experiments was collected directly from depth using a trace metal clean pumping system (refer to Chapter 3). Since there was no clean room on board the ship, all experiments were conducted in a portable laminar flow hood. Also, since no wet incubator was present, incubations were carried out in a dry incubator (Percival). Hours of light and dark exposure

corresponded to the natural patterns of sunrise and sunset (9h dark, 15h light). Light at 10 m depth was measured using a photometer at Station 78 and was determined to be ~30.2 μmol/m^2/s. The light intensity in the incubator was adjusted to 30-33 μmol/m^2/s by the use of a sheet of plexiglass in order to simulate light at 10 m depth. Incubated bottles were rotated periodically to ensure uniform exposure to light.

Due to logistical constraints, it was not possible to have radioactive ^{14}C on board the R/V *Lake Guardian*. Photosynthetic efficiency was therefore not determined in these experiments. The response of phytoplankton to various additions of Cu and Mn was instead assessed using chlorophyll *a* as a measure of phytoplankton biomass

4.2 Methods

4.21 ER-31M

From the experiment carried out at Station 23 in July 2002, it appears that the threshold for Cu toxicity lies between 20 nM and 60 nM Cu (since acute toxicity was observed with additions of 60 nM Cu but not with 20 nM Cu). In September 2002, we therefore added concentrations of Cu ranging from 0 nM to 70 nM in order to determine the threshold concentration of Cu that induced a toxic response in Lake Erie phytoplankton (Table 6).

Table 6: Metal concentrations (nM) added to water sampled from ER-31M in the Central Basin of Lake Erie in September 2002

Treatment number	Metal Additions
1	Control
2	10 nM Cu
3	20 nM Cu
4	30 nM Cu
5	40 nM Cu
6	50 nM Cu
7	60 nM Cu
8	70 nM Cu

Water was sampled from an 8 m depth on September 15th 2002. Polycarbonate bottles (24 x 300mL) were filled with <210 μm-filtered water, after being rinsed with <210 μm-filtered water. The samples were then subjected to additions of Cu ranging from 0 nM to 70 nM (refer to Table 6). Each treatment was prepared in triplicate. Bottles were then incubated for 24 hours at 21.5 °C (the water temperature at the time of sampling). Following incubation, 100 mL from each bottle were filtered onto a 0.2 μm-filter and 100 mL from each bottle were filtered onto a 2.0 μm-filter for chlorophyll *a* extraction and fluorometric analysis.

At ER-31M, 2 x 200 mL of lake water were filtered and frozen for future analysis of total dissolved Cu and Mn concentrations (refer to Chapter 2).

4.22 ER-78M

Unfortunately, due to time constraints, it was not possible to process the samples from the experiment at ER-31M prior to beginning the experiment at ER-78M. It was therefore decided that in order to determine whether higher concentrations of Mn could lessen the toxic effects of Cu, treatments would include additions of Cu ranging from 30 to 120 nM and additions of Mn of 60 or 120 nM (Table 7). It was hypothesized that since 60 nM of Cu was found to be toxic to phytoplankton at Station 23 in the Eastern Basin of Lake Erie in July 2002 (see Chapter 3), additions of Cu of 60 nM and 120 nM would induce toxic effects on phytoplankton at ER-78M. It was also hypothesized that additions of Mn of 60 and 120 nM would lessen the toxic effects of Cu.

Table 7 Metal concentrations (nM) added to water sampled from ER-78M in the Central Basin of Lake Erie in September 2002

Treatment number	Metal additions
1	control
2	30 nM Cu
3	60 nM Cu
4	120 nM Cu
5	60 nM Mn
6	120 nM Mn
7	30 nM Cu + 60 nM Mn
8	60 nM Cu + 60 nM Mn
9	120 nM Cu + 60 nM Mn
10	30 nM Cu + 120 nMMn
11	60 nM Cu + 120 nM Mn
12	120 nM Cu + 120 nM Mn

The methodology used for the experiment at ER-78M on September 16th 2002 was similar to that employed one day earlier at ER-31M (refer to previous section) with the following modifications:

-Water was sampled from a 10 m depth.

-Since the 210 μm-filter was pierced, polycarbonate bottles were rinsed and then filled with <20 μm-filtered lake water.

-At ER-78M, 2 × 250 mL were filtered and frozen for future GFAAS analysis of total dissolved Cu and Mn concentrations (refer to Chapter 2).

4.3 Results

4.31 ER-31M

Total phytoplankton assemblages were not affected by the metal additions; no significant effect ($p<0.05$) on phytoplankton biomass was detected (Figure16) (Appendix I1).

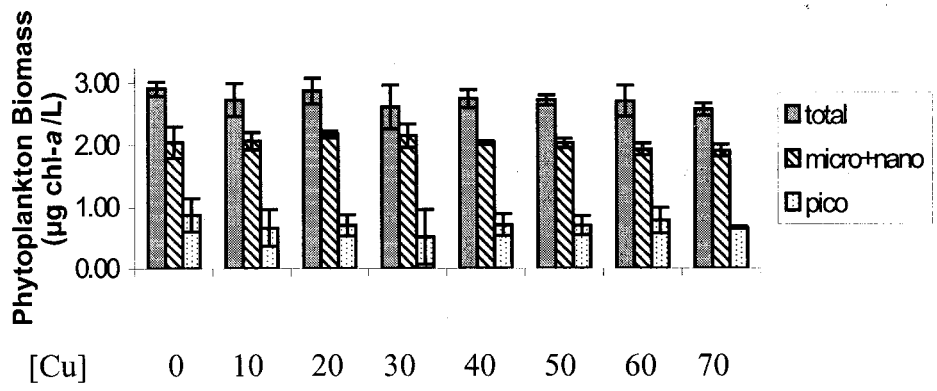

Figure 16: Response of phytoplankton (biomass) sampled from Station ER-31M in July 2002 and treated for 24 hours with various additions of Cu (nM). All values are mean ± standard deviation.

The nanoplankton + microplankton (>2.0 µm) and the picoplankton (0.2 µm-2.0 µm) size fractions were also unaffected by the metal additions; no significant effect ($p<0.05$) on phytoplankton biomass was detected (Appendix I2 and Appendix I3).

The total dissolved background concentrations of Cu and Mn at Station ER-31M in September 2002 as determined using GFAAS were 9.42 nM Cu and 5.18 nM Mn.

4.32 ER 78 M

The total phytoplankton assemblage was affected by the metal additions: a significant effect on phytoplankton biomass was detected ($p<0.05$). The treatments with 120 nM Cu added, 120 nM Cu + 60 nM Mn, and 120 nM Cu + 120 nM Mn added had significantly lower phytoplankton biomass than all other treatments ($p<0.05$). In addition, the treatments with 30 nM Cu + 60 nM Mn added, 60 nM Cu added and 60 nM Cu + 120 nM Mn added also had significantly lower phytoplankton biomass ($p<0.05$) than the control (Figure 17) (Appendix J1).

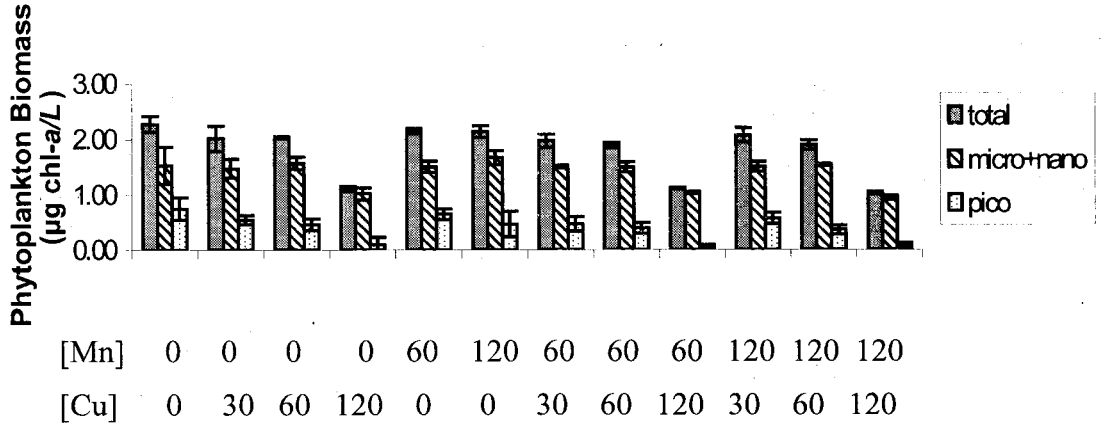

Figure 17: Response of phytoplankton (biomass) sampled from Station ER-78M in July 2002 and treated for 24 hours with various additions of Cu and Mn (nM). All values are mean ± standard deviation.

The nanoplankton assemblage (2.0 µm-20 µm) was also affected by the metal additions; the treatments with 120 nM Cu added, 120 nM Cu + 60 nM Cu, and 120 nM Cu + 120 nM Mn added had significantly lower phytoplankton biomass ($p<0.05$) than all other treatments (Appendix J2)

The picoplankton assemblage (0.2 µm-2.0 µm) was also affected by the metal additions; the treatments with 120 nM Cu added, 120 nM Cu + 60 nM Cu, and 120 nM Cu + 120 nM Mn added had significantly lower phytoplankton biomass than all other treatments. In addition, the treatments with 60 nM Cu added and 60 nM Cu + 120 nM Mn added had significantly lower phytoplankton biomass than the control (Appendix J3)

The total dissolved background concentrations of Cu and Mn at Station ER-78M in September 2002 as determined using GFAAS were 9.42 nM Cu and 65.90 nM Mn. However, these concentrations are probably overestimates since it is believed that the filter broke during the course of filtering.

4.33 Summary of results

The experiment conducted at ER-31M revealed that additions of Cu ranging from 0 to 70 nM had no effect on phytoplankton biomass and this occurred across all

size groups. As for the experiment conducted at ER-78M, a significant decrease in phytoplankton biomass was observed across all size groups in treatments that had 120 nM Cu added, 120 nM Cu +60 nM Mn added and 120 nM Cu+120 nM Mn added. The picoplankton and total phytoplankton assemblages also showed a significant decrease in chlorophyll *a* relative to only the controls with additions of 60 nM Cu + 60 nM Mn and 60 nM Cu + 120 nM Mn. Interestingly, the treatments with only 60 nM Cu added showed no significant decrease in chlorophyll *a* relative to the control.

4.4 Discussion

Based on the results obtained from Station 23 in July 2002, we had hypothesized that the threshold for phytoplankton Cu toxicity in Lake Erie lay between 20 nM and 60 nM of added Cu. However, the results from ER-31M do not support this hypothesis, since Cu concentrations of up to 70 nM added did not elicit a toxic response. This is interesting since a few months earlier, acute toxicity was observed in phytoplankton assemblages at Station 23 in the Eastern Basin with Cu additions of 60 nM. The lack of response at ER-31M is probably not due to the relative concentration of Mn since concentrations of total dissolved Cu and Mn were similar at Station 23 in July 2002 to those at ER-31M in September 2002 (~9 nM Cu and ~5 nM Mn). The lack of a toxic response to additions of 60 nM Cu at ER-31M could possibly be explained by the fact that the added Cu may not have been as biologically available at ER-31M in the Central Basin than at Station 23 in the Eastern Basin. The unavailability of Cu to the phytoplankton may be due to a possible increased concentration of particles in the Central Basin due to upwellings, or mixings.

It was also hypothesized that concentrations of Cu of 60 nM and 120 nM would elicit a toxic response in the phytoplankton and that Mn additions of 60 nM and 120 nM might lessen the toxic effect of the Cu. This hypothesis was not supported. The results from ER-78M do in fact indicate that 120 nM of added Cu induced toxicity in all sizes of phytoplankton, however, additions of up to 120 nM Mn had no effect on the toxicity of the Cu. Also, since in the picoplankton and total phytoplankton

assemblages, the treatments with 60 nM Cu + 60 nM Mn and 60nM Cu + 120 nM Mn resulted in significant decreases in chlorophyll *a* only relative to the control, and since the treatments with 60 nM Cu alone do not show significantly lower chlorophyll *a* concentrations, we cannot state that 60 nM Cu induced a toxic effect on phytoplankton under the tested conditions. At this time, we cannot explain the significant differences observed.

These results are in agreement with the Canadian Water Quality Guidelines (CCREM, 1987), since 60 nM Cu exceeds the concentration considered "safe" for all aquatic organisms (30 nM Cu) when the $CaCO_3$ concentration is between 60-120 mg/L (the $CaCO_3$ concentration in Lake Erie is approximately 90-95 mg/L {Rockwell *et al.*, 1985})

CHAPTER 5: CU AND MN ENRICHMENT EXPERIMENTS: PACIFIC OCEAN, TRANSECT FROM HONOLULU (HA) TO SAN DIEGO (CA), JUNE/JULY 2002

5.1 Introduction

Cu and Mn enrichment experiments were conducted during the summer of 2002 on a transect from Honolulu (HA) to San Diego (CA), aboard the Scripps Institute of Oceanography's R/V *Melville.* The response of phytoplankton to various additions of Cu and Mn was assessed using chlorophyll *a* as a measure of phytoplankton biomass. A methodology similar to the one used at Station 23 on Lake Erie in November 2001 (refer to Chapter 3) was employed. However, instead of the pumping system used in Lake Erie, water was collected from a 20 m depth using a Teflon®-coated 10 L Go-Flo bottle (General Oceanics), suspended from a non-metallic line. Also, since there was no clean room on board, all manipulations of water were carried out in a portable laminar flow hood. Although trace metal clean techniques were employed as much as possible aboard the R/V *Melville*, it should be noted that due to logistics, this cruise was not as "clean" as those carried out aboard the C.C.G.S. *Limnos* and the R/V *Guardian.*

5.2 Methods

5.21 Amendments to standard methodology

Since the Trace Metal Grade HCl (Fisher Scientific) did not arrive in Honolulu prior to our departure, once used, all labware was washed 3 times with Nanopure water, soaked for at least 24 hours in 10% Non Trace Metal Grade HCl (Fisher Scientific) and finally rinsed with Nanopure water 7 times.

