Investigating Factors that Control Trace Metal Toxicity in Coastal Systems: A Temporal and Spatial Analysis of Kinetically Inert Copper in Boston Harbor

by

Arthur G. Fitzmaurice III

S.B., Environmental Engineering Science (2003)
S.B., Chemical Engineering (2003)

Massachusetts Institute of Technology

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Signature of Author ....
Department of Civil and Environmental Engineering
August 20, 2004

Certified by ....
Bettina M. Voelker
Associate Professor of Civil and Environmental Engineering
Thesis Supervisor

Accepted by ....
Heidi M. Nepf
Chairman, Departmental Committee on Graduate Students

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ABSTRACT

Understanding how Cu reacts with constituents in coastal waters and interacts with biota is important for predicting Cu toxicity. It was recently shown that some of the Cu in coastal waters is present in kinetically inert forms that are not released from strongly bound compounds and do not bind to a strong synthetic ligand, remaining undetectable by competitive ligand exchange / adsorptive cathodic stripping voltammetry (CLE-ACSV) on time scales relevant to natural processes (48 hours). Inert Cu seems to be irreversibly bound to strong ligands or physically sequestered within organic and inorganic matrices such that it is likely non-toxic.

The primary objective of this work was to collect an extensive data set on one site, Boston Harbor, in order to examine the sources and sinks of inert Cu in this coastal system. Surface water samples were collected and filtered (0.2 μm) using trace-metal clean techniques from eleven locations in Boston Harbor every two to three weeks from June to November 2002. A strong ligand (salicylaldoxime) and increments of Cu were added to each sample to determine exchangeable Cu using CSV-ACSV. UV-treated samples were analyzed by CLE-ACSV to determine total dissolved Cu concentrations. Inert Cu concentrations were deduced by subtracting exchangeable from total Cu.

An average of 46.1% of the total dissolved Cu in Boston Harbor was determined to be inert Cu. Exchangeable Cu concentrations averaged 10.8 nM (σ=5.5 nM); total Cu, 20.6 nM (σ=5.7 nM); inert Cu, 10.7 nM (σ=3.1 nM). Although the fraction of inert Cu was variable among different sites and sampling dates, the concentration of exchangeable Cu remained within a smaller range (5-15 nM), implying that Cu inputs above this level mainly took the form of inert Cu. Correlations between Cu concentrations and total suspended solids at one sampling location suggested that sediment resuspension may be a source of inert Cu to coastal waters. Consistently significant inert Cu concentrations at a site near a salt marsh implied that the presence of sulfides may result in formation of inert Cu. No correlation between inert Cu and chlorophyll a was observed, suggesting that biological formation of inert Cu compounds *in situ* did not occur in this system. Whether Cu is added to surface waters as existing inert Cu compounds or as inert Cu that is formed when exchangeable Cu reacts with certain constituents in surface waters remains a subject for future work.

Thesis Supervisor: Bettina M. Voelker
Title: Associate Professor of Civil and Environmental Engineering

1 Introduction

Trace metals such as Cu can be toxic to aquatic organisms. At low concentrations Cu is an essential nutrient (Price *et al.* 1988/1989); however, at higher concentrations, Cu is toxic to many aquatic species (Hall *et al.* 1997). The Clean Water Act requires the United States Environmental Protection Agency to set maximum limits for the effluent discharge of various metals such as Cu to natural waters. However, current regulations do not properly reflect scientific understanding of metal toxicity, as they do not distinguish between toxic and nontoxic forms of the regulated metals (Shi *et al.* 1998). Even the recently developed Biotic Ligand Model proposed for predicting site-specific metal toxicity in freshwater systems (Paquin *et al.* 2000) does not provide a full description of Cu speciation and is probably not applicable to coastal systems. Current knowledge is not quite sufficient to explain the fate and transport of metals in complex natural systems. Further research to better understand the speciation of Cu and its impact on toxicity is needed to improve risk assessment and regulation of industrial effluents. The goal of this study is to assess whether relationships between different physico-chemical characteristics (e.g. chlorophyll a concentration, freshwater input) and Cu speciation exist in coastal systems which can ultimately be used to improve predictions of Cu toxicity.

1.1 Speciation of Cu

1.1.1 Dissolved Cu

Cu is found in aquatic systems in both dissolved and particulate forms. This study focuses on various forms of dissolved Cu. The speciation of dissolved Cu is conventionally described as the sum of two concentration terms:

$$[\mathrm{Cu}]_T = [\mathrm{Cu}^{2+}] + \Sigma[\mathrm{CuL}_i] \qquad (1)$$

That is, the total dissolved Cu is the sum of the concentrations of the free metal cupric ion Cu^{2+} and the Cu that is reversibly complexed with various organic and inorganic ligands, CuL_i. Complexation is a significant part of Cu speciation. More than 99.99% of the total dissolved Cu in coastal waters exists in complexes bound to strong ligands (Sunda and Guillard 1976). In oxygenated systems, the concentration of the free metal cuprous ion, Cu^+, is generally considered to be negligible.

1.1.2 Particulate Cu

Particulate Cu takes the form of solid Cu particles and Cu sorbed to other solid particles. In waters with suspended solids, Cu cations can sorb to the negatively-charged surfaces of particulate matter. Particulate Cu settles to the sediment where it remains until disturbed. Particularly in shallow areas, wind or currents can cause mixing throughout the water column. The turbulence resuspends the sediments and potentially frees the sorbed Cu back into dissolved form in the water column. Resuspension of contaminated sediments can be a significant source of dissolved Cu to surface waters (Cantwell 2002).

1.1.3 Kinetically Inert Cu

Filtration is commonly used to distinguish the concentrations of particulate, colloidal, and dissolved Cu in a sample, while techniques such as cathodic stripping voltammetry and atomic absorption spectroscopy are employed to determine Cu concentrations. The most commonly used variant of voltammetry involves competing ligand exchange, the addition of a synthetic ligand such as salicylaldoxime (SA) before analysis. Depending on the composition of the sample and the binding ability and concentration of the synthetic ligand (its "analytical competition strength"), it can outcompete natural ligands, and the Cu complexes with the synthetic ligand can be measured (see Methods). Since the ligand has a known conditional stability constant, the strength of the natural ligands can be characterized in this way.

Kogut and Voelker (2003) found that a fraction of the Cu in coastal waters does not appear to be exchanged from natural ligands to SA at concentrations of up to 1 mM (an analytical competition strength four to six orders of magnitude higher than is typically used) on time scales relevant to natural processes (48 hours). This fraction is bound by 1 mM SA in an aliquot of the same sample that has undergone UV-irradiation, implying that UV treatment destroys bonds that render Cu non-exchangeable. Kogut and Voelker proposed an additional classification of natural Cu compounds—"kinetically inert Cu"— to describe this form of Cu. They postulated that this Cu may be irreversibly bound to ligands or physically sequestered within organic or inorganic colloidal matrices (Kogut and Voelker 2003). Given this additional form, the speciation of dissolved Cu is more accurately described as:

$$[Cu]_T = [Cu^{2+}] + \Sigma[CuL_i] + [Cu]_{inert} \tag{2}$$

1.2 Cu Toxicity

The speciation of Cu into these various forms has certain implications for the toxicity of Cu in coastal systems. Sunda found that in the case of phytoplankton, Cu^{2+} is the major toxic form of Cu (Sunda 1988). Free ions are bioavailable—that is, they can bind to ligands at receptor sites on the cellular surface. These cellular complexes can then be actively transported through the lipid bilayer of the cell. Additionally, certain liposoluble complexes are considered bioavailable since they can passively diffuse into the cell. If Cu uptake raises Cu to substantially high intracellular concentrations, it can inhibit cellular processes and damage or even kill the cell (Moffett and Brand 1996).

Dissolved Cu can be toxic to multicellular organisms as well. Cu-binding to key receptors on the gill surfaces of fish can interfere with the exchange of gases and the established ionic balance, disrupting important physiological functions such as oxygen uptake for respiration (Lewis *et al.* 2002).

Both dissolved and particulate Cu can be toxic by other mechanisms as well (e.g. ingestion), although only these cellular receptor mechanisms are considered in the toxicity models discussed in this work.

1.3 Cu-complexing Ligands

Since toxicity of dissolved Cu is not simply a function of the total dissolved concentration, it is important to understand the relative contributions of the different forms of Cu in Eq.(2). The extent of complexation depends on the types and concentrations of ligands in the water. Inorganic ligands such as carbonate and hydroxide ions are abundant in seawater. However, coastal systems generally have a high concentration of stronger organic ligands, making inorganic complexes negligible. The structures of organic ligands that bind to Cu in coastal systems have not been determined. However, organic ligands are divided into two classes based on observed conditional binding constants—L_1 (10^{12} and greater) and L_2 (10^8 to 10^{11}).

1.3.1 L_1-class Ligands

Sources of L_1-class ligands include treated sewage effluent (Sedlak *et al.* 1997), diffusing sediment pore waters (Skrabal *et al.* 2000), and biota. Moffett *et al.* (1990) found that the cyanobacterium *Synechecoccus sp.* excretes a strong Cu-binding compound with a conditional stability constant of approximately $10^{13.2}$ under Cu stress. Ahner *et al.* (1998) found that the diatoms *Thalassoria weissflogii, T. parthenaia,* and *Emiliania huxleyi* produce polypeptide phytochelatins in response to Cu stress. This behavior is likely exhibited by other coastal species as well.

Sulfides and thiols have been proposed as potential L_1-class ligands. Although reduced sulfur species are susceptible to oxidation, even in aerobic environments Cu-sulfide complexes are able to resist oxidation on time scales of several days (Rozan and Benoit 1999). Al-Farawati and van den Berg (1999) found a conditional binding constant of 10^{13} for the Cu-bisulfide complex in seawater. Strong complexes have been found between Cu and thiols as well. For example, Dupont *et al.* found that a strong Cu-thiol complex is formed intracellularly and exuded by *E. huxleyi*; this nitrogen-rich thiol has a conditional binding constant of 10^{12} with Cu (Dupont *et al.* 2004). Other studies have also found that thiol compounds are produced by phytoplankton under Cu stress (Leal and van den Berg 1998; Ross *et al.* 2003; Vachet *et al.* 2003).

1.3.2 L_2-class Ligands

L_2-class ligands are weaker but often more abundant than L_1-class ligands. Skrabal *et al.* found pore waters to be a significant source of L_2-class ligands to the Chesapeake Bay (Skrabal *et al.* 2000). Other studies have identified terrestrial sources of L_2-ligands (Burton and Stratham 1988; Croue *et al.* 2004). In general, L_1-class ligands need to be saturated with Cu before L_2-class ligands significantly affect the speciation of Cu in the system. In relatively unimpacted coastal waters containing L_1-class ligands, the ambient Cu concentration is often less than the total concentration of L_1-class ligands, so the effect of L_2-class ligands is small (Byrne and Miller 1985).

1.3.3 Humic Substances

A potential source of both L_1- and L_2-class ligands in coastal waters is terrestrial humic substances (Kogut and Voelker 2001). Humic substances comprise a fraction of dissolved organic matter in aquatic systems and are generally derived from terrestrial plant material that decays into organic macromolecules. They are transported to receiving water bodies mainly through runoff, rivers, and sewage. As proposed structures have not proven adequate to describe these molecules, humic acids are likely composed of many different chemical structures with variable molar-mass distributions, substructures, and functional groups. Analyses imply that they have aromatic rings, carboxyl groups, alcohol groups, phenol groups, and aliphatic chains (Aiken *et al.* 1985; Averett *et al.* 1989). There is evidence of one or more nitrogenous binding sites as well (Croue *et al.* 2004). These variable functional groups result in humic substances having different conditional binding constants with Cu.