The method of chlorophyll *a* extraction was somewhat modified from that used in the experiments in Chapters 3 and 4. The filters were added to 5 mL of 90% acetone/10% $MgCO_3$ solution rather than 10 mL of 90% acetone/10% deionised water solution.

Due to logistical constraints, it was not possible to have radioactive ^{14}C on board the R/V *Melville.* A pulsed amplitude modulation (PAM) fluorometer was used as an alternative method for measuring photosynthetic efficiency for the first and

second experiments. However the PAM fluorometer was not sensitive enough to the low amounts of chlorophyll *a* present in the water samples and the results were deemed to be unreliable. Photosynthetic efficiency was therefore not calculated for these experiments.

5.22 Incubation of samples

Due to the scarcity of incubators on board, incubations were carried out in a child's plastic swimming pool (0.4 m deep and 2 m in diameter). Surface seawater was constantly pumped through the pool via a garden hose (7.62 m long and 0.016 m in diameter). Two layers of grey fiberglass mesh (Phifer Wire Products, Inc, Tuscaloosa, Alabama) were used to cover the pool in order to simulate natural light at a 20 m depth. Irradiance with depth was calculated using the following equation:

$$I_z = I_o \exp(-kz)$$

Where:

I_0 = surface irradiance calculated to be 1900 μmol $m^{-2}s^{-1}$ using a photometer

k = vertical attenuation coefficient assumed to be constant with depth and time and to be 0.06m^{-1} (Richardson *et al.,* 1998)

Z = depth in meters, in this case 20 m

I_z = photosynthetically available radiation (PAR) at depth z

Using the above equation, irradience at 20 m depth was calculated to be I_{20}=572.3. In this case, I_z is equal to 30.12%, which corresponds to 2 layers of gray fiberglass mesh; 30.12% is also a realistic irradience for 20 m depth in the Pacific, as this was also used by Coale when incubating his enrichment experiments (Coale, 1991).

Treatments from experiments in the Pacific were incubated for a much longer duration than those from Lake Erie and the Grand River. This was due to the fact that on the Pacific cruise, there were fewer time constraints since the cruise was of much longer duration (3 weeks versus 3-5 days). The incubation period was also longer in

the Pacific experiments since the planktonic biomass in the Pacific is lower than in Lake Erie and the Grand River and it would take longer for noticeable effects on phytoplankton biomass to manifest.

5.23 Determination of concentration of metal additions

For this cruise, it was decided that since the natural baseline concentrations of Cu and Mn are lower in the Pacific Ocean than in Lake Erie (refer to Chapter 1), the concentrations of metals added to the samples should reflect natural concentrations and should therefore also be lower. For the experiments carried out on the Pacific Ocean, samples were subjected to 5 different treatments in addition to a control (Table 8).

Table 8: Metal concentrations (nM) added to water sampled from Stations 4, 5 and 15 in the North Pacific in June-July 2002.

Treatment number	Metal Additions
1	Control
2	3 nM Mn
3	3 nM Cu
4	6 nM Cu
5	3 nM Cu + 3nM Mn
6	6nM Cu + 3nM Mn

These concentrations were chosen based on the fact that the concentration of Cu-complexing ions in the upper 100 m of the North Pacific averages approximately 1.8 nM, and by far exceeds the natural concentration of dissolved Cu which is about 0.5 nM (Bruland *et al*, 1991). Therefore, in order for Cu to be bioavailable and to induce toxicity in phytoplankton, the concentration of ionic Cu would have to be greater than the concentration of strong Cu-binding ligands present in the system. In Coale's enrichment experiments described in Chapter 1, additions of 3.9 nM of Cu did not elicit a toxic effect on the phytoplankton (Coale, 1991). It was therefore decided in these experiments to add concentrations of Cu up to 6 nM; the hypothesis being that

additions of 6 nM Cu would elicit a toxic response in the phytoplankton and that the addition of 3 nM Mn would lessen the toxic effect of the Cu.

5.24 Methodology for the Pacific experiments

Three Cu and Mn amendment experiments were conducted with water sampled from a 20 m depth from three different stations (Figure 18):

-Experiment 1:

Station 4: June 24th 2002 at 17:42 GMT (7:42 am local time on June 23rd 2002) at 28 01.058N. 159 00.078W.

-Experiment 2:

Station 5: June 25th 2002 at 15:05 GMT (5:05 am local time on June 26th 2002) at 27 59.976N. 157 59. 978W.

-Experiment 3:

Station 15: July 7th 2002 at 18:15 GMT (9:15 am local time on July 7th 2002) at 31 32.204N. 141 15.674W.

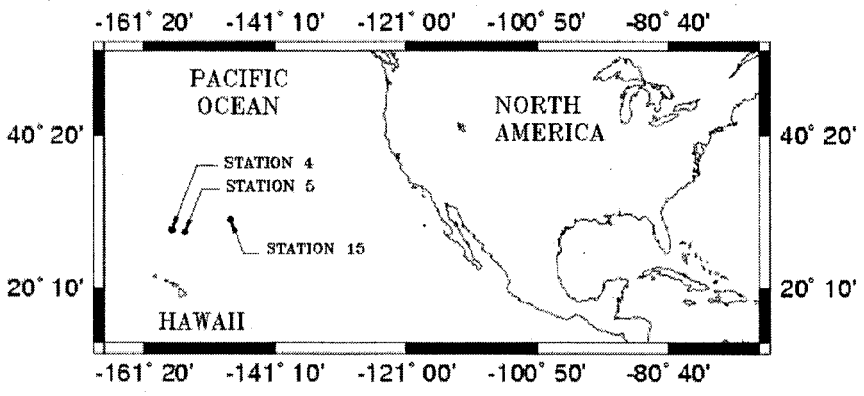

Figure 18: Map showing the location of study sites in the North Pacific Ocean.

At each station, at least 3 separate casts were made. Water was sampled from a 20 m depth and was transferred, using Teflon® tubing, from the Go-Flo bottle (General Oceanics) to a 20 L carboy (Nalgene) and a 10 L carboy (Nalgene) in order to homogenize the samples. The water was then transferred into polycarbonate bottles (18 × 1300 mL), which had been rinsed once with the sampled water prior to being

filled. The samples were then subjected to 5 different treatments in addition to a control: 3 nM Mn, 3 nM of Cu, 6 nM of Cu, 3 nM Cu + 3 nM Mn, 6 nM Cu + 3 nM Mn (Table 8, in previous section).

All bottles were sealed (Parafilm) in order to prevent contamination of the samples. Bottles were then incubated for 72 hours. Following incubation, 100 mL were then filtered from each bottle onto a 0.2 μm-filter and 200 mL from each bottle onto a 2.0 μm-filter for chlorophyll *a* extraction and fluorometric analysis. Following filtration, bottles were returned to the incubator for a further 3 days. Filtrations (in duplicate) and fluorometer readings were then repeated after a total incubation period of 6 days.

At each station, 2 × 400 mL was filtered for analysis of total dissolved Cu and Mn concentrations, the results of which are pending (refer to Chapter 2).

5.3 Results

5.31 Experiment 1

Total phytoplankton assemblages were not affected by the metal additions; no significant effect on phytoplankton biomass ($p<0.05$) was detected over both 3 day (Figure 19A) and 6 day incubation periods (Figure 19B) (Appendix K).

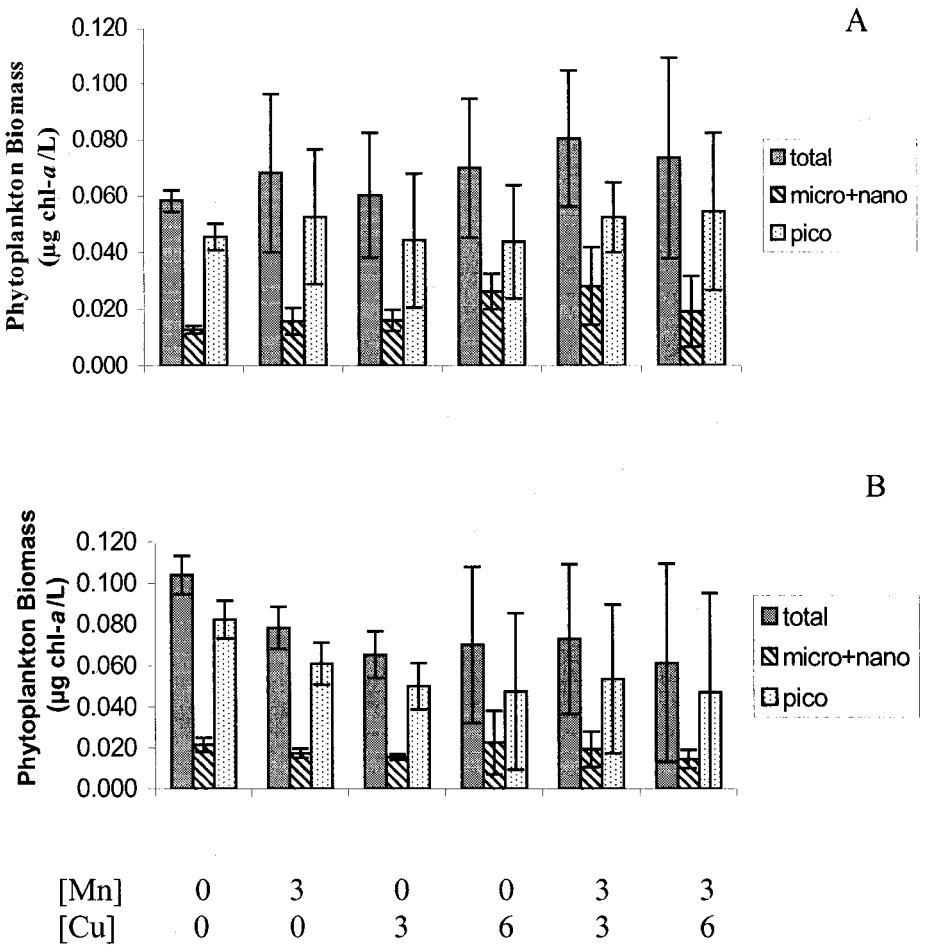

Figure 19: Response of phytoplankton (biomass) sampled from Station 4 in the North Pacificand treated for 3 days (Figure 19A) and treated for 6 days (Figure 19B) with various additions of Mn and Cu (nM). All values are mean ± standard deviation.

The picoplankton assemblage was also unaffected by metal additions; no significant effect on phytoplankton biomass ($p<0.05$) was detected following both the 3 day and the 6 day incubation periods (Appendix K).

The only size class that was affected by the metal additions was the nanoplankton + microplankton size fraction (>2.0 µm). It was found that following a 6 day incubation period, the treatment with 3 nM Cu + 3 nM Mn added had significantly greater phytoplankton biomass than the control ($p<0.05$) (Appendix K2).

5.32 Experiment 2

No phytoplankton assemblages were affected by the metal additions in Experiment 2; no significant effect on phytoplankton biomass ($p<0.05$) was detected across all size groups following both the 3 day and the 6 day incubation periods (Appendix L). Figure 20 shows the phytoplankton biomass for the total phytoplankton assemblages following both the 3 day (Figure 20A) and 6 day (Figure 20B) incubation periods.