1.3.4 Analytical Shortfalls

A major problem with analytical techniques that are commonly employed to characterize Cu-binding in natural waters is that they ignore the potential existence of inert Cu compounds and thus fail to distinguish between Cu-L_1 complexes and inert Cu compounds.

Competitive ligand exchange / adsorptive cathodic stripping voltammetry (CLE-ACSV) is a common method used for characterizing natural ligands in a sample. In this method, Cu is added incrementally to a sample with an added synthetic ligand (see Methods). As the natural and added ligands bind to added Cu, the concentration of the complex with the added ligand forms a curve when plotted against added Cu. When the natural ligands become saturated, this plot becomes linear as the added Cu binds to the added ligand only. Theoretically, the concentrations and conditional binding constants of natural ligands that are stronger than that of the added ligand can be determined from analysis of titration data based on the distinction between the curved and linear regions of the plot. Varying the analytical competition strength of the added ligand in the analysis of different aliquots of the same water can result in the characterization of both L_1- and L_2-class ligands in the sample.

However, Voelker and Kogut (2001) demonstrated that these common techniques are insufficient for accurately characterizing Cu speciation in some coastal waters. They found that in coastal waters with potentially many different ligands and inert Cu compounds, some of these can be misclassified (e.g. inert compounds are classified as L_1-class ligands) or disregarded from the analysis completely. While it appears that strong L_1-class ligands abound in some waters, in reality these waters may contain inert Cu compounds and zero to low concentrations of strong ligands.

1.4 The Effect of Cu Speciation on Cu Toxicity

An understanding of the dissolved Cu composition and the sources and sinks of Cu-binding ligands is vital to determine the speciation of Cu and its resulting toxicity in the system. As various Cu species try to maintain equilibrium, a balance is established among the free ion, complexes with ligands in the water, and complexes with cellular receptor ligands. Toxicity of dissolved Cu depends on the concentration of Cu ions at certain cellular receptor ligands (see 1.2 for examples of receptors). An equilibrium model can be used to predict how much Cu will be bound to these receptors and have potentially toxic effects.

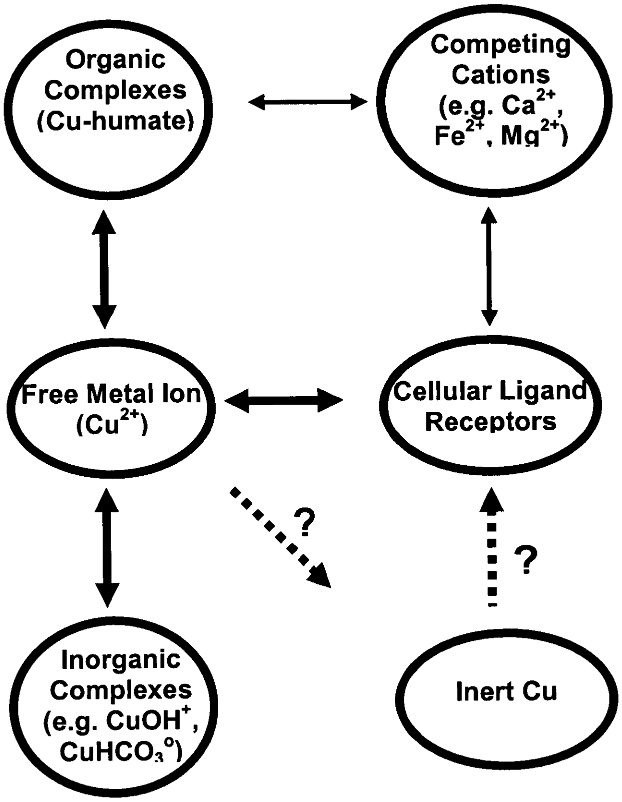


Figure 1. Cu speciation in aquatic systems. This simplified equilibrium model showcases the interactions among Cu, other metals, ligands, and biota (adapted from Pagenkopf 1983; Sunda 1988; Morel and Hering 1993; Paquin *et al.* 2000). How inert Cu fits into the model has not yet been determined.

Figure 1 depicts the interactions between Cu, ligands, and other metals in an aquatic system (adapted from Pagenkopf 1983; Sunda 1988; Morel and Hering 1993; Paquin *et al.* 2000). This model shows several compartments of chemical species and the equilibria existing between compartments (shown as double-headed arrows). The concentration of the free ion is controlled by the presence of both organic and inorganic ligands in the system. (In coastal systems, the effect of inorganic ligands is usually minor due to the strength and abundance of organic ligands.) By binding to and maintaining equilibria with the free metal ion according to inherent conditional stability constants, ligands can reduce the concentration of Cu that binds at the cellular ligands (proportional to free Cu ion), in effect reducing Cu bioavailability and/or toxicity. However, a source or sink of these ligands can disrupt the established balance and result in a change in the free ion concentration. Introduction of a ligand to the system can result in the formation of a Cu-ligand complex and in a decrease in the concentration of free Cu; on the other hand, destruction of a ligand can increase free Cu. Additionally, other metal ions such as Ca, Fe, Mg, Zn, and Mn ions can outcompete Cu in binding to ligands, thereby increasing the free Cu concentration. However, a more significant impact of other metals is that they can also outcompete Cu at the cellular surface and inhibit Cu binding to cellular ligands, thereby reducing Cu toxicity (Sunda and Huntsman 1983; Paquin *et al.* 2000). Notably, in the formulation of this model, free Cu is the only form of Cu that is able to bind to cellular receptors and thus considered directly bioavailable. This neglects the possibility that some compounds may also be bioavailable through other mechanisms (e.g. passive diffusion, ingestion).

1.4.1 Influence of Inert Cu on Cu Toxicity

The existence of inert Cu further complicates this model. The nature of inert Cu and its interactions with other system components is not known. One possibility is that inert Cu compounds serve as a sink of Cu available for equilibration, thereby decreasing the potential toxicity of Cu in the system. Another (much less likely) possibility is that inert

Cu compounds are directly bioavailable. Knowledge of the sources and sinks of inert Cu, including the factors that result in the formation or destruction of inert Cu, is vital for assessing the interactions among chemical species and determining the impact of inert Cu formation on the potential toxicity of Cu in a system.

1.5 Field Study Objectives

The only study that has been conducted on inert Cu involved the analysis of samples collected from five New England coastal locations on December 27, 2001, and January 4, 2002 (Kogut and Voelker 2003). For these ten data points, 10-60% of the total dissolved Cu was determined to be inert Cu. Retention of 0.2-μm-filtrable Cu by a 0.02-μm-pore size filter suggested that some of this inert Cu was physically sequestered in colloidal matrices. This study concluded that inert Cu exists in some coastal water samples, but the data set was too limited to be used to examine the factors that may result in the presence or absence of inert Cu.

The primary objective of the field study presented in this paper was to collect a much more extensive data set on one site, Boston Harbor, in order to examine the sources and sinks of inert Cu in this coastal system. This study was designed to test several hypotheses about the factors that result in the formation or destruction of inert Cu by examining correlations between the concentrations of inert Cu and certain physico-chemical parameters. A spatial distribution provided sampling locations at which different sources and sinks of Cu and Cu-binding compounds would potentially be dominant. A time series (June through November) provided variations in such physico-chemical parameters as turbidity, temperature, biological productivity, and freshwater inputs at each particular sampling location.

Sources of Cu to Boston Harbor, possibly as inert Cu, include industrial point-source pollution, river flow, runoff, combined sewer overflows, treated effluent discharge, shipping, and sediment resuspension. The possible effects of rivers, a shipping channel, and treated and untreated sewage outfalls on the concentrations of total and inert Cu are investigated at sampling locations in proximity to these sources. To examine possible effects of resuspension, concentrations of total suspended solids and sample turbidity are correlated with total dissolved, exchangeable, and inert Cu. Formation of inert Cu *in situ* by biota is considered through analysis of relationships between chlorophyll a (as an indicator of seasonal changes in biological activity) and concentrations of total dissolved, exchangeable, and inert Cu. Potential sulfide interactions with Cu are explored through close analysis of a site near a salt marsh.

2 Methods

2.1 Materials

Care was taken to prevent contamination in all aspects of this field study. Deionized distilled water (DDW) from a Millipore Q-H_2O system was used for diluting standards and for rinsing bottles and filters after acid-cleaning. OmniTrace nitric acid (69.0-70.0%) with less than 0.5 ppb Cu was used for acid-cleaning and sample acidification. Ultrex II Ultrapure ammonium hydroxide (20%) with less than 1 ppb Cu was used for neutralizing acidified samples.

A stock solution of 35 mM SA was made every two weeks by dissolving salicylaldoxime (SA) in DDW overnight. Solid SA was used as received from Aldrich Chemical Company and stored in the refrigerator. 1 M boric acid buffer was made by dissolving solid Superpure boric acid obtained from EN Science in DDW and adjusting to pH 8.0 with concentrated ammonium hydroxide. A standard solution of 1,025 μg/ml Cu in 1% nitric acid (Aldrich) was diluted with DDW to make a stock solution of 1 μM Cu. This stock solution was made at least daily.

5.0-grade nitrogen gas (BOC Gases) was used for purging samples during voltammetric analysis.

2.2 Sample Preparation

2.2.1 Sample Collection

Surface water samples were collected onboard a Massachusetts Water Resources Authority (MWRA) research vessel approximately every two to three weeks from June 2002 through November 2002 at eleven sites in and around Boston Harbor (Figure 2). These sampling locations, extending north to the mouth of the Mystic River and south to Hull, Massachusetts, are part of the MWRA's Weekly Water Quality Monitoring Program (Table 1). Auxiliary water quality data such as temperature, salinity, dissolved oxygen, total suspended solids, and chlorophyll a concentration at these locations were obtained from the MWRA.

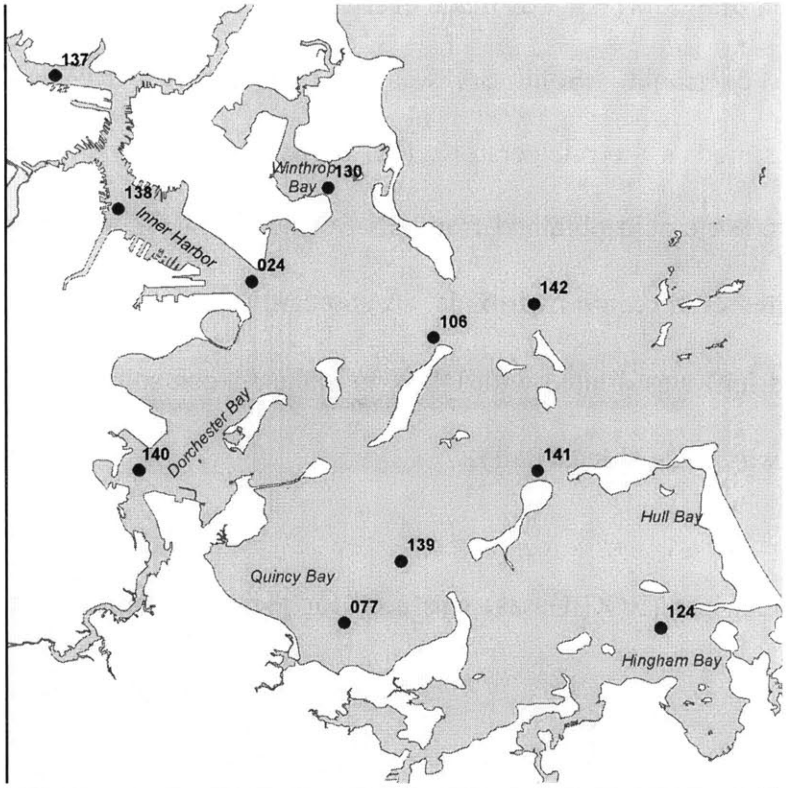


Figure 2. Boston Harbor sampling sites, June – November 2002.

Table 1. Boston Harbor sampling sites, June – November 2002.

MWRA Sampling Location	Location Description	GPS Location	
024	Inner Harbor - red buoy #10	42°20.59 N	71°00.48 W
077	Off Merrymount Park	42°16.51 N	70°59.31 W
106	Long Island – green can #17	42°20.00 N	70°57.60 W
124	Hingham Harbor – off Crow Point	42°16.36 N	70°53.86 W
130	Winthrop Bay - off Cottage Park Yacht Club - green can #1	42°21.80 N	70°59.25 W
137	Mystic River - 1/3 mile upstream Tobin Bridge	42°23.20 N	71°03.80 W
138	Inner Harbor - off New England Aquarium	42°21.59 N	71°02.82 W
139	Hangman's Island	42°17.20 N	70°58.10 W
140	Columbia Point / Savin Hill Cove	42°18.35 N	71°02.43 W
141	Peddocks Island	42°18.30 N	70°55.85 W
142	President Roads, South Channel - green can #13	42°20.35 N	70°55.89 W

Acid-cleaned Teflon containers were used for all samples, reagents, and experiments to minimize adsorption of Cu and ligands to bottle surfaces and leaching of phthalate plasticizers into the samples. After being acid-cleaned with 1% nitric acid for a minimum of twenty-four hours, the sampling bottles were stored full of DDW. At each sampling site, 500 ml of surface water was collected with a pole sampler consisting of a plexi-glass bottle holder, a twelve-foot PVC extension pole, and a 500-ml Teflon bottle fastened with Tygon tubing (Figure 3). Samples were collected off the bow outside the vessel's travel path to prevent contamination from

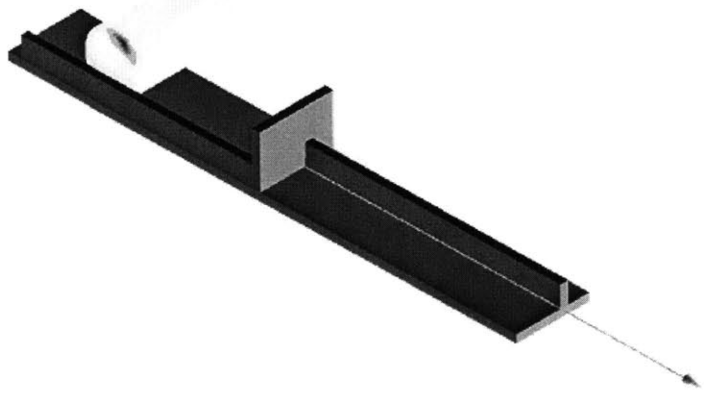


Figure 3. Plexi-glass sampling unit. Arrow indicates where the PVC extension pole was attached.

the boat. To further minimize the possibility of contamination, the collection bottle was rinsed with sample water three times before the sample was collected.

2.2.2 Cu Samples

At each sampling location, 240 ml of sample were syringe-filtered through 0.2-µm polycarbonate membrane filters (Nucleopore, 47 mm filter diameter) held in polycarbonate filter holders. The filters had been acid-cleaned and extensively rinsed with DDW before sample filtration. Contamination due to the addition of Cu or ligands from the filters or the sorption of Cu or ligands to these filters was previously found to be negligible (Kogut and Voelker 2003). The syringe and filters were rinsed with 60 ml of sample before the filtrate was retained.

Whenever possible, collected samples were filtered within fifteen minutes of collection. When this was not possible, samples were stored on ice and filtered as soon as possible. All samples were filtered and frozen in liquid nitrogen within four hours of collection to prevent bacterial activity from altering the state of Cu speciation in the samples. Liquid nitrogen was used for quick freezing to prevent coagulation or flocculation of organic matter in the samples (Kogut and Voelker 2003). Samples were stored in a freezer at – 20°C for later analysis.

2.2.3 Dissolved Organic Carbon Samples

At each sampling site, 20 ml of sample were filtered into pre-combusted amber glass vials using pre-combusted glass syringes and 0.2-μm polysulfonate cartridge filters. These materials were acid-cleaned and rinsed with 10 ml each of DDW and sample before filtrate was retained. These samples were stored in a freezer at –20°C for later analysis.

2.2.4 Sargasso Sea Samples

Samples from the Sargasso Sea were collected and filtered by J.W. Moffett using trace-metal clean sampling techniques. These samples were prepared for analysis in the same manner as those collected in this field study (described below).

2.3 Laboratory Methods

2.3.1 Ultraviolet-irradiation of Samples for Total Cu Analysis

30-ml aliquots of thawed Cu samples were transferred to acid-cleaned quartz test tubes. 20 μl of trace-metal clean concentrated nitric acid (69.0-70.0%) were added to each aliquot to act as an oxidant and to prevent Cu from adhering to the test tube surfaces. Samples were irradiated under ultraviolet light with a medium-pressure mercury lamp (Ace Glass, 1000 W) for 8 hours. This process destroyed the dissolved organic compounds in the sample by breaking C-C bonds, freeing any Cu complexed to organic ligands as well as any inert Cu sequestered within organic matrices. Since the combination of strong acid and light should have dissolved any iron oxide present, and hydroxyl radicals produced through nitrate photolysis should have oxidized both organic and reduced inorganic matrices (e.g. sulfides) in the samples, any remaining inert Cu in the sample should also have been released by UV-irradiation.

After 8 hours, the samples were removed from UV light, and DDW was added to replace the water that had evaporated during UV treatment (less than 3 ml). Concentrated ammonium hydroxide (20 %) was added to return the pH of the samples to about 8.0, and 24.0 ml of samples were retained and transferred to Teflon bottles for CLE-ACSV analysis (see below).

2.3.2 Competitive Ligand Exchange (CLE)

A competitive ligand exchange method was used to make samples measurable for concentrations of total dissolved and exchangeable Cu using a voltammetric method. SA was used as the strong ligand for these analyses, since it binds Cu strongly and is therefore expected to outcompete all of the natural ligands in the samples if used at sufficiently high concentrations (Kogut and Voelker 2001). This ligand was also used since the peak of its complex with Cu was not expected to be susceptible to voltammetric interference by other compounds.

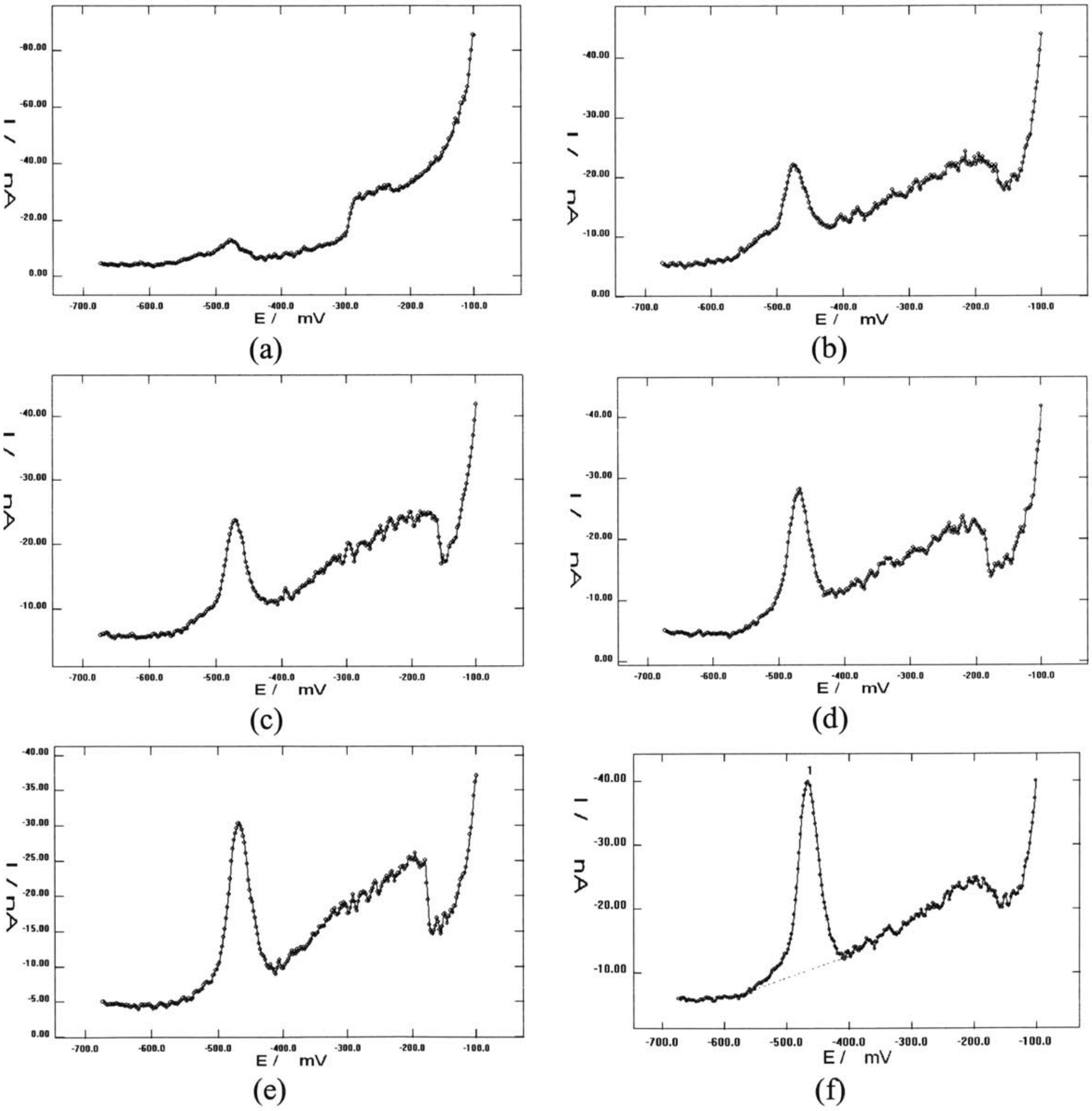
SA was added to 24.0-ml aliquots of samples to make final sample concentrations of 1 mM SA. 500 μl of UV-oxidized 1M boric acid buffer were added to the aliquots for a final buffer concentration of 20 mM. After addition of SA and buffer, the bottles were sealed in plastic bags and stored refrigerated in the dark. UV-irradiated samples were allowed to equilibrate overnight, and untreated samples were allowed to equilibrate for at least 48 hours before voltammetric analysis. No samples were measured after 96 hours.