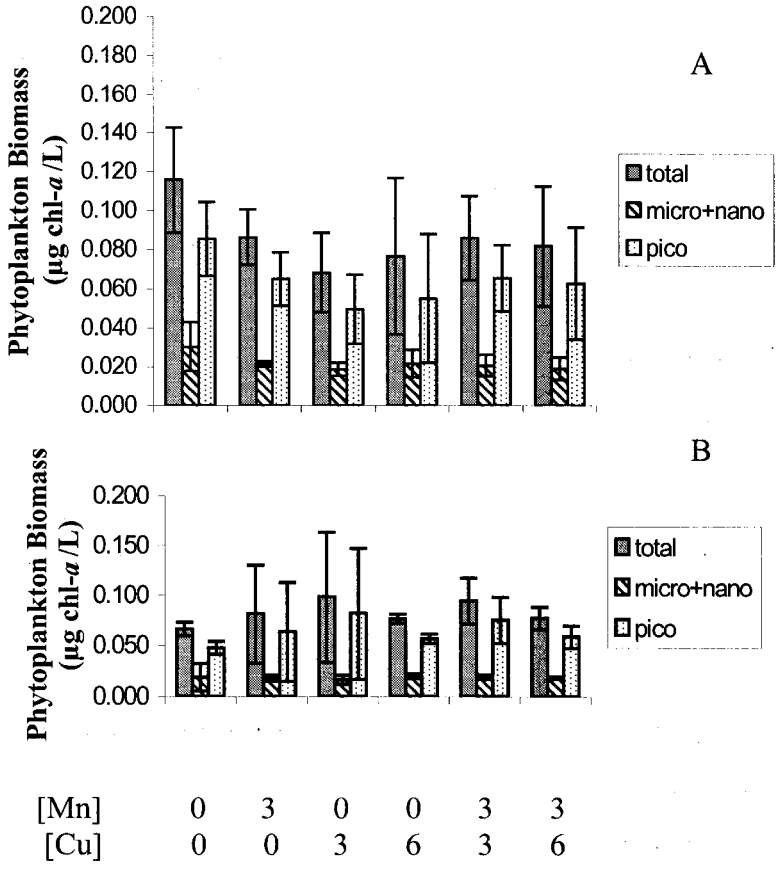

Figure 20: Response of phytoplankton (biomass) sampled at Station 5 in the North Pacific and treated for 3 days (Figure 20A) and 6 days (Figure 20B) with various additions of Mn and Cu (nM). Change in total phytoplankton biomass is indicated by chl-*a* concentrations. All values are mean ± standard deviation.

5.33 Experiment 3

3 day incubation

The total phytoplankton assemblage was affected by the metal additions; a significant effect ($p<0.05$) on phytoplankton biomass was detected. The treatment with 6 nM Cu added and the treatment with 6 nM Cu +3 nM Mn added had significantly greater phytoplankton biomass ($p<0.05$) than the control and the treatment with 3 nM Mn added (Figure 21) (Appendix M1).

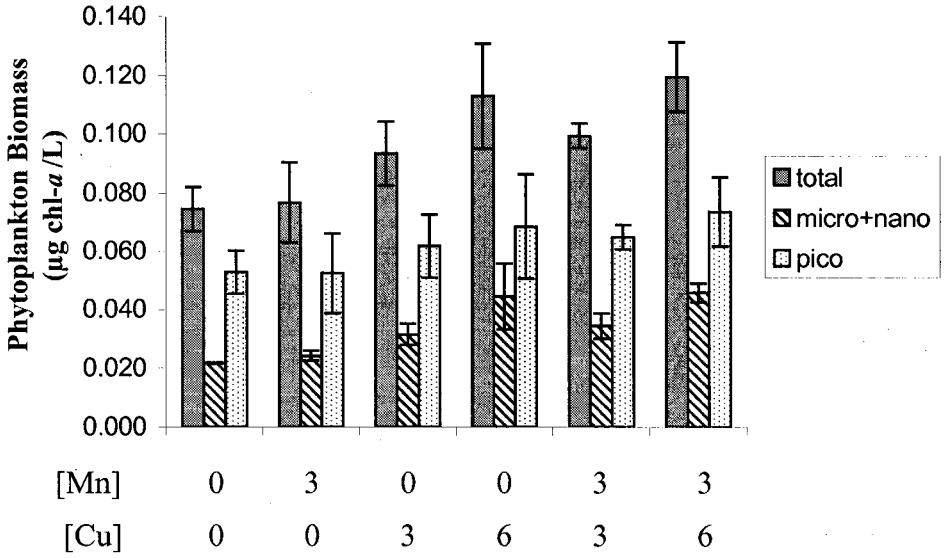

Figure 21: Response of phytoplankton (biomass) sampled from Station 15 in the North Pacific and treated for 3 days with various additions of Mn and Cu (nM). All values are mean ± standard deviation.

As for the nanoplankton + microplankton size fraction (>2.0 µm), phytoplankton in this size group were also affected by the metal additions; a significant effect on phytoplankton biomass was detected ($p<0.05$). The treatment with 6 nM Cu added had significantly greater phytoplankton biomass ($p<0.05$) than all other treatments with the exception of the treatment with 6 nM Cu + 3 nM Mn added. The treatment with 6 nM Cu + 3 nM Mn added had significantly greater chlorophyll *a* concentrations ($p<0.05$) than the control, the treatment with 3 nM Mn added, and the treatment with 3 nM Cu added (Appendix M2).

The picoplankton assemblage was not affected by the metal additions; no significant effect on phytoplankton biomass was detected following the 3 day incubation period (Appendix M3).

6 day incubation

The total phytoplankton assemblage was affected by the metal additions; a significant effect ($p<0.05$) on phytoplankton biomass was detected. The treatment with 6 nM Cu + 3 nM Mn added had significantly greater phytoplankton biomass ($p<0.05$) than the control and the treatment with 6 nM Cu added. In addition, the treatment with 3 nM Mn added, had significantly greater phytoplankton biomass ($p<0.05$) than the treatment with 6 nM Cu added (Figure 22) (Appendix M4).

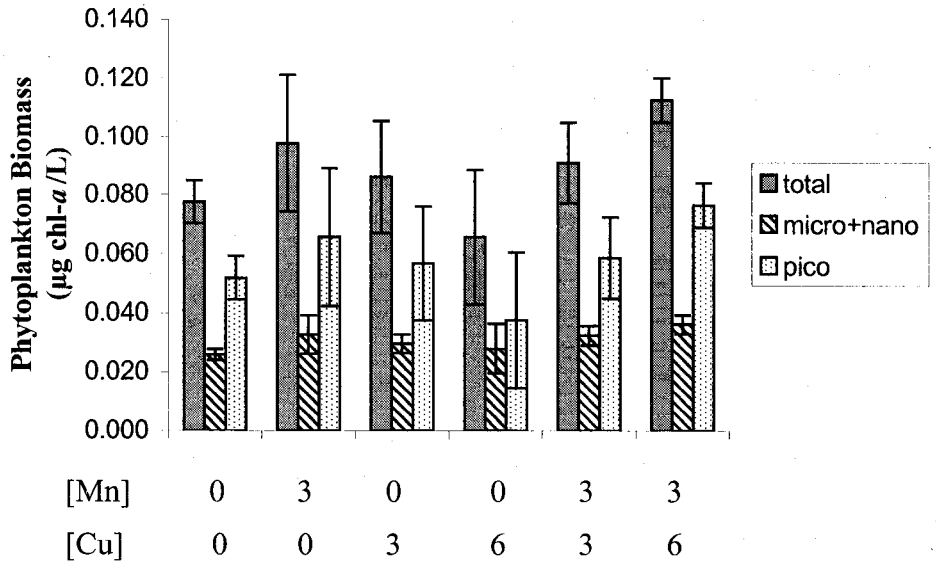

Figure 22: Response of phytoplankton (biomass) sampled from Station 15 in the North Pacific and treated for 6 days with various additions of Mn and Cu (nM). All values are mean ± standard deviation

The nanoplankton + microplankton size fraction (>2.0 µm) was also affected by the metal additions; a significant effect ($p<0.05$) on phytoplankton biomass was detected. The treatment with 6 nM Cu and 3 nM Mn added had significantly greater phytoplankton biomass than the control ($p<0.05$)(Appendix M5).

In the picoplankton size fraction (0.2 μm-2.0μm) was also affected by the metal additions; a significant effect ($p<0.05$) on phytoplankton biomass was detected. The treatment with 6 nM Cu and 3 nM Mn added had significantly phytoplankton biomass than the treatment with 6 nM Cu added ($p<0.05$) (Appendix M6).

5.34 Summary of Results

The results from all three experiments indicate that Cu additions of up to 6 nM do not show a significant decrease in phytoplankton biomass relative to the control for any size group, following both 3 and 6 day incubations. Experiments 1 and 2 showed no significant differences amongst the treatments for phytoplankton biomass amongst all size groups following 3 and 6 day incubations. The only exception was the nanoplankton + microplankton size fraction, in which, following a 6 day incubation period, the treatment with 3 nM Cu +3 nM Mn added showed significantly greater phytoplankton biomass than the control. As for Experiment 3, when significant differences are observed between treatments, treatment 6 (with 6 nM Cu +3 nM Mn added) consistently had significantly higher phytoplankton biomass than various other treatments, across all size groups, and this, following both 3 and 6 day incubations.

5.5 Discussion

The working hypothesis for these experiments was that additions of 6 nM Cu (a concentration that is higher than expected under natural conditions, even in the deep waters of the Pacific) would elicit a toxic response in the phytoplankton population and that 3 nM Mn would possibly lessen the toxic effect of Cu. However, based on the previously described results, we can conclude that in contrast with our hypothesis, additions of Cu of up to 6 nM did not elicit a toxic response from phytoplankton under the tested conditions. Although no clear trend can be observed, additions of metals seem to be stimulating growth in certain cases; as evidenced by the fact that whenever a significant difference between treatments was observed in Experiments 1 and 3, a significant **increase** in phytoplankton biomass was observed relative to the control, rather than a decrease. When Coale (1991) observed a significant increase in phytoplankton productivity following additions of Mn, he attributed the increase to Mn limitation, which may also be the case in the present study. However, when Coale

(1991) observed a significant increase in phytoplankton productivity following additions of Cu, he attributed the increase to a decrease in grazing by the microzooplankton (refer to Chapter 1), which may also be the case in the present study. However, as stated previously, no clear trend can be observed with regards to the significant differences amongst the treatments in Experiment 3. Since the treatments with 6 nM Cu +3 nM Mn added have consistently higher phytoplankton biomass than various other treatments, across all size groups, and this, following both 3 and 6 day incubations, one would expect that if Mn were limiting, the treatments with 3 nM Mn added to also show significant increases in phytoplankton biomass. This was not the case. We are therefore unable to conclude that Mn was limiting in Experiment 3.

CHAPTER 6: SUMMARY OF RESULTS, CONCLUSIONS, AND RECOMMENDATIONS

6.1 Summary of results and conclusions

Many laboratory studies have established that Cu toxicity and the effect of the antagonistic relationship between Cu and Mn on phytoplankton are indeed of importance under controlled laboratory conditions (Van den Berg *et al.*, 1979; Sunda and Guillard, 1976; Brand *et al.*, 1986; Gerringa *et al.*, 1995; Sunda *et al.*, 1981; Sunda and Huntsman, 1986, 1995, 1998a, 1998b; Twiss *et al.*, 2003). The significance of this study is that it is one of the first to examine whether the importance of Cu toxicity and the interaction between Cu and Mn in the laboratory is also observable under natural conditions. We examined the effect of additions of Cu and Mn on phytoplankton in three different natural environments: a river, a lake, and an ocean. The short-term response of phytoplankton to the metal additions was observed in Lake Erie and the Grand River, whereas the experiments in the Pacific studied the response of phytoplankton over longer time periods. Our results indicate that:

-Cu toxicity was not observed at the mouth of the Grand River. Additions of up to 200 nM Cu had no negative on phytoplankton biomass or photosynthetic efficiency, both in November 2001 and again in July 2002.

-Only relatively high concentrations of Cu (60 nM to 120 nM) induced a toxic response in phytoplankton in Lake Erie, and the threshold for Cu toxicity seems to vary between the Eastern and Central Basins of Lake Erie. In the Eastern Basin of Lake Erie, acute toxicity was observed with additions of 60 nM Cu whereas this concentration had no effect on phytoplankton sampled in the Central Basin of Lake Erie. It is also interesting to note that when Cu toxicity was observed, additions of Mn did not lessen the toxic effect of the Cu.

not elicit a toxic response in phytoplankton sampled from three separate stations on a transect from Honolulu, HA to San Diego, CA.

We can therefore conclude that Cu toxicity and Cu and Mn interactions in these three natural environments cannot necessarily be predicted from laboratory studies. Although Cu toxicity may be observed under controlled conditions in the laboratory, there are many modifying factors present in natural environments that may influence the speciation of Cu and the sensitivity of phytoplankton to Cu. One must acknowledge that although Cu toxicity to phytoplankton is important, it may only be relevant under certain circumstances. In fact, of the three different environments studied, Cu toxicity was only observed in Lake Erie and this, only at relatively high levels.

There is one caveat associated with the experiments conducted in the Grand River and in Lake Erie: the experiments were conducted over short incubation times of approximately 24 hours. Although acute toxicity was observed when 60 nM Cu was added to samples from the Eastern Basin of Lake Erie and 120 nM Cu was added to samples from the Central Basin of Lake Erie, lower concentrations of Cu may have a negative effect on phytoplankton over longer incubation periods.

6.2 Recommendations

Since our conclusions regarding Cu and Mn in the Grand River and Lake Erie are based on short-term experiments, it is recommended that our experiments be repeated over a longer incubation time than 24 hours, in order to determine if the short exposure period affected the results.

In addition to longer incubation periods, it is also recommended that future experiments use differential pulse anodic stripping voltametry (DPASV) to determine the speciation of Cu and Mn during the course of the experiments. Repeating our experiments while knowing the speciation of Cu and Mn would provide insight into whether the lack of toxicity observed in the Grand River, and the high threshold of Cu

toxicity in Lake Erie compared with Lake Superior, was due to low biovailabilty of Cu.

REFERENCES

Achterberg, E.P., van den Berg, C.M.G., Boussemart, M., Davison, W. 1997. Speciation and cycling of trace metals in Esthwaite water: a productive English lake with seasonal deep water anoxia. Geochim. Cosmochim. Acta 61 (24): 5233-5255.