2.3.3 Adsorptive Cathodic Stripping Voltammetry (ACSV)

Differential pulse ACSV was used to measure the concentration of Cu-SA complexes, $[Cu(SA)_x]$. 11.0-ml aliquots of the equilibrated samples were analyzed with an EG&G PAR 394 analyzer in the instrument's Teflon sample cup. Instrument settings were as follows: adsorption potential, -0.08 V (vs Ag/AgCl electrode); scan range, –100 to –700 mV; scan rate, 6.66667 mV/s; scan increment, 2 mV; drop time, 0.3 s; electrode area, 1 cm^2; pulse height, 20 mV.

This method involved an adsorption step followed by a potential scan. During the adsorption step, $Cu(SA)_x$ complexes sorbed onto a PAR 303A static mercury drop electrode. The deposition time was set to be 30 seconds, although the actual experimental deposition time was approximately 60 seconds longer before the instrument scan reached the desired voltage. During the scan, potential was applied to the Hg in the negative direction. Cu in the sorbed complexes was reduced, and the resulting current was measured. This created a well-defined peak current, I, between –410 and –480 mV.

The 1 μM Cu standard was used to add increments of 7 to 8 nM Cu to the sample cup with 4 to 9 Cu additions to reach a final added Cu concentration of 30 to 72 nM depending on the sample. Before the first peak current measurement, the sample was purged with nitrogen gas for 300 seconds to remove oxygen from the sample. After additions of Cu, the sample was purged with nitrogen for 210 seconds for the same reason as well as to provide sufficient time for the added Cu to equilibrate with SA before the peak current measurement.



(a) (b) (c) (d) (e) (f)

Figure 4. ACSV potential scans from a representative sample analysis at $[Cu]_{added}$ = 0.0, 7.7, 15.2, 22.7, 30.0, 37.2 nM.

Figure 4 shows the voltammetric analysis of a representative sample at added Cu concentrations of 0.0, 7.7, 15.2, 22.7, 30.0, and 37.2 nM, respectively. The peak height was determined from each of these plots (Figure 5). Linear regression analysis was then used to determine the slope and intercept of the titration for use in determining total dissolved and exchangeable Cu concentrations.

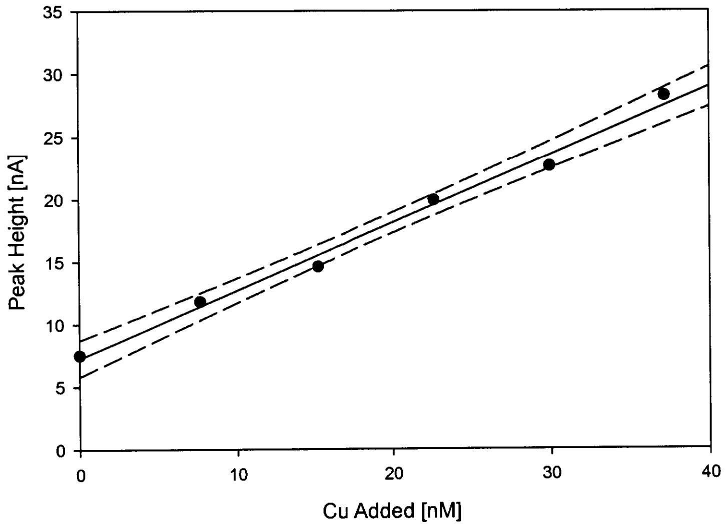


Figure 5. Plot of titration data from sample analyzed in Figure 4. Dashed lines show 95% confidence interval of linear regression (solid line, R^2=0.9910). $[Cu]_{sample}$ is determined by dividing the y-intercept by the slope of the regression line.

2.4 *Data Analysis*

2.4.1 Determination of Cu Concentrations

The concentration of $Cu(SA)_x$ sorbed to the Hg drop is proportional to $[Cu(SA)_x]$ in the sample. The ratio of I and $[Cu(SA)_x]$ is called the sensitivity, S:

$$S = I / [Cu(SA)_x] \qquad (3)$$

S varies depending on the instrument settings and the composition of the sample. Cu was added incrementally by standard addition to determine S and deduce Cu concentrations in the samples (Kogut and Voelker 2001). Since the SA was present at sufficiently high concentrations to outcompete any natural ligands present in the samples (Kogut and Voelker 2001), all of the added Cu, $\Delta[\mathrm{Cu}]_T$, complexed with SA during each titration step such that:

$$\Delta[\mathrm{Cu}]_T = \Delta[\mathrm{Cu(SA)}_x] \tag{4}$$

Eq.(4) can be applied to Eq.(3) to show that S is then given by the slope of a plot of I versus total Cu added, $\Delta[\mathrm{Cu}]_T$. The y-intercept of the plot divided by S gives $[\mathrm{Cu(SA)}_x]$ initially in the sample before the Cu additions.

During equilibration of an untreated (not UV-irradiated) sample, the SA partially outcompetes the non-inert (exchangeable) natural ligands that were bound to Cu, so that the expression for Cu speciation in the sample becomes:

$$[\mathrm{Cu}]_T = [\mathrm{Cu}^{2+}]_{\mathrm{untreated}} + \Sigma[\mathrm{CuL}_i]_{\mathrm{untreated}} + [\mathrm{Cu(SA)}_x]_{\mathrm{untreated}} + [\mathrm{Cu}]_{\mathrm{inert}} \tag{5}$$

where SA forms both *mono* and *bis* complexes with Cu:

$$[\mathrm{Cu(SA)}_x] = [\mathrm{Cu(SA)}] + [\mathrm{Cu(SA)}_2] \tag{6}$$

For high concentrations of strong competing ligand (1 mM SA in this study), $[\mathrm{Cu(SA)_x}]_{\mathrm{untreated}}$ dominates Cu speciation and $[\mathrm{Cu^{2+}}]_{\mathrm{untreated}}$ and $\Sigma\,[\mathrm{CuL_i}]_{\mathrm{untreated}}$ are negligible such that Eq.(5) becomes:

$$[\mathrm{Cu}]_{\mathrm{T}} \cong [\mathrm{Cu(SA)_x}]_{\mathrm{untreated}} + [\mathrm{Cu}]_{\mathrm{inert}} \qquad (7)$$

After UV irradiation, Cu contributing to $[\mathrm{Cu}]_{\mathrm{inert}}$ is released and also binds to SA, such that the expression for Cu speciation in the sample becomes:

$$[\mathrm{Cu}]_{\mathrm{T}} \cong [\mathrm{Cu(SA)_x}]_{\mathrm{UV\text{-}irradiated}} \qquad (8)$$

Thus, measurement of $[\mathrm{Cu(SA)_x}]$ in the untreated sample gives the exchangeable Cu concentration (the Cu that will be bound by added SA; approximately $[\mathrm{Cu^{2+}}]+\Sigma[\mathrm{CuL_i}]$ in Eq.(2)), while the UV-irradiated sample gives the total dissolved Cu concentration. The difference between the two gives the inert Cu concentration:

$$[\mathrm{Cu}]_{\mathrm{inert}} = [\mathrm{Cu(SA)_x}]_{\mathrm{untreated}} - [\mathrm{Cu(SA)_x}]_{\mathrm{UV\text{-}irradiated}} \qquad (9)$$

2.4.2 Error Analysis

For each titration in this study, the uncertainties of the slope and y-intercept of the linear regression were calculated as a 95% confidence interval (J.C. Miller and J.N. Miller 1993). As the total or exchangeable Cu concentration was calculated by dividing the y-intercept by the slope from the appropriate sample titration regression, the uncertainty of either was determined by error propagation through division. The uncertainty of the inert Cu concentration was the square root of the sum of the squares of the uncertainties of total and exchangeable Cu concentrations. Whenever they were obtained, replicate measurements were averaged before calculating inert Cu. Error bars associated with replicate averages (see Results) were calculated as the standard deviation of the set of measured values.

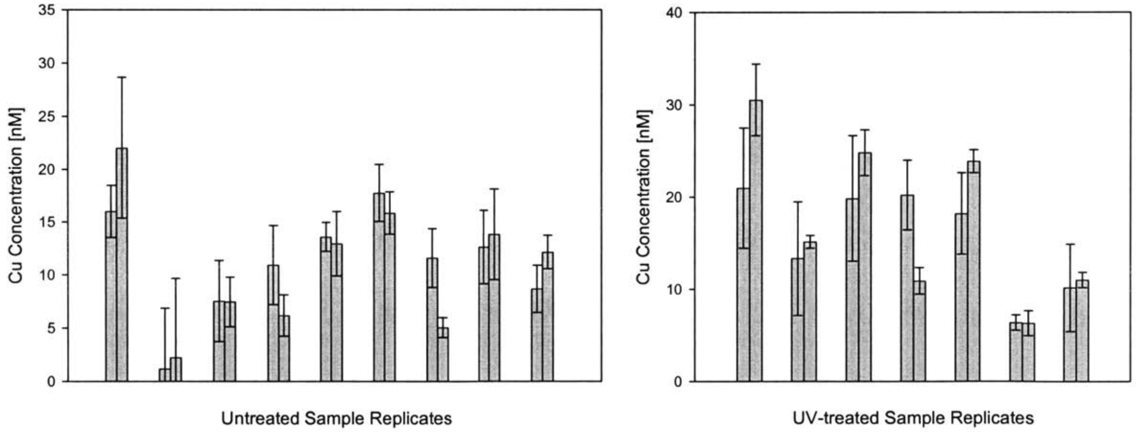
3 Results

Appendices A and B show exchangeable and total Cu concentrations for each sampling location over the duration of this field study. R^2 from linear regressions of titrations used to derive these values ranged from 0.9674 to 1.0000 (see 2.4.2 for analytical method). The inert Cu concentration was determined for each of 80 date/location pairs by subtracting the exchangeable Cu concentration from the total Cu concentration (Appendix A).

3.1 Data Quality Control

Two measures were taken to test the accuracy and precision of the CSV measurements. First, samples of untreated and UV-irradiated Sargasso Sea water were measured periodically to check for contamination problems in the analytical procedure. This water was selected for its availability and low Cu content. Moffett found total Cu concentrations in the Sargasso Sea to range from 0.9 to 2 nM (Moffett 1995); our work yielded concentrations of 0.1±2.1 to 3.7±2.4 nM for Sargasso Sea water. Although these samples were not from the same time and precise location, the absence of significant contamination problems was validated by the similarity in measured concentrations.

As a second check on the analytical technique, two different types of replicates were measured. For one type, twice the amount of sample needed for one voltammetric analysis was equilibrated with SA and buffer, and then two separate titrations with voltammetric analyses were performed on the same day. In other cases, two aliquots of the same collected sample were prepared for analysis (equilibrated with SA and buffer) on two different days; one or more replicates of each were then analyzed. Figure 6 shows replicates for 9 exchangeable Cu measurements and 7 total Cu measurements. Error bars for all raw measurements reflect our estimated 95% confidence interval based on the uncertainties of the slope and intercept of each individual titration (see Methods). 14 of 16, or 88%, of the replicates show agreement within the calculated error bars. This demonstrates that our 95% confidence interval is a reasonable estimate of the precision of this method. The variation of error bar magnitudes from different analyses of a given sample also demonstrates the variable behavior of the instrument; in general an effort was made to reduce use of the instrument on days when error bars were particularly large.



(a)

(b)

Figure 6. Comparisons of replicate measurements. (a) 8 of 9 sets of exchangeable Cu and (b) 6 of 7 sets of total Cu concentrations are within 95% confidence. This demonstrates the precision of the method.

3.2 *General Trends*

3.2.1 Fraction of Inert Cu

A significant portion of the Cu in this coastal system was found to be kinetically inert, although the fraction of inert Cu varied considerably from sample to sample, ranging from an average of 29.7% at location 138 to 65.2% at location 130 (Figure 7). The average % inert Cu was 46.1%.

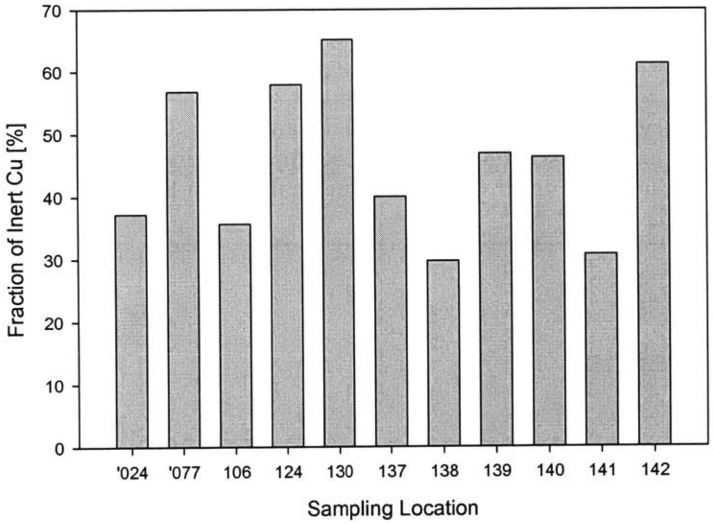


Figure 7. Average % inert Cu at each sampling location. The fraction of Cu that is inert Cu varies from site to site. Location 130 has the highest % inert Cu (65.2%); 138 has the lowest (29.7%).

Figure 8 shows the distribution of % inert Cu among analyzed samples. 9 samples with less than 0% inert Cu and 9 with more than 100% were omitted from the figure. The errors associated with % inert Cu (not shown) demonstrate that these data points were not significantly far from 0% and 100% and thus reflect samples with nearly no or all inert Cu, respectively.

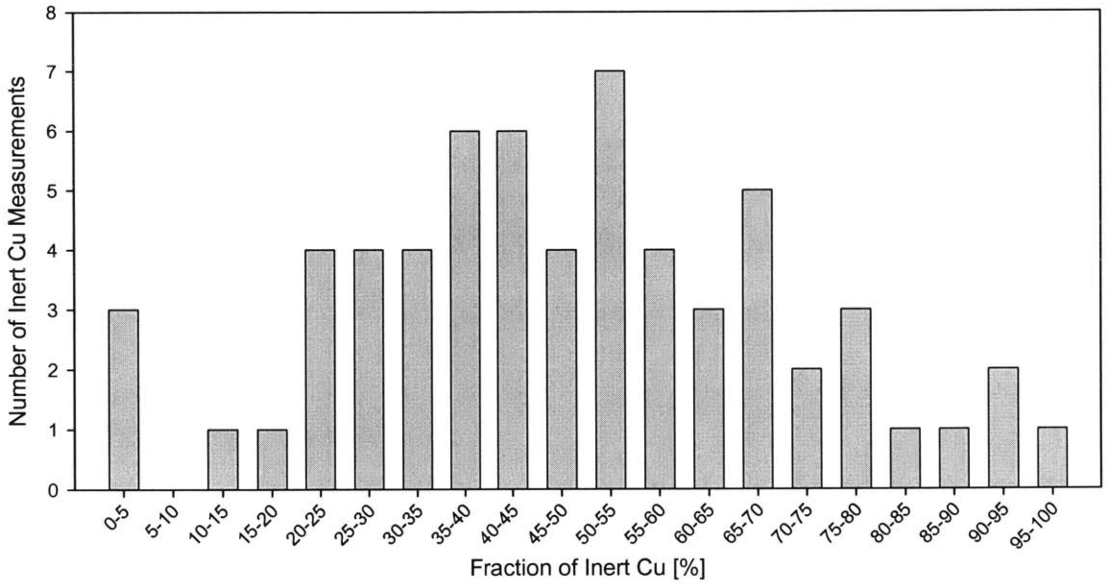


Figure 8. Frequency of samples with specified ranges of % inert Cu. A significant number of samples have substantial % inert Cu, implying that coastal waters typically have a fraction of Cu that is inert Cu. (9 samples not shown likely have no inert Cu; 9 likely are entirely inert Cu.)

3.2.2 Temporal Trends

There is a general decrease in the fraction of inert Cu from June to November (Figure 9). Each data point in Figure 9 represents the average of % inert Cu at all locations sampled on the given day. Not all sampling locations were analyzed for each date and thus different samples were included in each average value. However, this should not skew the averages since the sites were not skipped in a systematic way. The same trend can be seen by averaging the data from only the samples that have analyses for 78% (7 of 9) or more of the days in the field study (Figure 9).

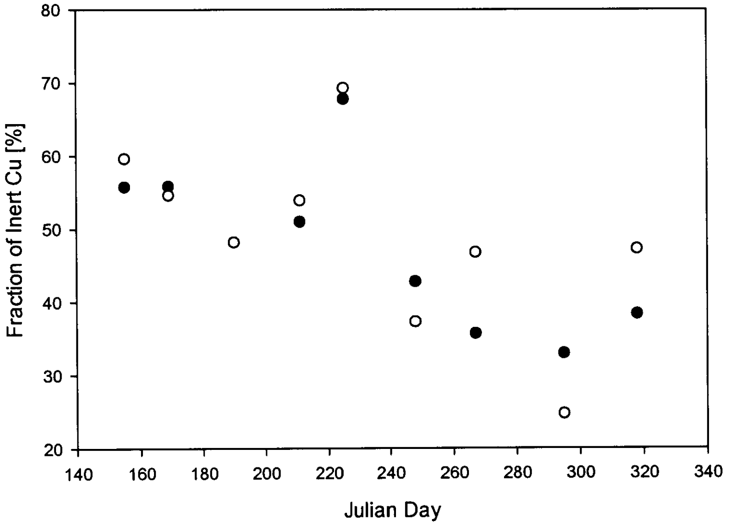


Figure 9. Temporal trend of % inert Cu. Each data point represents the average value for all site samples analyzed on the corresponding date. Dark circles show the averages for all given data; hollow circles show averages of data from samples for which at least 7 of 9 dates were analyzed. For either case, % inert Cu decreases from June to November.

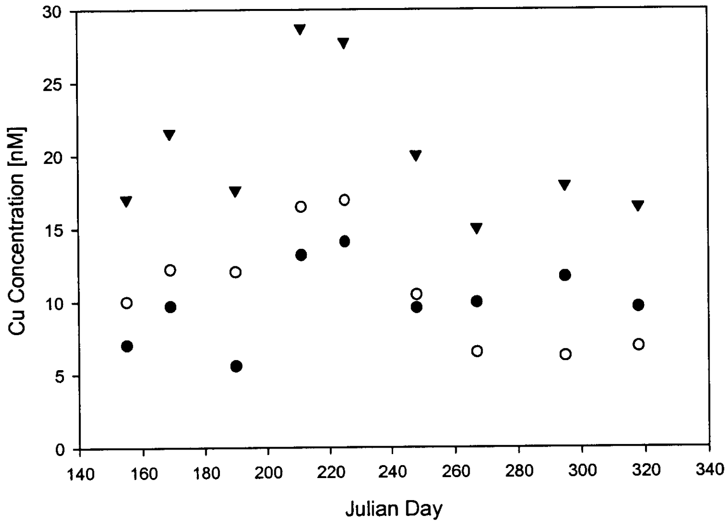
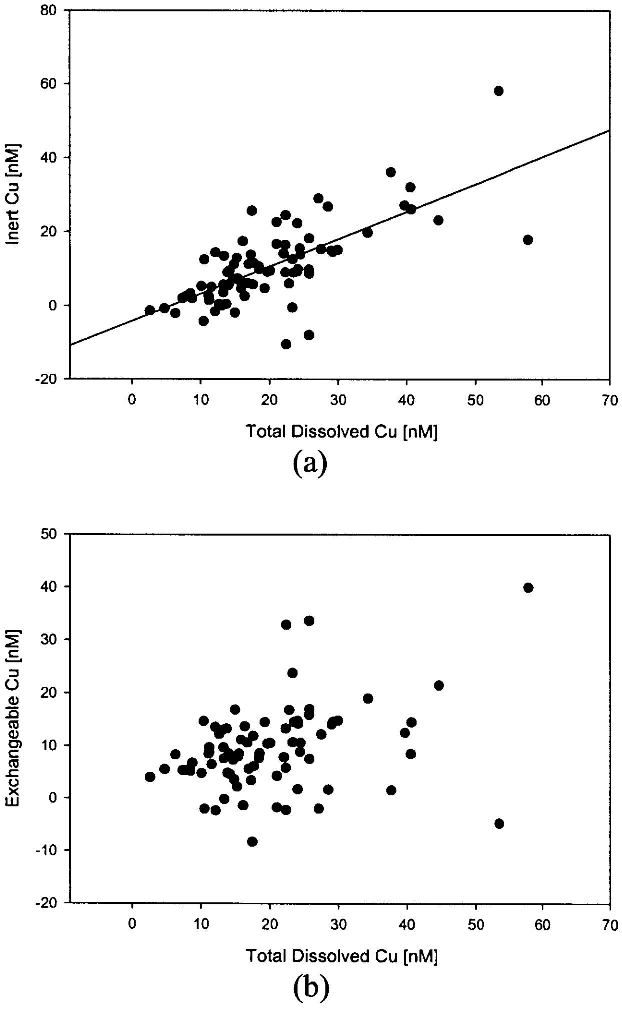


Figure 10. Temporal trends of exchangeable (dark circles), inert (hollow circles), and total Cu (triangles) concentrations. Each data point represents the average value for all site samples analyzed on the corresponding date. All Cu concentrations increase to a mid-summer peak before subsiding.

Total, exchangeable, and inert Cu concentrations peak in the middle of the summer (Figure 10). Again, we assume that these trends are temporal and not an artifact of biased site-sampling.

3.2.3 Correlations Among Cu Species

The concentrations of inert Cu and total dissolved Cu in this system are somewhat correlated (Figure 11-a, R^2=0.5078). In contrast, the concentration of exchangeable Cu is not well correlated with that of total Cu (Figure 11-b, R^2=0.1134).



(a)

(b)

Figure 11. Relationships among inert Cu, exchangeable Cu, and total dissolved Cu. (a) Inert Cu is relatively correlated with total Cu (R^2=0.5078); (b) exchangeable Cu is not well-correlated with total Cu (R^2=0.1134). When total Cu in the system changes, inert Cu changes and exchangeable Cu remains within a small range.