ATSDR 2000. Toxicological profile for copper. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Springfield.

Brand, L.E., Sunda, W.G., Guillard, R.L. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. J. Exp. Mar. Biol. Ecol. 96: 225-250.

Bruland, K.W., Donat, J.R., Hutchins, D.A. 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. Limnol. Oceanogr. 36: 1551-1577.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: a critique of the Free-Ion Activity Model in Metal Speciation and Bioavailability in Aquatic Systems. Edited by Tessier and Turner. John Wiley & Sons Ltd.

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian Water Quality Guidelines (Guidelines for the Protection of Aquatic Life).

Coale, K.H. and Bruland, K.W. 1988. Copper complexation in the northeast Pacific. Limnol. Oceangr. 33(5):1084-1101.

Coale, K.H. and Flegal, A.R. 1989. Copper, zinc, cadmium and lead in surface waters of Lakes Erie and Ontario. Sci. Tot. Environ. 87/88: 297-304.

Coale, K.H. and Bruland, K.W. 1990. Spatial and temporal variability in copper complexation in the North Pacific. Deep-Sea Research. 37(2): 317-336.

Coale, K.H. 1991. Effects of iron, manganese, copper and zinc enrichments on productivity and biomass in the subarctic Pacific. Limnol. Oceanogr. 36:1851-1864.

Croot, P.L., Moffett, J.W., and Brand, L.E. 2000. Production of extracellular Cu complexing ligands by eucaryotic phytoplankton in response to Cu stress. Limnol. Oceanogr. 45 (3): 619-627.

Croot, P.L., Karlson, B, van Elteren, J.T., and Kroon, J.J. 2003. Uptake and efflux of 64Cu by the marine cyanobacterium Synechococcus (WH7803). Limnol. Oceanogr. 48 91): 179-188.

Falkowski, P.G. and Raven, J.A. 1997. Aquatic Photosynthesis. Blackwell Science.

Fisher, N.S. and Reinfelder J.R. 1995. The trophic transfer of metals in marine systems in Metal Speciation and Bioavailability in Aquatic Systems. Edited by Tessier and Turner. John Wiley & Sons Ltd.

Forstner, U. and Wittman, G.T.W. 1983. Metal Pollution in the Aquatic Environment. Springer Verlag.

Fuller, K. and Shear, H (eds) 1995 (3rd edition). The Great Lakes: An Environmental Atlas and Resource Book. Government of Canada and United States Environmental Protection Agency.

Gerringa, L.J.A., Rijstenbil, J.W., Poortvliet, T.C.W., van Drie, J., Schot, M.C. Speciation of copper and responses of the marine diatom *Ditylum brightwellii* upon increasing copper concentrations. Aquatic Toxicology 31: 77-90.

Grand River Conservation Authority. 1979. Environmental Assessment of Water Control Structures in the Grand River Basin.

Hunter, K.A. and Boyd, P. 1999. Biogeochemistry of trace metals in the ocean. Mar. Freshwater Res., 50: 739-753.

Kolak, J.J., Long, D.T., Beals, T.M., Eisenreich, S.J. and Swackhamer, D.L. 1998. Anthropogenic inventories and historical and present accumulation rates of copper in Great Lakes sediments. Applied Geochemistry. 13: 59-75.

Lum, K.R. and Leslie, J.K. 1983. Dissolved and particulate metal chemistry of the central and eastern basins of Lake Erie. Sci. Tot. Environ. 30: 99-109.

Mansilla-Rivera, I. and Nriagu., J.O. 1999. Copper chemistry in freshwater ecosystems: An overview. J. Great Lakes Res. 25 (4):599-610.

Moffett, J.W., Zika, R.G. and Brand, L.E. 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso Sea. Deep-Sea Research. 37 (1): 27-36.

Moffett, J.W. 1995. Temporal and spatial variability of copper complexation by strong chelators in the Sargasso Sea. Deep-Sea Research. 42 (8): 1273-1295.

Moffett, J.W., Brand, L.E., and Barbeau, K.A. 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. Limnol. Oceanogr. 42 (5):789-799.

Mota, A.M. and Correia Dos Santos, M.M. 1995. Trace Metal Speciation of Labile Chemical Species in Natural Waters: Electrochemical Methods. in Metal Speciation and Bioavailability in Aquatic Systems. Edited by Tessier and Turner. John Wiley & Sons Ltd.

Nicholls, K.H., Taylor, R. and Hamdy, Y. The influence of the Grand River on phytoplankton near the northeastern shore of Lake Erie during 1979. 1983. Arch. Hydrobiol. 146–172.

Nimmo,M., van den Berg, C.M.G. and Brown, J. 1989. The chemical speciation of dissolved nickel, copper, vanadium and iron in Liverpool bay, Irish Sea. Estuarine, Coastal and Shelf Science .Vol 29:57-74.

Nicholls, K.H. 1997. Planktonic green algae in western Lake Erie; the importance of temporal scale in the interpretation of change. Freshwater Biology. 38: 419-425.

Nriagu, J.O. 1990. Global Metal Pollution: Poisoning the biosphere? Environment 32(7): 6-11, 28-33.

Nriagu, J.O. 1996. A history of global metal pollution. Science 272: 223-224.

Nriagu, J.O., Lawson, G., Wong, KT, Azcu, J.M. 1993. A protocol for minimizing contamination in the analysis of trace metals in Great Lakes waters. J. Great Lakes Res., 19(1): 175-182.

Nriagu, J.O., Lawson, G., Wong, H.K.T., Cheam, V. 1996. Dissolved trace metal in Lakes Superior, Erie, and Ontario. Environ. Sci.Technol., 30: 178-187.

Raven, J.A., Evans, M.C.W, and Korb, R.E. 1999. The role of trace metals in photosynthetic electron transport in O2-evolving organisms. Photosynthesis Research 60: 111-149.

Richardson, T., Cullen, J. 1998. Potential contributions of vertically migrating Rhizosolenia to nutrient cycling and new production in the open ocean. J. Plankton Res., 20(2):219-241.

Rockwell, D.C., Salisbury, D.K., and Lesht, B.M. 1989. Water Quality in the Middle Great Lakes: Results of the 1985 USEPA Survey of Lakes Erie, Huron, and Michigan. GLNPO report no. 4, EPA-905/6/89-001

Rossman, R and Barres, J. 1988. Trace element concentrations in near-surface waters of the Great Lakes and methods of collection, storage and analysis. Internat. Assoc. Great Lakes Res. 14(2):188-204.

Rott, E. Duthie, H.C., Pipp, E. 1998. Monitoring organic pollution and eutrophication in the Grand River, Ontario, by means of diatoms. Can. J. Fish.Aquat. Sci. Vol 55:1443-1453.

Rott, E. 1995. Diatoms of the Grand River, Ontario, Canada restudied after 25 years. Limnologica 25 (2): 165-192.

Sunda, W.G., and Guillard, R.L. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res.. 34:5 11-529.

Sunda, W.G., Barber, R.T. and Huntsman, S.A. 1981. Phytoplankton growth in nutrient rich seawater: importance of copper-manganese cellular interactions. J. Mar. Res. 39: 567-586.

Sunda, W.G., Huntsman, S.A. 1983. Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. Limnol. Oceanogr. Vol 28(5): 924-934.

Sunda, W.G., Huntsman, S.A. 1995. Regulation of copper concentrations in the oceanic nutricline by phytoplankton uptake and regeneration cycles. Limnol. Oceanogr. 40 (1): 132-137.

Sunda, W.G., Huntsman, S.A 1998a. Processes regulating cellular metal accumulation and physiological effects: phytoplankton as a model system. Sci. Tot. Environ. 219:165-181

Sunda, W.G., Huntsman, S.A. 1998b. Interaction among Cu^{2+}, Zn^{2+} and Mn^{2+} in controlling cellular Mn, Zn, and growth rate in the coastal alga *Chlamydomonas*. Limnol. Oceanogr. Vol 43(6): 1055-1064.

Sunda, W.G., Huntsman, S.A. 1998c. Interactive effects of external manganese, the toxic metals copper and zinc, and light in controlling cellular manganese and growth in a coastal diatom. Limnol. Oceanogr. 43(7): 1467-1475.

Sweet, C.W., Weiss, A., Vermette, S.J. 1998. Atmospheric deposition of trace metals at three sites near the Great Lakes. Water, Air, and Soil Pollution. 103: 423-439.

Sze, P. 1998. A biology of the algae. WCB/McGraw-Hill 3rd edition.

Tessier, A. and Turner, D.R.(eds).1995. Metal Speciation and Bioavailability in Aquatic Systems. John Wiley & Sons Ltd.

Turner, D.R.,Whitfield, M., and Dickson, A.G. 1981. The equilibrium speciation of dissolved components in freshwater and seawater at 25°C and 1 atm pressure. Geochim. Cosmochim. Acta, 45: 885-881.

Twiss, M.R., Campbell, P.G.C., Auclair, J.-C. 1996. Regeneration, recycling, and trophic transfer of trace metals by microbial food-web organisms in the pelagic surface waters of Lake Erie. Limnol. Oceanogr. 41:1425-1437.

Twiss, M.R., and Campbell, P.G.C. 1998. Trace metal cycling in the surface waters of Lake Erie; linking ecological and geochemical fates. J. Great Lakes Res. 24(4): 791-807.

Twiss, M.R., Rattan, K.J., Sherell, R., McKay, R.M.L. 2003. Sensitivity of phytoplankton to copper in Lake Superior. In press.

Van den Berg, C.M.G., Wong, P.T.S. and Chau, Y.K. 1979. Measurement of complexing materials excreted from algae and their ability to ameliorate copper toxicity. Journal of Fish. Res. Board Can., 36: 901-905.

Welschmeyer, N.A and Lorenzen, C.J. 1984. Carbon-14 labeling of phytoplankton carbon and chlorophyll *a* carbon: Determination of specific growth rates. Limnol. Oceanogr. 29:135-145.

Welschmeyer, N.A. 1994. Fluometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. 39(8):1985-1992.

Whitfield, M. 2001. Interactions between phytoplankton and trace metals in the ocean. Advances in Marine Biology 41: 1-124.

Witter, A.E., Mabury, S.A., Jones, A.D. 1998. Copper (II) complexation in northern California rice field waters: an investigation using differential pulse anodic and cathodic stripping voltammetry. Sci. Tot. Environ. 212:21-37.

Xue, H., Oestreich, A., Kistler, D., Sigg, L. 1996. Free cupric ion concentrations and Cu complexation in selected Swiss lakes and rivers. Aquatic Sciences. 59(1): 69-87.

APPENDIX

Appendix A: Method development for chlorophyll *a* analysis

Since fieldwork can often be very intensive, the schedule does always permit samples to be analyzed for chlorophyll *a* after exactly 24 hours of extraction. Logistics may occasionally allow for analysis only after 22 or 26 hours extraction. In order to determine whether shorter or longer extraction times influence chlorophyll *a* readings, the following method development for chlorophyll *a* extraction was carried out. Water was collected from Lake Erie on 17th September 2002 and 100 mL was filtered onto a 2.0μm-filter, added to a 90% acetone solution and refrigerated, this was performed 21 times. Filters were submitted to seven different extraction times ranging form 4 hours to 28 hours and then analyzed for chlorophyll *a*. The mean chlorophyll *a* reading for each given extraction time is presented in Table 1. An ANOVA test revealed that there was no significant difference amongst chlorophyll *a* readings following extraction periods ranging from 4 hours to 28 hours; $F(6,14)=1.78$; $p=0.1746$.

Table 1: Mean chlorophyll *a* readings following various extraction times ranging from 4 hours to 28 hours.

Extraction time	Chl-*a* μg/100mL	Standard Deviation	N
1 (4 hours)	11.58667	0.369098	3
2 (8 hours)	12.00333	0.318800	3
3 (12 hours)	12.54667	0.645316	3
4 (16 hours)	12.86333	0.807548	3
5 (20 hours)	12.24667	0.225906	3
6 (24 hours)	13.00333	1.040497	3
7 (28 hours)	12.31667	0.624527	3

It was therefore concluded that if the samples were extracted for 22 or 26 hours rather than the conventional 24 hours, it would not significantly affect the chlorophyll *a* readings.