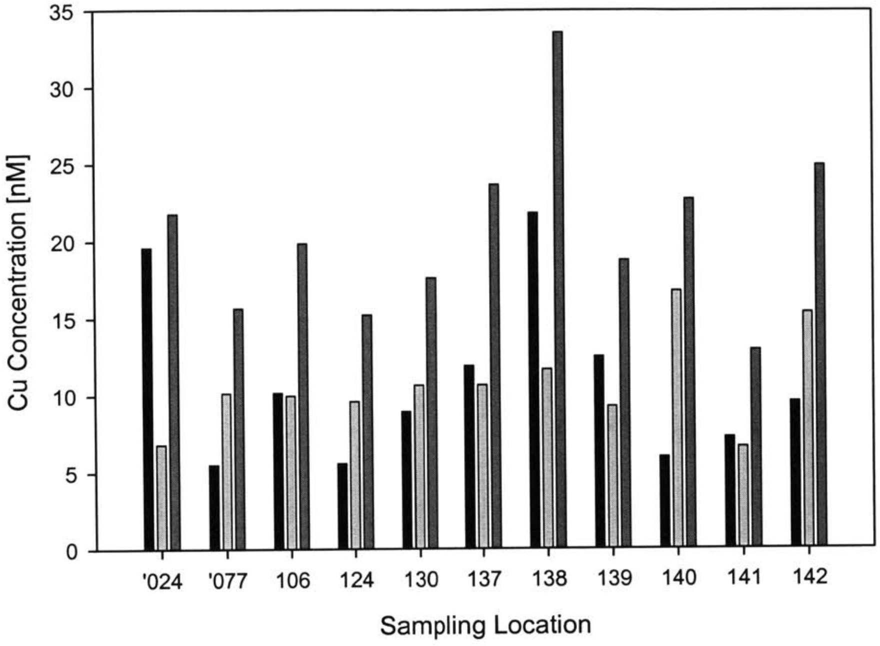


Figure 12. Average exchangeable (black), inert (light gray), and total dissolved Cu (dark gray) measured at each site.

3.3 *Sources of Cu*

3.3.1 Rivers

Three of the four highest site average concentrations of total and inert Cu are at locations associated with river mouths, suggesting that there may be riverine sources of Cu to Boston Harbor (Figure 12). Sampling locations 138, 137, and 140 are near the mouths of the Charles, Mystic, and Neponset rivers, respectively, and exhibit average total Cu concentrations (33.5, 23.7, 22.8 nM) that are higher than the average total Cu concentration measured in this study (20.6 nM). Additionally, these locations have significant inert Cu concentrations, averaging 11.7, 10.7, and 16.8 nM. The exchangeable Cu concentrations at 137 and 138 (11.9, 21.9 nM) are also above the average for the study (10.8 nM), while that at 140 (6.0 nM) is one of the smallest in the study. Moreover, location 024 at the opening of the Inner Harbor to the Outer Harbor, influenced by the Charles and Mystic rivers, measured 21.8 nM total Cu. The salinities at these sites are lower than at other sites, revealing that these four locations are most influenced by freshwater river inputs.

3.3.2 Sediment Resuspension

One possible source of Cu to Boston Harbor via rivers is sediment resuspension. Auxiliary data show that location 140 experiences average to high concentrations of total suspended solids (TSS), ranging from 3.5 to 9.7 mg/l during the field study. The range of variation and the average and maximum TSS are all higher than at any other location (Figure 13). It is possible that turbulent mixing near the mouth of the Neponset River stirs up sediments and contributes to increased Cu concentrations there. This is supported

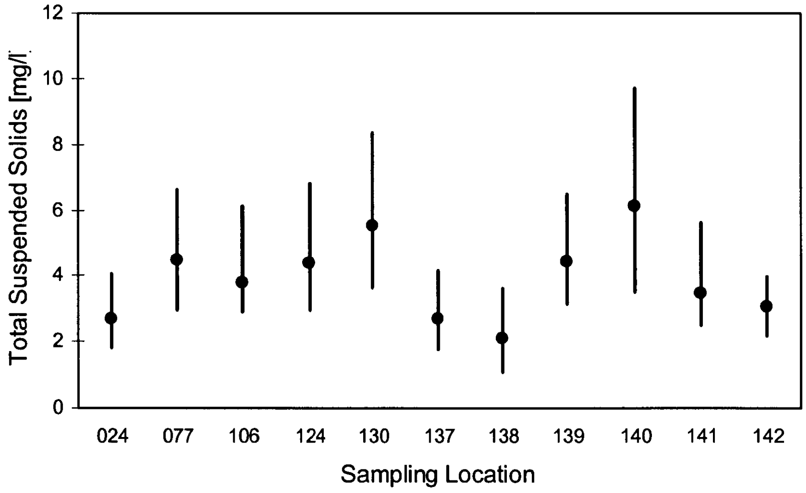


Figure 13. Range and mean (circle) of TSS measured at each sampling location over the duration of the field study. 140 exhibits a high range and mean of TSS.

by an observed increase in both total dissolved Cu and inert Cu (although not exchangeable Cu) with increasing total suspended solids concentration at this location, although it is important to note that this correlation is based on a small set of sample points (Figure 14).

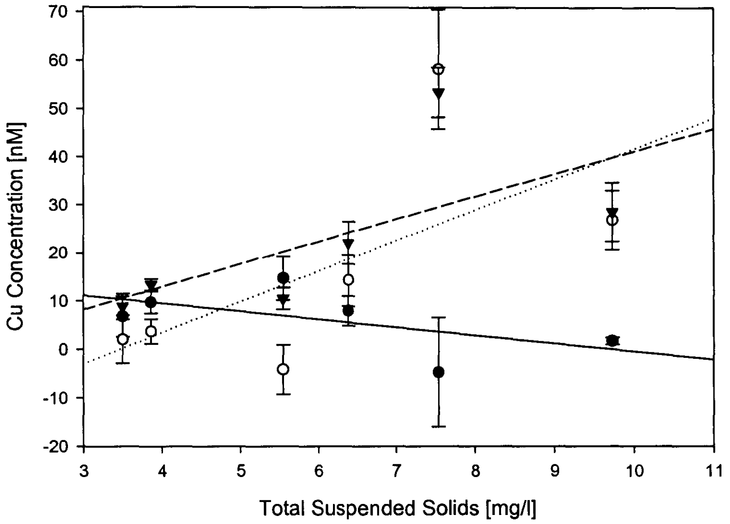


Figure 14. Effect of sediment resuspension on Cu concentrations at location 140. Exchangeable Cu (dark circles, solid line) remains about constant while inert Cu (hollow circles, dotted line) and total Cu (triangles, dashed line) concentrations increase with increasing TSS. This implies that sediment resuspension is a source of inert Cu at this site.

3.3.2.1 Influence of Currents

It is also possible that sediment resuspension at the river mouths is caused by tidal currents. Data from the United States Geological Survey 011055566 Neponset River flow gauge (Figure 15) reflect that high TSS is not associated with high river flow (semi-log scale R^2=0.0997, Figure 16).

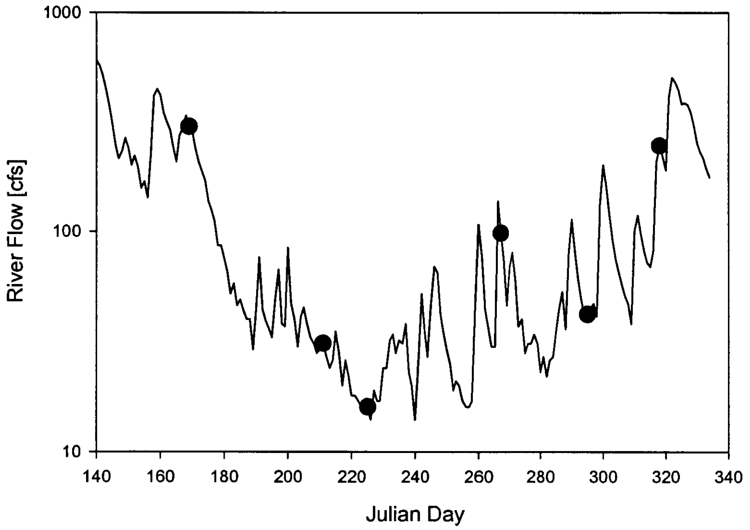
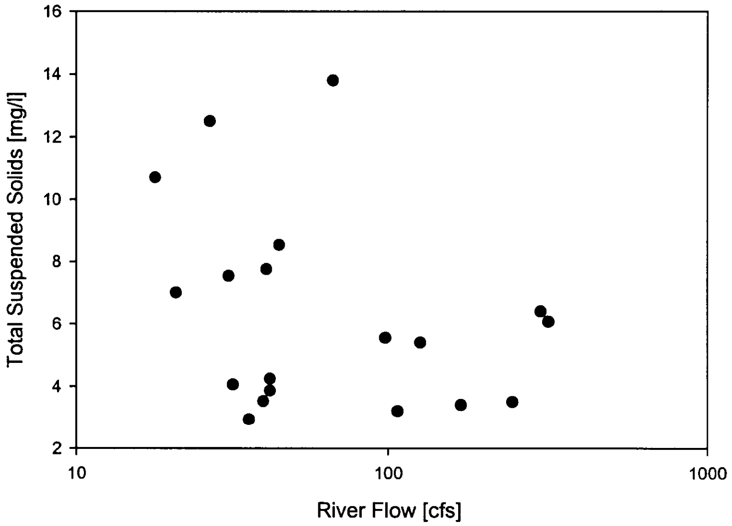


Figure 15. Daily flow of the Neponset River throughout this field study. Circles show the days for which exchangeable, inert, and total dissolved Cu were measured at location 140.

Figure 16. Relationship between TSS at location 140 and daily flow of Neponset River for available data throughout field study. Scattered data (R^2=0.0997) implies that resuspension at 140 is not caused by river flow.



Studying the shallowest sampling locations reveals that sediment resuspension may only be substantial at site 140. Locations 077, 124, 130, and 139 range in depth from 2.4 to 7.9 meters depending on the tide (Figure 17). These shallow locations may be more susceptible than deeper locations to sediment resuspension by turbulent mixing caused by wind. This is demonstrated by the range of TSS at these locations (Figure 13). Notably, there is no relationship between the concentration of TSS and the Cu concentrations at these shallow locations (Figure 18). However, TSS does not reach high levels as in the analysis of location 140 (Figure 13). The maximum TSS at these sites does not exceed 6.8 mg/l, while that at location 140 peaks at 9.7 mg/l.

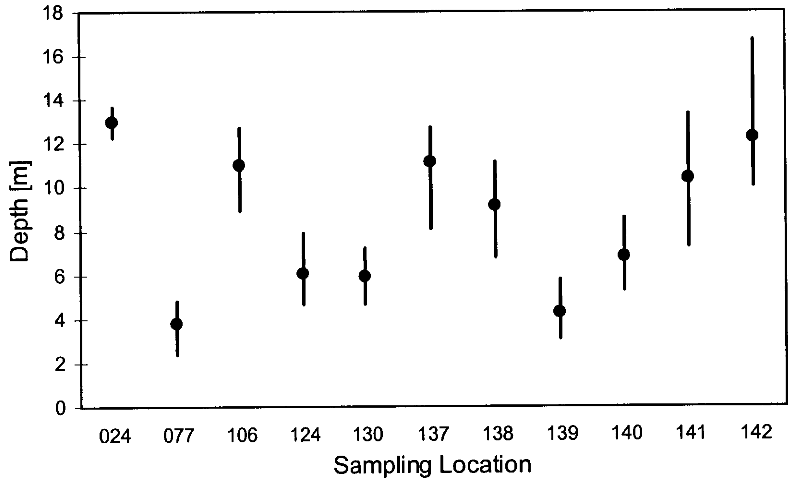


Figure 17. Range and mean (circle) of depths measured at each sampling location over the duration of the field study.

3.3.3 Offshore Sources

The second highest site average concentrations of total (24.9 nM) and inert Cu (15.4 nM) are at the location (142) that is farthest from the shore (Figure 12).

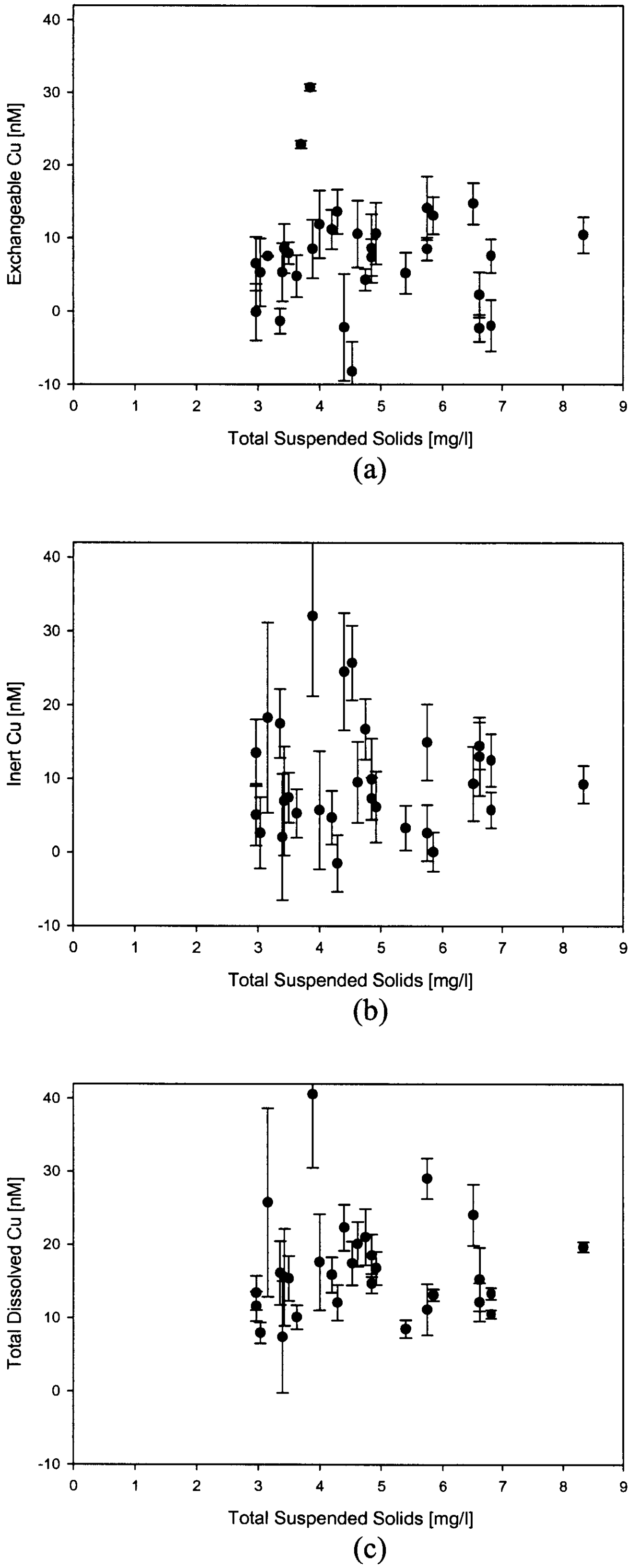


Figure 18. Relationship between TSS and concentrations of (a) exchangeable, (b) inert, and (c) total dissolved Cu at the shallowest locations (077, 124, 130, 139). These data are not well-correlated, implying that the resuspension observed at location 140 is caused by more than wind shear.

3.4 *Influences on Cu Speciation*

3.4.1 Biota

Since it was expected that biological activity was responsible for the formation of a fraction of inert Cu, correlations with chlorophyll a as a metric for phytoplankton productivity were investigated. Chlorophyll a exhibited considerable variability in this field study and followed the expected trend of peaking in mid-summer (Figure 19).

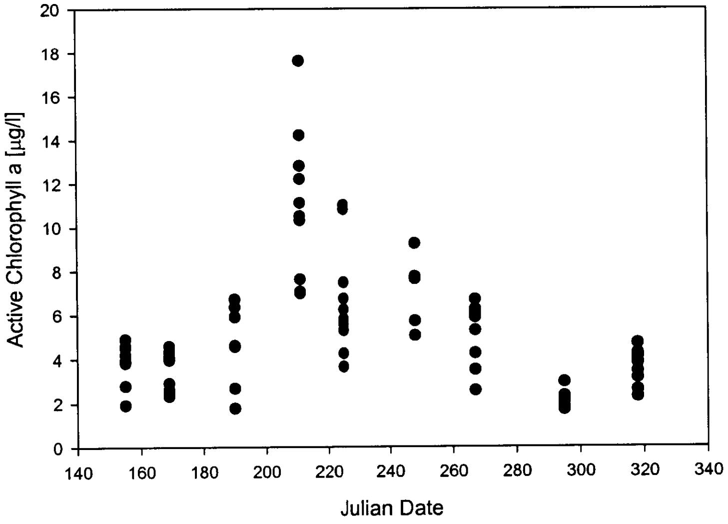


Figure 19. Temporal trend of chlorophyll a. There is considerable variation in chlorophyll a in the samples analyzed. A bloom is observed on days 211, 225, and 248.

There is not a good correlation between chlorophyll a and inert Cu (R^2=0.0274, Figure 20-a), exchangeable Cu (R^2=0.0362, Figure 20-b), or total Cu (R^2=0.0814, Figure 20-c) for the field study as a whole. Examination of individual or pooled locations with particularly high peak chlorophyll a concentrations also failed to reveal a correlation between inert Cu and chlorophyll a.

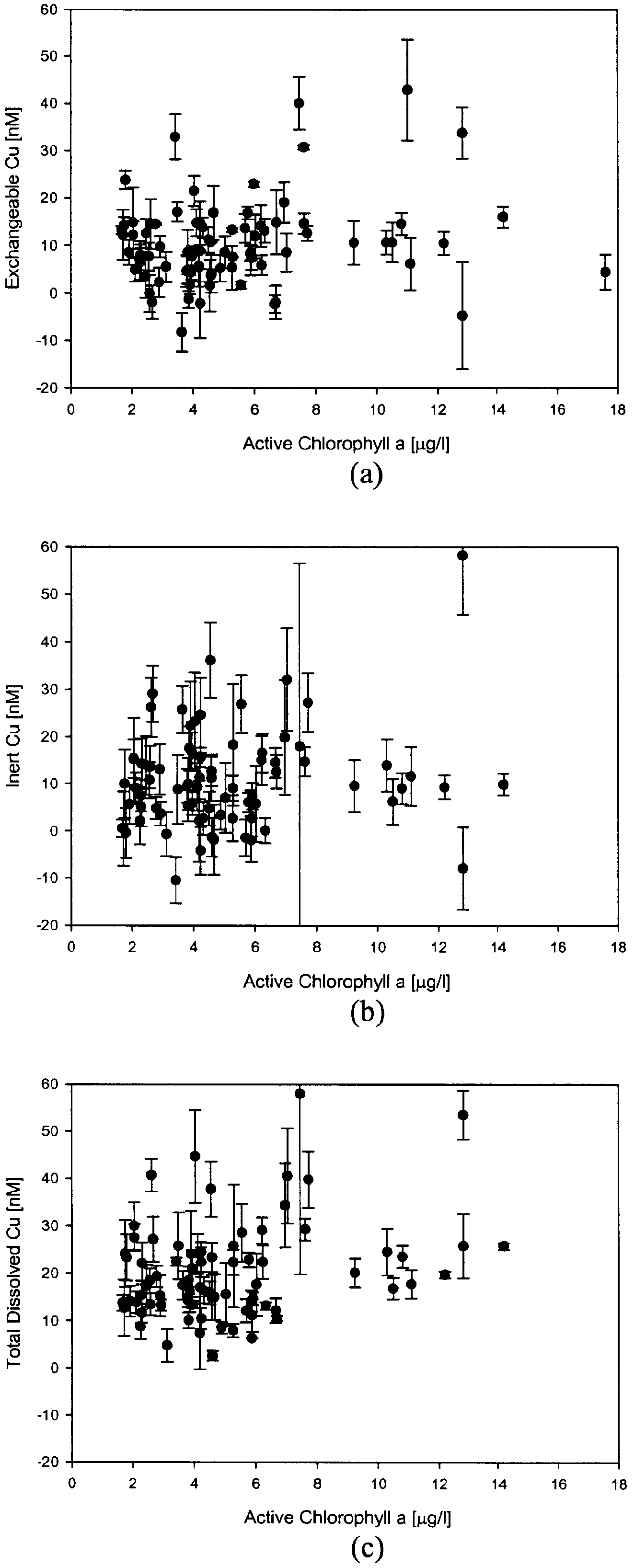
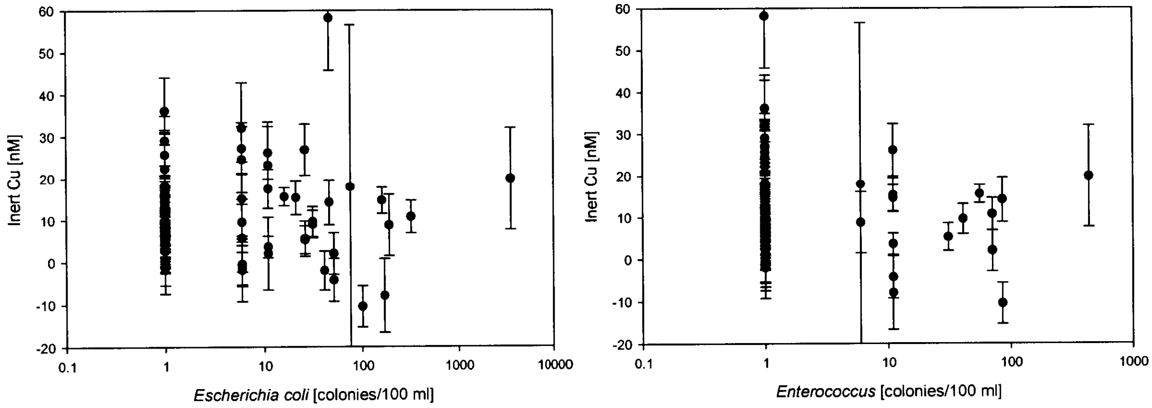


Figure 20. Relationship between chlorophyll a and concentrations of (a) exchangeable, (b) inert, and (c) total dissolved Cu. Chlorophyll a is a metric for phytoplankton production. There does not seem to be a correlation between biological activity and exchangeable, inert, or total dissolved Cu.

3.4.2 Sewage

There is also no correlation between inert Cu and the presence of *Escherichia coli* (semi-log scale R^2=0.0009, Figure 21-a) or *Enterococcus* (semi-log scale R^2=0.0075, Figure 21-b), two organisms associated with fecal matter and thus inputs of raw sewage to the coastal system.



(a) (b)

Figure 21. Relationship between inert Cu concentration and presence of (a) *Escherichia coli* and (b) *Enterococcus*. These organisms are not correlated with and do not seem to affect Cu concentrations.