Appendix B: Statistical analysis of results from Station 23 in November 2001

B1: Total Phytoplankton Assemblage (>0.2 μm)

Table B1a: Mean phytoplankton biomass from total phytoplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.60	0.08	2
2 (10nM Mn)	0.59	0.03	3
3 (5nM Cu)	0.58	0.14	3
4 (20nM Cu)	0.60	0.14	3
5 (5nM Cu+10nM Mn)	0.61	0.04	3
6 (20nM Cu+10nM Mn)	0.59	0.12	3

Table B1b: Mean photosynthetic efficiency from total phytoplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	4.03×10^{-7}	1.24×10^{-7}	2
2 (10nM Mn)	3.72×10^{-7}	7.61×10^{-9}	3
3 (5nM Cu)	3.34×10^{-7}	1.94×10^{-8}	3
4 (20nM Cu)	2.91×10^{-7}	5.03×10^{-8}	3
5 (5nM Cu+10nM Mn)	2.75×10^{-7}	2.60×10^{-8}	3
6 (20nM Cu+10nM Mn)	2.79×10^{-7}	3.84×10^{-8}	3

Table B1e ANOVA results from total phytoplankton assemblage at Station 23 in November 2001, using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	11	0.01	0.34	1.00

Table B1dANOVA results from total phytoplankton assemblage at Station 23 in November 2001 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	11	0.00	3.10	0.05

B2: Microplankton Assemblage (> 20 μm)

Table B2a: Mean phytoplankton biomass of microplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-a/L)	Standard Deviation	N
1 (control)	0.21	0.01	2
2 (10nM Mn)	0.16	0.05	3
3 (5nM Cu)	0.15	0.06	3
4 (20nM Cu)	0.14	0.06	3
5 (5nM Cu+10nM Mn)	0.12	0.09	3
6 (20nM Cu+10nM Mn)	0.10	0.04	3

Table B2b: Mean photosynthetic efficiency of microplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/μg chl-*a*/hour)**	**Standard Deviation**	**N**
1(control)	3.08×10^{-7}	1.97×10^{-8}	2
2(10nM Mn)	3.77×10^{-7}	1.64×10^{-7}	3
3(5nM Cu)	5.27×10^{-7}	1.50×10^{-7}	3
4(20nM Cu)	5.50×10^{-7}	2.27×10^{-7}	3
5(5nM Cu+10nM Mn)	1.01×10^{-6}	9.60×10^{-7}	3
6(20nM Cu+10nM Mn)	8.29×10^{-7}	2.14×10^{-7}	3

Table B2c: ANOVA results from microplankton assemblage at Station 23 in November 2001, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	11	0.00	1.05	0.44

Table B2e: ANOVA results from total phytoplankton assemblage at Station 23 in November 2001 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	11	0.00	1.03	0.45

B3: Nanoplankton Assemblage (2.0 μm-20 μm)

Table B3a: Mean phytoplankton biomass from nanoplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.31	0.09	2
2 (10nM Mn)	0.24	0.01	3
3 (5nM Cu)	0.42	0.12	3
4 (20nM Cu)	0.43	0.07	3
5 (5nM Cu+10nM Mn)	0.48	0.01	3
6 20nM Cu+10nM Mn)	0.44	0.14	3

Table B3b: Mean photosynthetic efficiency of nanoplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	4.30×10^{-7}	5.37×10^{-8}	2
2 (10nM Mn)	4.27×10^{-7}	8.53×10^{-8}	3
3 (5nM Cu)	3.44×10^{-7}	7.80×10^{-8}	3
4 (20nM Cu)	2.44×10^{-7}	7.50×10^{-8}	3
5 (5nM Cu+10nM Mn)	2.24×10^{-7}	9.14×10^{-8}	3
6 (20nM Cu+10nM Mn)	1.92×10^{-7}	4.36×10^{-8}	3

Table B3c ANOVA results from nanoplankton assemblage at Station 23 in November 2001using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.02	11	0.01	2.93	0.06

Table B3d ANOVA results from nanoplankton assemblage at Station 23 in November 2001 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	11	0.00	5.36	**0.01**

Table B3e: Probabilities for Newman-Keuls post hoc tests for nanoplankton assemblage at Station 23 in November 2001, using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable (the numbers 1 through 6, at the top and at the left of the table refer to Treatments 1 through 6)

	{1}	{2}	{3}	{4}	{5}	{6}
{1}		0.96	0.40	0.06	**0.05**	**0.03**
{2}	0.96		0.22	**0.04**	**0.04**	**0.02**
{3}	0.40	0.22		0.14	0.19	0.13
{4}	0.06	**0.04**	0.14		0.76	0.69
{5}	**0.05**	**0.04**	0.19	0.76		0.61
{6}	**0.03**	**0.02**	0.13	0.69	0.61	

B4: Picoplankton Assemblage (0.2 μm- 2.0 μm)

Table B4a: Mean phytoplankton biomass from picoplankton assemblage at Station 23 in November 2001 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.08	0.00	2
2 (10nM Mn)	0.18	0.04	3
3 (5nM Cu)	0.08	0.12	3
4 (20nM Cu)	0.05	0.05	3
5 (5nM Cu+10nM Mn)	0.02	0.04	3
6(20nM Cu+10nM Mn)	0.10	0.09	3

Table B4b: ANOVA results from picoplankton assemblage at Station 23 in November 2001, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.01	11	0.01	1.65	0.23

NOTE: It was not possible to run a ANOVA on the photosynthetic efficiency data from the picoplankton assemblage at Station 23 in November 2001 since in most cases, the mol C fixed attributable to the picoplankton size fraction was less than, or equal to 0; thus the photosynthetic activity measured as a ratio of mol C fixed/μg chlorophyll *a*/h would give an unrealistic value.

Appendix C: Statistical analysis of results from Station 938 in November 2001

C1: Total Phytoplankton Assemblage (>0.2 μm)

Table C1: Mean phytoplankton biomass from total phytoplankton assemblage at Station 938 in November 2001 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.76	0.09	6
2 (10nM Mn)	0.97	0.10	6
3 (5nM Cu)	0.67	0.12	6
4 (20nM Cu)	0.87	0.11	6
5 (5nM Cu+10nM Mn)	0.67	0.12	6
6 (20nM Cu+10nM Mn)	0.57	0.16	6

Table C2: Mean photosynthetic efficiency from total phytoplankton assemblage at Station 938 in November 2001 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	3.43×10^{-7}	4.40×10^{-8}	2
2 (10nM Mn)	3.21×10^{-7}	2.10×10^{-8}	3
3 (5nM Cu)	3.61×10^{-7}	4.96×10^{-8}	3
4 (20nM Cu)	2.83×10^{-7}	8.34×10^{-8}	3
5 (5nM Cu+10nM Mn)	3.64×10^{-7}	5.95×10^{-8}	3
6 (20nM Cu+10nM Mn)	3.16×10^{-7}	3.69×10^{-8}	3

Table C4: ANOVA results from total phytoplankton assemblage at Station 938 in November 2001using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.13	30	0.01	9.34	**0.00**

Table C5: ANOVA results from total phytoplankton assemblage at Station 938 in November 2001 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	11	0.00	2.04	0.10

Table C6: Probabilities for Newman-Keuls post hoc tests for the total phytoplankton assemblage at Station 938 in November 2001 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 6 at the top and left of the table refer to treatments 1 through 6)

	1	2	3	4	5	6
1}		**0.01**	0.22	0.10	0.39	**0.04**
2}	**0.01**		**0.00**	0.18	**0.00**	**0.00**
3}	0.22	**0.00**		**0.02**	0.94	0.29
4}	0.10	0.17	**0.02**		**0.02**	**0.00**
5}	0.39	**0.00**	0.94	0.02		0.16
6}	**0.04**	**0.00**	0.29	**0.00**	0.16	

Appendix D: Statistical analysis of results from the Grand River in November 2001

D1: Total Phytoplankton Assemblage (>0.2 μm)

Table D1: Mean phytoplankton biomass from total phytoplankton assemblage in the Grand River in November 2001 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	11.40	1.01	9
2 (10nM Mn)	11.20	0.59	8
3 (20 nM Cu)	10.94	0.55	9
4 (200 nM Cu)	10.87	0.32	9
5 (20 nM Cu+10nM Mn)	10.59	0.38	9
6 (200 nM Cu+10nM Mn)	11.84	0.49	9

Table D2: Mean photosynthetic efficiency from total phytoplankton assemblage at Grand River in November 2001 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	8.70×10^{-8}	1.69×10^{-8}	6
2 (10nM Mn)	9.15×10^{-8}	2.24×10^{-8}	3
3 (5nM Cu)	8.72×10^{-8}	9.41×10^{-9}	6
4 (20nM Cu)	8.80×10^{-8}	1.27×10^{-8}	6
5 (5nM Cu+10nM Mn)	9.13×10^{-8}	1.06×10^{-8}	6
6 (20nM Cu+10nM Mn)	8.00×10^{-8}	8.84×10^{-9}	6

Table D3: ANOVA results from total phytoplankton assemblage in the Grand River in November 2001, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	1.74	47	0.36	4.82	**0.00**

Table D4: ANOVA results from total phytoplankton assemblage in the Grand River in November 2001 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	27	0.00	0.55	0.74

Table D5: Probabilities for Newman-Keuls post hoc tests for total phytoplankton assemblage in the Grand River in November 2001 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 6 at the top and left of the table refer to treatments 1 through 6)

	1	2	3	4	5	6
1}		0.25	0.26	0.26	0.05	0.13
2}	0.25		0.69	0.78	0.37	**0.02**
3}	0.26	0.69		0.79	0.44	**0.01**
4}	0.26	0.78	0.79		0.34	**0.01**
5}	0.05	0.37	0.44	0.34		**0.00**
6}	0.13	**0.02**	**0.02**	**0.01**	**0.00**	

Appendix E: Statistical analysis of results from Station 23 in July 2002

E1:Total Phytoplankton Assemblage (>0.2 μm)

Table E1a: Mean phytoplankton biomass from total phytoplankton assemblage at Station 23 in July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	1.41	0.01	3
2 (10 nM Mn)	1.52	0.15	3
3 (5 nM Cu)	1.44	0.27	3
4 (20 nM Cu)	1.36	0.13	3
5 (5 nM Cu+10 nM Mn)	1.48	0.03	3
6 (20 nM Cu+10 nM Mn)	1.49	0.14	3
7 (60 nM Cu)	0.80	0.03	3
8 (60 nM Cu +30 nM Mn)	0.79	0.07	3

Table E1b: Mean photosynthetic efficiency from total phytoplankton assemblage at Station 23 in July 2002 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	5.63×10^{-7}	4.70×10^{-8}	3
2 (10 nM Mn)	5.98×10^{-7}	1.52×10^{-7}	3
3 (5 nM Cu)	6.86×10^{-7}	2.15×10^{-7}	3
4 (20 nM Cu)	7.20×10^{-7}	2.64×10^{-7}	3
5 (5 nM Cu+10 nM Mn)	1.01×10^{-7}	4.16×10^{-7}	3
6 (20 nM Cu+10 nM Mn)	5.41×10^{-7}	3.04×10^{-8}	3
7 (60 nM Cu)	4.90×10^{-7}	3.95×10^{-8}	3
8 (60 nM Cu+30 nM Mn)	5.47×10^{-7}	2.53×10^{-8}	3

Table E1c: ANOVA results from total phytoplankton assemblage at Station 23 in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.28	16	0.02	14.93	**0.00**

Table E1d: ANOVA results from total phytoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.00	16	0.00	2.08	0.11

Table E1e: Probabilities for Newman-Keuls post hoc tests for the total phytoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 8 at the top and left hand side of the table refer to treatments 1 through 8)

	1	2	3	4	5	6	7	8
1}		0.88	0.81	0.65	0.82	0.90	**0.00**	**0.00**
2}	0.88		0.90	0.72	0.94	0.80	**0.00**	**0.00**
3}	0.81	0.90		0.76	0.72	0.90	**0.00**	**0.00**
4}	0.65	0.72	0.76		0.71	0.78	**0.00**	**0.00**
5}	0.82	0.94	0.72	0.71		0.94	**0.00**	**0.00**
6}	0.90	0.80	0.90	0.78	0.94		**0.00**	**0.00**
7}	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**		0.92
8}	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.92	

E2: Microplankton+ Nanoplankton Assemblage (>2.0 μm)

Table E2a: Mean phytoplankton biomass from micoplankton + nanoplankton assemblage at Station 23 in July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.60	0.06	3
2 (10 nM Mn)	0.77	0.08	3
3 (5 nM Cu)	0.65	0.15	3
4 (20 nM Cu)	0.63	0.08	3
5 (5 nM Cu+10 nM Mn)	0.64	0.06	3
6 (20 nM Cu+10 nM Mn)	0.68	0.05	3
7 (60 nM Cu)	0.40	0.01	3
8 (60 nM Cu +30 nM Mn)	0.40	0.05	3

Table E2b: Mean photosynthetic efficiency from the microplankton + nanoplankton assemblage at Station 23 in July 2002 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	7.77×10^{-7}	3.24×10^{-7}	3
2 (10 nM Mn)	6.49×10^{-7}	9.47×10^{-8}	3
3 (5 nM Cu)	7.98×10^{-7}	4.78×10^{-7}	3
4 (20 nM Cu)	9.05×10^{-7}	4.07×10^{-7}	3
5 (5 nM Cu+10 nM Mn)	1.50×10^{-7}	1.09×10^{-6}	3
6 (20 nM Cu+10 nM Mn)	5.44×10^{-7}	6.83×10^{-8}	3
7 (60 nM Cu)	5.37×10^{-7}	1.81×10^{-7}	3
8 (60 nM Cu+30 nM Mn)	7.19×10^{-7}	1.06×10^{-7}	3

Table E2c: ANOVA results from microplankton + nanoplankton assemblage at Station 23 in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.05	16	0.00	9.01	**0.00**

Table E2d: ANOVA results from microplankton + nanoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.00	16	0.00	1.30	0.31

Table E2e: Probabilities for Newman-Keuls post hoc tests for the micorplankton + nanoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 8 at the top and left side of the table refer to the Treatments 1 through 8)

	1	2	3	4	5	6	7	8
1}		0.12	0.82	0.63	0.76	0.66	**0.01**	**0.01**
2}	0.12		0.18	0.21	0.22	0.19	**0.00**	**0.00**
3}	0.82	0.18		0.92	0.88	0.64	**0.01**	**0.01**
4}	0.63	0.21	0.92		0.83	0.82	**0.01**	**0.01**
5}	0.76	0.22	0.88	0.82		0.80	**0.01**	**0.01**
6}	0.66	0.19	0.64	0.82	0.80		**0.00**	**0.00**
7}	**0.01**	**0.00**	**0.01**	**0.01**	**0.01**	**0.00**		0.96
8}	**0.01**	**0.00**	**0.01**	**0.01**	**0.01**	**0.00**	0.96	

E3: Picoplankton Assemblage (0.2 μm -2.0 μm)

Table E3a: Mean phytoplankton biomass from picoplankton assemblage at Station 23 in July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.81	0.14	3
2 (10 nM Mn)	0.75	0.14	3
3 (5 nM Cu)	0.79	0.15	3
4 (20 nM Cu)	0.73	0.05	3
5 (5 nM Cu+10 nM Mn)	0.84	0.07	3
6 (20 nM Cu+10 nM Mn)	0.81	0.12	3
7 (60 nM Cu)	0.40	0.03	3
8 (60 nM Cu +30 nM Mn)	0.40	0.08	3

Table E3b: Mean photosynthetic efficiency from the picoplankton assemblage at Station 23 in July 2002 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	4.31×10^{-7}	1.49×10^{-7}	3
2 (10 nM Mn)	5.66×10^{-7}	3.14×10^{-7}	3
3 (5 nM Cu)	6.16×10^{-7}	3.61×10^{-8}	3
4 (20 nM Cu)	5.62×10^{-7}	1.40×10^{-7}	3
5 (5 nM Cu+10 nM Mn)	6.85×10^{-7}	1.75×10^{-7}	3
6 (20 nM Cu+10 nM Mn)	5.31×10^{-7}	8.46×10^{-8}	3
7 (60 nM Cu)	4.45×10^{-7}	1.58×10^{-7}	3
8 (60 nM Cu+30 nM Mn)	3.75×10^{-7}	5.82×10^{-8}	3

Table E3c: ANOVA results from picoplankton assemblage at Station 23 in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.10	16	0.01	8.59	**0.00**

Table E3d: ANOVA results from picoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.00	16	0.00	1.23	0.34

Table E3e: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 8 at the top and left of the table refer to treatments 1 through 8)

	1	2	3	4	5	6	7	8
1}		0.87	0.95	0.87	0.79	0.92	**0.00**	**0.00**
2}	0.87		0.66	0.85	0.83	0.79	**0.00**	**0.01**
3}	0.95	0.66		0.80	0.94	0.83	**0.00**	**0.00**
4}	0.87	0.85	0.80		0.82	0.83	**0.00**	**0.00**
5}	0.79	0.83	0.94	0.82		0.93	**0.00**	**0.00**
6}	0.92	0.79	0.83	0.83	0.93		**0.00**	**0.00**
7}	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**		0.92
8}	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	0.92	

Appendix F: Statistical analysis of results from the Station GR-1 on the Grand River in July 2002

F1: Total Phytoplankton Assemblage (>0.2 μm)

Table F1a: Mean phytoplankton biomass from total phytoplankton assemblage at Station GR-1 on the Grand River in July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	88.91	0.31	2
2 (20 nM Cu)	90.77	0.31	2
3 (100 nM Cu)	97.58	0.14	2
4 (200 nM Cu)	85.69	6.70	2

Table F1b: Mean photosynthetic efficiency from total phytoplankton assemblage at at Station GR-1 on the Grand River in July 2002 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	2.68×10^{-7}	1.58×10^{-8}	2
2 (20 nM Cu)	2.45×10^{-7}	2.00×10^{-8}	2
3 (100 nM Cu)	2.30×10^{-7}	8.20×10^{-9}	2
4 (200nM Cu)	2.35×10^{-7}	4.56×10^{-9}	2

Table F1c: ANOVA results from total phytoplankton assemblage at Station GR-1 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	50.43	4	11.27	4.47	0.09

Table F1d: ANOVA results from total phytoplankton assemblage at Station GR-1 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	4	0.00	3.16	0.15

F2: Microplankton and Nanoplankton Assemblage (>2.0 μm)

Table F2a: Mean phytoplankton biomass from the microplankton + nanoplankton assemblage at Station GR-1 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	45.06	1.41	2
2 (20 nM Cu)	49.96	6.65	2
3 (100 nM Cu)	53.23	1.47	2
4 (200 nM Cu)	48.70	5.52	2

Table F2b: Mean photosynthetic efficiency from total the microplankton + nanoplankton assemblage at at Station GR-1 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/μg chl-*a*/hour)**	**Standard Deviation**	**N**
1 (control)	3.07×10^{-7}	2.78×10^{-8}	2
2 (20 nM Cu)	2.61×10^{-7}	6.53×10^{-8}	2
3 (100 nM Cu)	2.62×10^{-7}	3.95×10^{-8}	2
4 (200nM Cu)	2.57×10^{-7}	3.77×10^{-8}	2

Table F2c: ANOVA results from the microplankton + nanoplankton assemblage at Station GR-1 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	22.83	4	19.72	1.16	0.43

Table F2d: ANOVA results from the microplankton + nanoplankton assemblage at Station GR-1 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	4	0.00	0.57	0.66

F3: Picoplankton assemblage (0.2 µm-2.0 µm)

Table F3a: Mean phytoplankton biomass from the picoplankton assemblage at Station GR-1 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	43.85	1.09	2
2 (20 nM Cu)	22.87	32.34	2
3 (100 nM Cu)	44.34	1.33	2
4 (200 nM Cu)	36.99	1.17	2

Table F3b: Mean photosynthetic efficiency from total the picoplankton assemblage at at Station GR-1 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/µg chl-*a*/hour)**	**Standard Deviation**	**N**
1 (control)	2.29×10^{-7}	5.73×10^{-9}	2
2 (20 nM Cu)	2.12×10^{-7}	N/A	1
3 (100 nM Cu)	1.92×10^{-7}	2.72×10^{-8}	2
4 (200nM Cu)	2.09×10^{-7}	5.78×10^{-8}	2

Table F3c: ANOVA results from the picoplankton assemblage at Station GR-1 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	200.44	4	262.5	0.76	0.57

Table F3d: ANOVA results from the picoplankton assemblage at Station GR-1 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	3	0.00	0.33	0.81

Appendix G: Statistical analysis of results from Station GR-2 on the Grand River in July 2002

G1: Total Phytoplankton Assemblage (>0.2 μm)

Table G1a: Mean phytoplankton biomass from total phytoplankton assemblage at Station GR-2 on the Grand River in July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	72.22	0.49	2
2 (20 nM Cu)	71.02	0.71	2
3 (100 nM Cu)	72.74	1.95	2
4 (200 nM Cu)	67.24	6.37	2

Table G1b: Mean photosynthetic efficiency from total phytoplankton assemblage at Station GR-2 on the Grand River in July 2002 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	2.28×10^{-7}	2.05×10^{-8}	2
2 (20 nM Cu)	2.07×10^{-7}	1.02×10^{-8}	2
3 (100 nM Cu)	2.17×10^{-7}	5.15×10^{-9}	2
4 (200nM Cu)	2.33×10^{-7}	2.16×10^{-8}	2

Table G1c: ANOVA results from total phytoplankton assemblage at Station GR-2 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	12.34	4	11.29	1.09	0.45

Table G1d: ANOVA results from total phytoplankton assemblage at Station GR-2 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	4	0.00	1.08	0.45

G2: Microplankton and Nanoplankton Assemblage (>2.0 µm)

Table G2a: Mean phytoplankton biomass from the microplankton + nanoplankton assemblage at Station GR-2 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	41.31	1.72	2
2 (20 nM Cu)	36.01	1.00	2
3 (100 nM Cu)	39.52	2.34	2
4 (200 nM Cu)	39.47	2.08	2

Table G2b: Mean photosynthetic efficiency from total the microplankton + nanoplankton assemblage at at Station GR-2 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/µg chl-*a*/hour)**	**Standard Deviation**	**N**
1 (control)	2.45×10^{-7}	2.62×10^{-8}	2
2 (20 nM Cu)	2.49×10^{-7}	3.15×10^{-8}	2
3 (100 nM Cu)	2.51×10^{-7}	1.52×10^{-9}	2
4 (200nM Cu)	2.16×10^{-7}	5.79×10^{-9}	2

Table G2c: ANOVA results from the microplankton + nanoplankton assemblage at Station GR-2 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	9.83	4	3.45	2.85	0.17

Table G2d: ANOVA results from the microplankton + nanoplankton assemblage at Station GR-2 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	4	0.00	1.23	0.41

G3: Picoplankton assemblage (0.2 µm-2.0 µm)

Table G3a: Mean phytoplankton biomass from the picoplankton assemblage at Station GR-2 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	30.91	2.22	2
2 (20 nM Cu)	35.01	1.72	2
3 (100 nM Cu)	33.22	0.40	2
4 (200 nM Cu)	16.88	23.87	2

Table G3b: Mean photosynthetic efficiency from total the picoplankton assemblage at Station GR-2 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/µg chl-*a*/hour)**	**Standard Deviation**	**N**
1 (control)	2.05×10^{-7}	1.54×10^{-8}	2
2 (20 nM Cu)	1.65×10^{-7}	8.32×10^{-9}	1
3 (100 nM Cu)	1.76×10^{-7}	1.02×10^{-8}	2
4 (200nM Cu)	2.16×10^{-7}	N/A	1

Table G3c: ANOVA results from the picoplankton assemblage at Station GR-2 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	136.43	4	144.39	0.94	0.50

Table G3d: ANOVA results from the picoplankton assemblage at Station GR-2 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	3	0.00	6.55	0.08

Appendix H: Statistical analysis of results from Station GR-3 on the Grand River in July 2002

H1: Total Phytoplankton Assemblage (>0.2 μm)

Table H1a: Mean phytoplankton biomass from total phytoplankton assemblage at Station GR-3 on the Grand River in July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	48.04	3.05	2
2 (20 nM Cu)	50.07	5.65	2
3 (100 nM Cu)	45.67	7.28	2
4 (200 nM Cu)	48.49	1.18	2

Table H1b: Mean photosynthetic efficiency from total phytoplankton assemblage at Station GR-3 on the Grand River in July 2002 (N= number of replicates).

Treatment	Photosynthetic Efficiency (mol C/μg chl-*a*/hour)	Standard Deviation	N
1 (control)	2.99×10^{-7}	N/A	1
2 (20 nM Cu)	2.50×10^{-7}	1.81×10^{-8}	2
3 (100 nM Cu)	2.72×10^{-7}	3.82×10^{-8}	2
4 (200nM Cu)	2.41×10^{-7}	3.51×10^{-8}	2

Table H1c: ANOVA results from total phytoplankton assemblage at Station GR-3 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	6.65	4	23.91	0.28	0.84

Table H1d: ANOVA results from total phytoplankton assemblage at Station GR-3 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	3	0.00	0.90	0.53

H2: Microplankton and Nanoplankton Assemblage (>2.0 µm)

Table H2a: Mean phytoplankton biomass from the microplankton + nanoplankton assemblage at Station GR-3 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	25.11	0.87	2
2 (20 nM Cu)	27.23	2.84	2
3 (100 nM Cu)	23.97	3.71	2
4 (200 nM Cu)	27.09	4.13	2

Table H2b: Mean photosynthetic efficiency from total the microplankton + nanoplankton assemblage at at Station GR-3 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/µg chl-*a*/hour)**	**Standard Deviation**	**N**
1 (control)	3.30×10^{-7}	N/A	1
2 (20 nM Cu)	2.56×10^{-7}	2.08×10^{-8}	2
3 (100 nM Cu)	2.97×10^{-7}	1.98×10^{-8}	2
4 (200nM Cu)	2.46×10^{-7}	6.10×10^{-8}	2

Table H2c: ANOVA results from the microplankton + nanoplankton assemblage at Station GR-3 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	5.01	4	9.93	0.50	0.70

Table H2d: ANOVA results from the microplankton + nanoplankton assemblage at Station GR-3 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	3	0.00	1.40	0.39

H3: Picoplankton assemblage (0.2 μm-2.0 μm)

Table H3a: Mean phytoplankton biomass from the picoplankton assemblage at Station GR-3 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	21.69	3.93	2
2 (20 nM Cu)	22.84	2.81	2
3 (100 nM Cu)	21.69	3.56	2
4 (200 nM Cu)	11.74	16.61	2

Table H3b: Mean photosynthetic efficiency from total the picoplankton assemblage at Station GR-3 on the Grand River in July 2002 (N= number of replicates).

Treatment	**Photosynthetic Efficiency (mol C/μg chl-*a*/hour)**	**Standard Deviation**	**N**
1 (control)	2.70×10^{-7}	N/A	2
2 (20 nM Cu)	2.43×10^{-7}	1.47×10^{-8}	1
3 (100 nM Cu)	2.45×10^{-7}	5.82×10^{-8}	2
4 (200nM Cu)	2.43×10^{-7}	N/A	1

Table H3c: ANOVA results from the picoplankton assemblage at Station GR-3 on the Grand River in July 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
3	58.37	4	77.98	0.75	0.58

Table H3d: ANOVA results from the picoplankton assemblage at Station GR-3 on the Grand River in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable

df Effect	MS Effect	df Error	MS Error	F	p-level
3	0.00	2	0.00	0.11	0.95

Appendix I: Statistical analysis of results from Station ER-31M in September 2002

I1:Total Phytoplankton Assemblage (>0.2 μm)

Table I1a: Mean phytoplankton biomass from total phytoplankton assemblage at Station ER-31M in September 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	2.91	0.11	3
2 (10 nM Cu)	2.73	0.26	3
3 (20 nM Cu)	2.87	0.21	3
4 (30 nM Cu)	2.61	0.35	3
5 (40 nM Cu)	2.75	0.15	3
6 (50 nM Cu)	2.72	0.09	3
7 (60 nM Cu)	2.71	0.25	3
8 (70 nM Cu)	2.57	0.10	3

Table I1b: ANOVA results from total phytoplankton assemblage at Station ER-31M in September 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.04	16	0.04	0.91	0.52

I2: Microplankton+ Nanoplankton Assemblage (>2.0 μm)

Table I2a: Mean phytoplankton biomass from micoplankton + nanoplankton assemblage at Station ER-31M in September 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	2.04	0.25	3
2 (10 nM Cu)	2.07	0.14	3
3 (20 nM Cu)	2.17	0.05	3
4 (30 nM Cu)	2.15	0.19	3
5 (40 nM Cu)	2.04	0.03	3
6 (50 nM Cu)	2.03	0.07	3
7 (60 nM Cu)	1.93	0.09	3
8 (70 nM Cu)	1.91	0.09	3

Table I2b: ANOVA results from microplankton + nanoplankton assemblage at Station ER-31M in September 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.02	16	0.02	1.42	0.26

I3: Picoplankton Assemblage (0.2μm -2.0 μm)

Table I3a: Mean phytoplankton biomass from picoplankton assemblage at Station ER-31M in September 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.87	0.27	3
2 (10 nM Cu)	0.87	0.27	3
3 (20 nM Cu)	0.66	0.30	3
4 (30nM Cu)	0.70	0.17	3
5 (40 nM Cu)	0.51	0.45	3
6 (50 nM Cu)	0.71	0.17	3
7 (60 nM Cu)	0.70	0.15	3
8 (70 nM Cu)	0.78	0.21	3

Table I3b: ANOVA results from picoplankton assemblage Station ER-31M in September 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	0.04	16	0.07	0.58	0.76

Appendix J: Statistical analysis of results from Station ER-78M in September 2002

J1: Total Phytoplankton Assemblage (>0.2 μm)

Table J1a: Mean phytoplankton biomass from total phytoplankton assemblage at ER-78M in September 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	2.29	0.14	3
2 (30 nM Cu)	2.03	0.23	3
3 (60 nM Cu)	2.04	0.02	3
4 (120 nM Cu)	1.12	0.05	3
5 (60 nM Mn)	2.16	0.05	3
6 (120 nM Mn)	2.15	0.11	3
7 (30 nM Cu+60 nM Mn)	1.98	0.12	3
8 (60 nM Cu+60 nM Mn)	1.90	0.05	3
9 (120 nM Cu+60 nM Mn)	1.12	0.02	3
10 (30 nM Cu +120 nM Mn)	2.08	0.13	3
11 (60 nM Cu+120 nM Mn)	1.90	0.08	3
12 (120 nM Cu+120 nM Mn)	1.03	0.03	3

Table J1b: ANOVA results from total phytoplankton assemblage at at ER-78M in September 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
11	0.61	24	0.01	57.63	**0.00**

Table J1c: Probabilities for Newman-Keuls post hoc tests for the total phytoplankton assemblage at ER-78M in September 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable (the numbers refer to the Treatments 1 through 8)

	1	2	3	4	5	6	7	8	9	10	11	12
1)		0.05	0.05	**0.00**	0.14	0.24	**0.02**	**0.00**	**0.00**	0.09	**0.00**	**0.00**
2}	0.05		0.90	**0.00**	0.55	0.52	0.57	0.43	**0.00**	0.82	0.30	**0.00**
3}	0.05	0.90		**0.00**	0.50	0.43	0.76	0.47	**0.00**	0.63	0.38	**0.00**
4}	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**	**0.00**	**0.00**	0.98	**0.00**	**0.00**	0.32
5}	0.14	0.55	0.50	**0.00**		0.88	0.31	0.08	**0.00**	0.63	0.07	**0.00**
6}	0.24	0.52	0.43	**0.00**	0.88		0.31	0.09	**0.00**	0.44	0.08	**0.00**
7}	**0.02**	0.57	0.76	**0.00**	0.31	0.31		0.61	**0.00**	0.64	0.36	**0.00**
8}	**0.00**	0.43	0.47	**0.00**	0.08	0.09	0.61		**0.00**	0.30	0.97	**0.00**
9}	**0.00**	**0.00**	**0.00**	0.98	**0.00**	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**	0.57
10}	0.09	0.82	0.63	**0.00**	0.63	0.44	0.64	0.30	**0.00**		0.25	**0.00**
11}	**0.00**	0.30	0.38	**0.00**	0.07	0.08	0.36	0.97	**0.00**	0.25		**0.00**
12}	**0.00**	**0.00**	**0.00**	0.32	**0.00**	**0.00**	**0.00**	**0.00**	0.57	**0.00**	**0.00**	

J2: Microplankton+ Nanoplankton Assemblage (>2.0 µm)

Table J2a: Mean phytoplankton biomass from micoplankton + nanoplankton assemblage at ER-78M in September 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	1.54	0.34	3
2 (30 nM Cu)	1.48	0.17	3
3 (60 nM Cu)	1.58	0.10	3
4 (120 nM Cu)	1.02	0.11	3
5 (60 nM Mn)	1.51	0.10	3
6 (120 nM Mn)	1.68	0.13	3
7 (30 nM Cu+60 nM Mn)	1.51	0.02	3
8 (60 nM Cu+60 nM Mn)	1.50	0.09	3
9 (120 nM Cu+60 nM Mn)	1.04	0.02	3
10 (30 nM Cu +120 nM Mn)	1.51	0.09	3
11 (60 nM Cu+120 nM Mn)	1.54	0.02	3
12 (120 nM Cu+120 nM Mn)	0.95	0.04	3

Table J2b: ANOVA results from microplankton + nanoplankton assemblage at ER-78M in September 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
11	0.19	24	0.02	10.60	**0.00**

Table J2c: Probabilities for Newman-Keuls post hoc tests for the microplankton + nanoplankton assemblage at ER-78M in September 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable (the numbers refer to the Treatments 1 through 12)

	1	2	3	4	5	6	7	8	9	10	11	12
1}		1.00	0.92	**0.00**	0.97	0.57	0.84	1.00	**0.00**	0.99	0.99	**0.00**
2}	1.00		0.99	**0.00**	0.99	0.69	1.00	0.87	**0.00**	0.97	1.00	**0.00**
3}	0.92	0.99		**0.00**	0.97	0.37	0.93	0.99	**0.00**	0.99	0.71	**0.00**
4}	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**	**0.00**	**0.00**	0.91	**0.00**	**0.00**	0.47
5}	0.97	0.99	0.97	**0.00**		0.64	0.97	1.00	**0.00**	0.99	0.99	**0.00**
6}	0.57	0.69	0.37	**0.00**	0.64		0.56	0.73	**0.00**	0.71	0.41	**0.00**
7}	0.84	1.00	0.93	**0.00**	0.97	0.56		1.00	**0.00**	1.00	0.97	**0.00**
8}	1.00	0.87	0.99	**0.00**	1.00	0.73	1.00		**0.00**	0.95	1.00	**0.00**
9}	**0.00**	**0.00**	**0.00**	0.91	**0.00**	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**	0.68
10}	0.99	0.97	0.99	**0.00**	0.99	0.71	1.00	0.95	**0.00**		1.00	**0.00**
11}	0.99	1.00	0.71	**0.00**	0.99	0.41	0.97	1.00	0.00	1.00		**0.00**
12}	**0.00**	**0.00**	**0.00**	0.47	**0.00**	**0.00**	**0.00**	**0.00**	0.68	**0.00**	**0.00**	

J3: Picoplankton Assemblage (0.2μm -2.0 μm)

Table J3a: Mean phytoplankton biomass from picoplankton at ER-78M in September 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.75	0.20	3
2 (30 nM Cu)	0.54	0.09	3
3 (60 nM Cu)	0.46	0.11	3
4 (120 nM Cu)	0.10	0.13	3
5 (60 nM Mn)	0.65	0.09	3
6 (120 nM Mn)	0.47	0.23	3
7 (30 nM Cu+60 nM Mn)	0.47	0.13	3
8 (60 nM Cu+60 nM Mn)	0.40	0.10	3
9 (120 nM Cu+60 nM Mn)	0.08	0.02	3
10 (30 nM Cu +120 nM Mn)	0.57	0.09	3
11 (60 nM Cu+120 nM Mn)	0.36	0.08	3
12 (120 nM Cu+120 nM Mn)	0.09	0.05	3

Table J3b: ANOVA results from picoplankton assemblage at ER-78M in September 2002, using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
11	0.15	24	0.02	9.65	**0.00**

Table J3c: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage at Station 23 in July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable (the numbers 1 through 12 a the top and at the left hand side refer to Treatments 1 through 12).

	1	2	3	4	5	6	7	8	9	10	11	12
1}		0.20	0.10	**0.00**	0.32	0.07	0.09	**0.03**	**0.00**	0.20	**0.02**	**0.00**
2}	0.20		0.84	**0.00**	0.57	0.45	0.71	0.59	**0.00**	0.80	0.48	**0.00**
3}	0.10	0.84		**0.01**	0.45	1.00	0.97	0.53	**0.01**	0.81	0.60	**0.01**
4}	**0.00**	**0.00**	**0.01**		**0.00**	**0.02**	**0.01**	**0.02**	0.97	**0.00**	**0.02**	0.85
5}	0.32	0.57	0.45	**0.00**		0.30	0.39	0.20	**0.00**	0.45	0.13	**0.00**
6}	0.07	0.45	1.00	**0.02**	0.30		0.98	0.89	**0.01**	0.57	0.83	**0.01**
7}	0.09	0.71	0.97	**0.01**	0.39	0.98		0.77	**0.01**	0.73	0.74	**0.01**
8}	**0.03**	0.59	0.53	**0.02**	0.20	0.89	0.77		**0.03**	0.53	0.75	**0.02**
9}	**0.00**	**0.00**	**0.01**	0.97	**0.00**	**0.01**	**0.01**	**0.03**		**0.00**	**0.04**	0.96
10}	0.20	0.80	0.81	**0.00**	0.45	0.57	0.73	0.53	**0.00**		0.41	**0.00**
11}	**0.02**	0.48	0.60	**0.02**	0.13	0.83	0.74	0.75	**0.04**	0.41		**0.03**
12}	**0.00**	**0.00**	**0.01**	0.85	**0.00**	**0.01**	**0.01**	**0.02**	0.96	**0.00**	**0.03**	

Appendix K: Statistical analysis of results from Experiment 1 (Station 4) in the North Pacific, June-July 2002

K1: Total Phytoplankton Assemblage (>0.2 μm) following 3 days incubation

Table K1a: Mean phytoplankton biomass, after 3 days incubation, from total phytoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.10	0.01	3
2 (3 nM Mn)	0.08	0.01	3
3 (3 nM Cu)	0.07	0.01	3
4 (6 nM Cu)	0.07	0.04	3
5 (3 nM Cu+3 nM Mn)	0.07	0.04	3
6 (6 nM Cu+3 nM Mn)	0.06	0.05	3

Table K1b: ANOVA results from total phytoplankton assemblage after 3 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.78	0.58

K2: Microplankton + Nanoplankton Assemblage (>2.0μm) following 3 days incubation

Table K2a: Mean phytoplankton biomass after 3 days incubation, from microplankton+nanoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.022	0.003	3
2 (3 nM Mn)	0.017	0.002	3
3 (3 nM Cu)	0.015	0.001	3
4 (6 nM Cu)	0.023	0.016	3
5 (3 nM Cu+3 nM Mn)	0.019	0.009	3
6 (6 nM Cu+3 nM Mn)	0.015	0.005	3

Table K2b: ANOVA results from microplankton+nanoplankton assemblage after 3 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.53	0.75

K3: Picoplankton Assemblage (<0.2 μm) following 3 days incubation

Table K3a: Mean phytoplankton biomass from the picoplankton assemblage after 3 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.082	0.012	3
2 (3 nM Mn)	0.061	0.008	3
3 (3 nM Cu)	0.050	0.010	3
4 (6 nM Cu)	0.047	0.022	3
5 (3 nM Cu+3 nM Mn)	0.054	0.028	3
6 (6 nM Cu+3 nM Mn)	0.047	0.044	3

Table K3b: ANOVA results from the picoplankton assemblage after 3 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.94	0.49

K4: Total Phytoplankton Assemblage (>0.2 μm) following 6 days incubation

Table K4a: Mean phytoplankton biomass from total phytoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.058	0.004	6
2 (3 nM Mn)	0.068	0.028	6
3 (3 nM Cu)	0.060	0.022	6
4 (6 nM Cu)	0.070	0.025	6
5 (3 nM Cu+3 nM Mn)	0.080	0.024	6
6 (6 nM Cu+3 nM Mn)	0.073	0.036	6

Table K4b: ANOVA results from total phytoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.65	0.66

K5: Microplankton + Nanoplankton Assemblage (>2.0μ) following 6 days incubation

Table K5a: Mean phytoplankton biomass from microplankton+nanoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.013	0.001	6
2 (3 nM Mn)	0.016	0.005	6
3 (3 nM Cu)	0.016	0.004	6
4 (6 nM Cu)	0.026	0.006	6
5 (3 nM Cu+3 nM Mn)	0.028	0.014	6
6 (6 nM Cu+3 nM Mn)	0.019	0.013	6

Table K5b: ANOVA results from microplankton+nanoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	3.29	**0.02**

Table K5c: Probabilities for Newman-Keuls post hoc tests for the microplankton+nanoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002: using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable (the numbers 1 through 6 a the top and at the left of the table refer to Treatments 1 through 6).

	1	2	3	4	5	6
1		0.546	0.777	0.063	**0.036**	0.566
2	0.546		0.946	0.151	0.102	0.768
3	0.777	0.946		0.104	0.080	0.535
4	0.063	0.151	0.104		0.702	0.147
5	**0.036**	0.102	0.080	0.702		0.164
6	0.566	0.768	0.535	0.147	0.164	

K6: Picoplankton Assemblage (<0.2 μm) following 6 days incubation

Table K6a: Mean phytoplankton biomass from the picoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.046	0.005	6
2 (3 nM Mn)	0.053	0.024	6
3 (3 nM Cu)	0.044	0.024	6
4 (6 nM Cu)	0.044	0.020	6
5 (3 nM Cu+3 nM Mn)	0.052	0.012	6
6 (6 nM Cu+3 nM Mn)	0.054	0.029	6

Table K6b: ANOVA results from the picoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	0.34	0.89

Appendix L: Statistical analysis of results from Experiment 2 (Station 5) in the North Pacific, June-July 2002

L1: Total Phytoplankton Assemblage (>0.2 μm) following 3 days incubation

Table L1a: Mean phytoplankton biomass from total phytoplankton assemblage after 3 days incubation in Experiment 2 (Station 5) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.066	0.001	3
2 (3 nM Mn)	0.082	0.049	3
3 (3 nM Cu)	0.098	0.065	3
4 (6 nM Cu)	0.076	0.005	3
5 (3 nM Cu+3 nM Mn)	0.094	0.023	3
6 (6 nM Cu+3 nM Mn)	0.077	0.011	3

Table L1b: ANOVA results from total phytoplankton assemblage after 3 days incubation in Experiment 2 (Station 5) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.35	0.87

L2: Microplankton + Nanoplankton Assemblage (0.2 µm-2.0 µm) following 3 days incubation

Table L2a: Mean phytoplankton biomass from microplankton+nanoplankton assemblage after 3 days incubation in Experiment 2 (Station 5) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.019	0.014	3
2 (3 nM Mn)	0.018	0.003	3
3 (3 nM Cu)	0.017	0.004	3
4 (6 nM Cu)	0.020	0.002	3
5 (3 nM Cu+3 nM Mn)	0.019	0.002	3
6 (6 nM Cu+3 nM Mn)	0.018	0.001	3

Table L2b: ANOVA results from microplankton+nanoplankton assemblage after 3 days incubation in Experiment 2 (Station 5) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.10	0.99

L3: Picoplankton Assemblage (0.2 μm-2.0 μm) following 3 days incubation

Table L3a: Mean phytoplankton biomass from the picoplankton assemblage after 3 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.048	0.007	3
2 (3 nM Mn)	0.064	0.046	3
3 (3 nM Cu)	0.082	0.061	3
4 (6 nM Cu)	0.057	0.005	3
5 (3 nM Cu+3 nM Mn)	0.075	0.021	3
6 (6 nM Cu+3 nM Mn)	0.059	0.012	3

Table L3b: ANOVA results from the picoplankton assemblage after 3 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	0.44	0.81

L4: Total Phytoplankton Assemblage (>0.2 μm) following 6 days incubation

Table L4a: Mean phytoplankton biomass from total phytoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.116	0.027	6
2 (3 nM Mn)	0.086	0.014	6
3 (3 nM Cu)	0.068	0.020	6
4 (6 nM Cu)	0.077	0.040	6
5 (3 nM Cu+3 nM Mn)	0.086	0.022	6
6 (6 nM Cu+3 nM Mn)	0.081	0.031	6

Table L4b: ANOVA results from total phytoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	2.16	0.08

L5: Microplankton + Nanoplankton Assemblage (0.2 µm-2.0µm) following 6 days incubation

Table L5a: Mean phytoplankton biomass from microplankton+nanoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (µg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.030	0.013	6
2 (3 nM Mn)	0.021	0.001	6
3 (3 nM Cu)	0.019	0.003	6
4 (6 nM Cu)	0.022	0.007	6
5 (3 nM Cu+3 nM Mn)	0.021	0.006	6
6 (6 nM Cu+3 nM Mn)	0.019	0.006	6

Table L5b: ANOVA results from microplankton+nanoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	2.29	0.07

L6: Picoplankton Assemblage (0.2 μm- 2.0 μm) following 6 days incubation

Table L6a: Mean phytoplankton biomass from the picoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.085	0.019	6
2 (3 nM Mn)	0.064	0.014	6
3 (3 nM Cu)	0.050	0.018	6
4 (6 nM Cu)	0.055	0.033	6
5 (3 nM Cu+3 nM Mn)	0.065	0.017	6
6 (6 nM Cu+3 nM Mn)	0.062	0.025	6

Table L6b: ANOVA results from the picoplankton assemblage after 6 days incubation in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	1.90	0.12

Appendix M: Statistical analysis of results from Experiment 3 (Station 15) in the North Pacific, June-July 2002

M1: Total Phytoplankton Assemblage (>0.2 μm) following 3 days incubation

Table M1a: Mean phytoplankton biomass from total phytoplankton assemblage after 3 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.075	0.007	3
2 (3 nM Mn)	0.077	0.014	3
3 (3 nM Cu)	0.093	0.011	3
4 (6 nM Cu)	0.113	0.018	3
5 (3 nM Cu+3 nM Mn)	0.099	0.004	3
6 (6 nM Cu+3 nM Mn)	0.119	0.012	3

Table M1b: ANOVA results from total phytoplankton assemblage after 3 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	7.19	**0.00**

Table M1c: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 6 a the top and at the left of the table refer to Treatments 1 through 6).

	1	2	3	4	5	6
1}		0.823	0.168	**0.013**	0.099	**0.006**
2}	0.827		0.111	**0.013**	0.088	**0.006**
3}	0.168	0.111		0.151	0.547	0.081
4}	**0.013**	**0.013**	0.151		0.188	0.515
5}	0.099	0.088	0.547	0.188		0.139
6}	**0.006**	**0.006**	0.081	0.515	0.139	

M2: Microplankton + Nanoplankton Assemblage (>2.0 μm) following 3 days incubation

Table M2a: Mean phytoplankton biomass from microplankton+nanoplankton assemblage after 3 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.022	0.000	3
2 (3 nM Mn)	0.024	0.002	3
3 (3 nM Cu)	0.032	0.004	3
4 (6 nM Cu)	0.044	0.011	3
5 (3 nM Cu+3 nM Mn)	0.034	0.004	3
6 (6 nM Cu+3 nM Mn)	0.046	0.003	3

Table M2b: ANOVA results from microplankton+nanoplankton assemblage after 3 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	10.49	**0.00**

Table M2c: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 6 a the top and at the left of the table refer to Treatments 1 through 6).

	1	2	3	4	5	6
1}		0.577	0.105	**0.002**	0.056	**0.002**
2}	0.577		0.122	**0.003**	0.089	**0.003**
3}	0.105	0.122		0.030	0.516	**0.030**
4}	**0.002**	**0.003**	**0.030**		**0.041**	0.765
5}	0.056	0.089	0.516	**0.041**		0.056
6}	**0.002**	**0.003**	**0.030**	0.765	0.056	

M3: Picoplankton Assemblage (0.2 μm- 2.0 μm) following 3 days incubation

Table M3a: Mean phytoplankton biomass from the picoplankton assemblage after 3 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	**Phytoplankton Biomass (μg chl-*a*/L)**	**Standard Deviation**	**N**
1 (control)	0.053	0.007	3
2 (3 nM Mn)	0.052	0.013	3
3 (3 nM Cu)	0.062	0.008	3
4 (6 nM Cu)	0.068	0.007	3
5 (3 nM Cu+3 nM Mn)	0.065	0.003	3
6 (6 nM Cu+3 nM Mn)	0.074	0.009	3

Table M3b: ANOVA results from the picoplankton assemblage after 3 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	12	0.00	3.07	0.05

M4: Total Phytoplankton Assemblage (>0.2 μm) following 6 days incubation

Table M4a: Mean phytoplankton biomass from total phytoplankton assemblage after 6 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.078	0.007	6
2 (3 nM Mn)	0.098	0.023	6
3 (3 nM Cu)	0.086	0.019	6
4 (6 nM Cu)	0.066	0.023	6
5 (3 nM Cu+3 nM Mn)	0.091	0.014	6
6 (6 nM Cu+3 nM Mn)	0.112	0.008	6

Table M4b: ANOVA results from total phytoplankton assemblage after 6 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	5.39	**0.00**

Table M4c: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the independent variable and phytoplankton biomass as the dependent variable (the numbers 1 through 6 a the top and at the left of the table refer to Treatments 1 through 6).

	1	2	3	4	5	6
1}		0.197	0.391	0.236	0.378	**0.011**
2}	0.197		0.481	**0.022**	0.499	0.145
3}	0.391	0.481		0.111	0.633	0.056
4}	0.236	**0.022**	0.111		0.070	**0.001**
5}	0.378	0.499	0.633	0.070		0.091
6}	**0.011**	0.145	0.056	**0.001**	0.091	

M5: Microplankton + Nanoplankton Assemblage (>2.0μm) following 6 days incubation

Table M5a: Mean phytoplankton biomass from microplankton+nanoplankton assemblage after 6 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.026	0.002	6
2 (3 nM Mn)	0.032	0.007	6
3 (3 nM Cu)	0.030	0.003	6
4 (6 nM Cu)	0.028	0.008	6
5 (3 nM Cu+3 nM Mn)	0.032	0.003	6
6 (6 nM Cu+3 nM Mn)	0.036	0.003	6

Table M5b: ANOVA results from microplankton+nanoplankton assemblage after 6 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	3.16	**0.02**

Table M5c: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the independent variable and photosynthetic efficiency as the dependent variable (the numbers 1 through 6 a the top and at the left of the table refer to Treatments 1 through 6).

	1	2	3	4	5	6
1}		0.152	0.402	0.414	0.174	**0.014**
2}	0.152		0.390	0.379	0.908	0.356
3}	0.402	0.390		0.635	0.590	0.129
4}	0.414	0.379	0.635		0.469	0.070
5}	0.174	0.908	0.590	0.469		0.211
6}	**0.014**	0.356	0.129	0.070	0.211	

M6: Picoplankton Assemblage (0.2 μm- 2.0μm) following 6 days incubation

Table M6a: Mean phytoplankton biomass from the picoplankton assemblage after 6 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 (N= number of replicates).

Treatment	Phytoplankton Biomass (μg chl-*a*/L)	Standard Deviation	N
1 (control)	0.052	0.008	6
2 (3 nM Mn)	0.066	0.018	6
3 (3 nM Cu)	0.057	0.018	6
4 (6 nM Cu)	0.040	0.022	6
5 (3 nM Cu+3 nM Mn)	0.059	0.013	6
6 (6 nM Cu+3 nM Mn)	0.076	0.008	6

Table M6b: ANOVA results from the picoplankton assemblage after 6 days incubation in Experiment 3 (Station 15) in the North Pacific, June-July 2002 using concentration of added metal as the dependent variable and phytoplankton biomass as the dependent variable.

df Effect	MS Effect	df Error	MS Error	F	p-level
5	0.00	30	0.00	3.89	**0.01**

Table M6c: Probabilities for Newman-Keuls post hoc tests for the picoplankton assemblage in Experiment 1 (Station 4) in the North Pacific, June-July 2002 using concentration of added metal as the independent variable and phytoplankotn biomass as the dependent variable (the numbers 1 through 6 a the top and at the left of the table refer to Treatments 1 through 6).

	1	2	3	4	5	6
1}		0.406	0.586	0.201	0.724	0.062
2}	0.406		0.567	0.052	0.426	0.230
3}	0.586	0.567		0.169	0.829	0.133
4}	0.201	0.052	0.169		0.184	**0.003**
5}	0.724	0.426	0.829	0.184		0122
6}	0.062	0.230	0.133	**0.003**	0.122	