3.4.2 Sulfide Species

Data from location 130 suggest that sulfides may form some inert Cu compounds. This location is near the Belle Isle Marsh, a 241-acre salt marsh, and is likely affected by marsh chemistry—that is, it may have a higher concentration of sulfide species. The average fraction of inert Cu at this site over the time studied is 65.2%—higher than that at any other site (Figure 7). Figure 22 shows the measurements of both total and exchangeable Cu concentrations for 7 sampling dates at this location. The error bars indicate that with 95% confidence every sample has a significant fraction of kinetically inert Cu. That is, taking into account the error bars, the exchangeable Cu never constitutes all of the total dissolved Cu measured in these samples. This is not the case for any other location in this study (Appendix B).

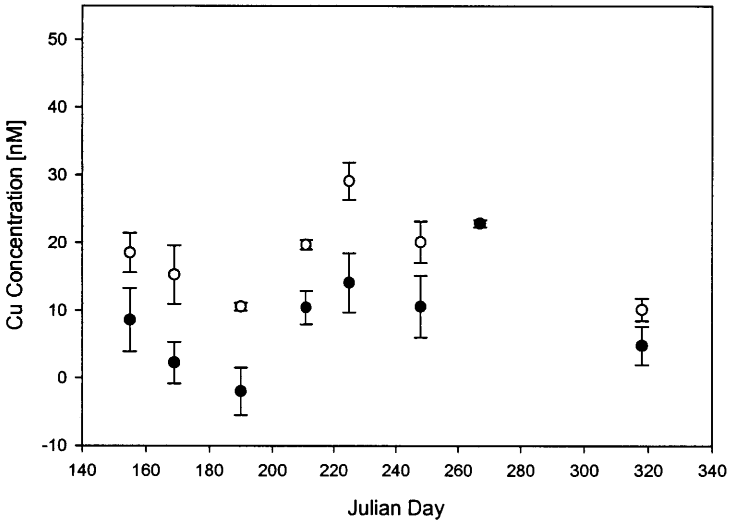


Figure 22. Total (hollow circles) and exchangeable Cu concentrations (dark circles) measured at location 130. Considerable inert Cu is present throughout the duration of the field study. The presence of sulfides at this site near the Belle Isle Marsh likely results in the formation of inert Cu compounds. (No total Cu measurement was made on day 267.)

4 Discussion

This field study was designed to provide an analysis of a complex coastal system with various physicochemical features characteristic of coastal systems such as temporal variations in sources of Cu, sediment resuspension, freshwater inputs, and biological activity, as well as proximity to other water systems such as rivers and marshes. While correlations are observed among Cu speciation and various other parameters, these relationships, if causal, would likely be stronger given a simpler system with fewer potential sources and sinks of Cu and Cu-binding ligands. However, factors that are potentially resulting in the formation of inert Cu compounds can still be identified from this work. Information from this work is a first step in explaining the nature of inert Cu and determining implications of inert Cu formation on Cu toxicity in future studies.

4.1 Data Accuracy

Certain considerations were taken to ensure that sample contamination did not occur in this work (see Results). Few studies have been conducted on dissolved Cu in Boston Harbor; those that exist further confirm the likelihood that these data are accurate. Sunda and Huntsman (1989) used a chemiluminescence technique with the added ligand EDTA to deduce an average detectable Cu concentration of 14.6 nM (ranging from 9.0 to 30.2 nM) for eight sites throughout the same research area (near locations 106, 130, 137, 138, 139, two near 024, and in Dorchester Bay). This was likely an underestimate of total dissolved Cu, as they found higher total Cu (average 22.6 nM; ranging from 18.2 to 27.4 nM) when they acidified (to pH 2) and UV-irradiated the same samples before analysis.

In a later study, Sunda and Huntsman (1990) found an average of 19.7 nM total dissolved Cu at these sample locations. (These samples were acidified to pH 2 and UV-irradiated.) Shea and Kelly (1992) observed a Cu concentration of approximately 25 nM at an unspecified location in Boston Harbor. Kogut and Voelker (2003) used the same CLE-ACSV technique described in this paper to find exchangeable Cu concentrations averaging 6.0 and 5.4 nM, and total dissolved Cu concentrations of 9.4 and 11.1 nM, near locations 077 and 140, respectively, in the winter. These values are close to many of the measurements made at these sites in the present study, although some higher values were also observed (Appendix A, B). Shine and Wallace (1995) used graphite furnace atomic absorption spectrometry to determine total dissolved Cu concentrations ranging from 1.3 to 7.2 nM 5 m below the surface at a location in Massachusetts Bay approximately 15 km east of the mouth of Boston Harbor. This is comparable to the 9.6 nM exchangeable Cu found in this work at location 142.

4.2 General Trends

A fraction of dissolved Cu is typically present as inert Cu (Figure 8). This fraction varies among sites and sampling dates according to certain trends. The exchangeable Cu concentration at each site in this work generally remains within a relatively small range even when total dissolved Cu changes more dramatically (Appendix B). Figure 11 further shows a lack of correlation between exchangeable and total Cu concentrations. When the Cu concentration at a site increases, it is not the exchangeable Cu but the inert Cu concentration that increases (Figure 11). This implies that either the source of Cu is inert Cu or something effects the formation of inert Cu from an input of exchangeable Cu such that the level of exchangeable Cu is maintained at a certain level.

The concentrations of total dissolved, exchangeable, and inert Cu increase to a mid-summer peak (Figure 10). This is likely due to increased industrial activity and waste stream volumes during the summer months. The fraction of dissolved Cu that is inert Cu generally decreases over the duration of this study. Kogut and Voelker (2003) observed that the average % inert Cu near locations 139 and 140 during mid-winter was 44.7%. This is in line with the observed trend in this work (Figure 9).

4.3 Sources of Cu

4.3.1 River Flow

A primary source of Cu seems to be riverine, as most of the highest total Cu concentrations were measured near the mouths of the Charles, Mystic, and Neponset rivers. Possible sources of riverine Cu include industrial waste from combined sewer overflows (CSOs) as well as from storm water runoff. The MWRA regulates industries in the watershed that have significant Cu in their waste streams with a maximum effluent loading limit of 1 mg/l (approximately 15.7 nM) dissolved Cu. On days with heavy precipitation, CSOs can transport industrial waste contaminated with Cu to the rivers that flow into Boston Harbor. Some of the pipes in the sewer system are made of Cu, so when acidic rains and sewage are diverted they may corrode the pipes and transport dissolved Cu as well. Furthermore, municipalities use additives to control odor and prevent plant root growth in sewer systems. One such additive contains Cu sulfate; other additives are highly corrosive to Cu pipes. Either process adds Cu to the harbor when water flows through these pipes into the rivers or harbor. Sources such as these may be responsible for increased total Cu concentrations, in particular at locations 137, 138, and 140.

Dams may be partially responsible for elevated Cu levels near the Charles, Mystic, and Neponset river mouths. Suspended solids settle near river dams. Some Cu sorbs to these particles and settles, building up high concentrations in the sediment. These particles are later resuspended, periodically releasing pulses of dissolved Cu to surface waters in addition to ambient concentrations. This may result in high total dissolved Cu concentrations at these three sampling locations during sediment resuspension events.

To determine the extent of fluvial sources, a transect study should be conducted along each river. Determining a correlation over a large salinity range could reveal more about sources of Cu including if it is transported directly as inert Cu or if it becomes inert Cu when it reaches coastal waters.

4.3.2 Sediment Resuspension

While the Charles and Mystic rivers are significant sources of total dissolved Cu, the Neponset River (location 140) is specifically a source of inert Cu to Boston Harbor. (The average exchangeable Cu concentration is low at this site (6.0 nM).) Something distinguishes this location from those near the other two rivers. It is likely that sediment resuspension provides a source of inert Cu to surface waters. High levels of TSS correlated with high total and inert Cu concentrations (Figure 14) imply that Cu is either released directly from the sediments as inert Cu or is released with other substances that bind to render the Cu inert.

TSS may serve as an indicator of constituents (e.g. organic material) in resuspended sediments that may result in higher inert Cu concentrations. Kogut and Voelker (2003) postulated that inert Cu includes colloidal matrices that sequester Cu. Inert Cu compounds at location 140 may be formed in colloidal matrices or by strong Cu-binding to organic material, for example. The release of DOC coupled with the release of Cu from resuspended sediments may result in the immediate formation of inert Cu. The samples prepared for DOC determination will be analyzed in the future to determine whether the concentration of inert Cu is correlated with DOC.

TSS is a metric for the turbulence in the vertical profile at a given site. TSS is quite low at locations 137 and 138 (Figure 13), implying that little resuspension occurs at these locations. This lack of resuspension near the Charles and Mystic rivers plus the lack of correlation observed between high TSS at location 140 and Neponset River flow (Figure 16) indicate that another mechanism such as tidal currents or currents caused by winds is responsible for sediment resuspension near the Neponset River (140).

TSS (and thus sediment resuspension) is higher at the shallower locations in this study (Figures 13, 17). This implies that currents caused by wind are strong enough to resuspend sediments at shallow but not deep locations. Locations 137 and 138 are among the deepest sampling locations in this study, while location 140 is among the shallowest. It is not surprising that much more TSS (i.e. resuspension) is observed at location 140 than at 137 or 138. Additionally, the shallow locations (077, 124, 130, 139) have high variability in TSS. However, data from these locations do not demonstrate a strong correlation between TSS and total, exchangeable, or inert Cu concentration (Figure 18). This may imply that the sediments near these locations do not have high concentrations of Cu so increased suspension does not result in higher Cu levels.

Substantial sediment resuspension is necessary for Cu to be released to surface waters from sediments. The shallow locations exhibit less resuspension as reflected in lower TSS than at location 140. Location 140 near the mouth of the Neponset River experiences stronger tidal currents than the other shallow locations. Strong tidal currents coupled with wind shears provide sufficient energy for resuspension to occur and exhibit the correlation between TSS and total and inert Cu at location 140 (Figure 14).

4.3.3 Shipping

Another source of Cu seems to exist at location 142. As this location is the furthest from shore and thus from industrial activity, it was expected that it would have low Cu concentrations. However, location 142 had the second highest total dissolved (24.9 nM) and inert Cu concentrations (15.4 nM), as well as a high exchangeable Cu concentration (9.6 nM) (Figure 12). As this location is part of a major shipping channel, it is possible that shipping is a source of Cu to this coastal system, although there may be a different explanation for these elevated levels.

4.4 Formation of Inert Cu

4.4.1 Biological Productivity

Under Cu stress, various microorganisms exude strong ligands to bind to Cu and render it nontoxic. Although some of these biologically-produced ligands have been characterized as L_1-class ligands, it is possible that these are actually inert Cu compounds. A correlation between seasonal variations in biological activity and the fraction of inert Cu might therefore be expected but was not observed in this work (Figure 20).

It is possible that certain biota are able to create inert Cu compounds but that this behavior was masked by other interactions in the system. One possible explanation is that inert Cu formation is species-specific. While some cyanobacteria have been reported to produce L_1-class ligands in response to Cu stress, others have been revealed to produce L_2-class ligands or to not be able to protect itself from Cu stress at all (Moffett *et al.* 1990). Various phytoplankton species are represented by the chlorophyll a measurement; some of these may obscure a correlation between biological activity and the formation of inert Cu. Another possibility is that biological activity is not really responsible for forming inert Cu. The production of L_1-class ligands may be sufficient for survival.

4.4.2 Sulfide Complexes

The hypothesis that Cu becomes inert in the presence of sulfides is supported by inert Cu concentrations determined from the sampling location (130) near the Belle Isle Marsh (Figure 22). Salt marshes are generally characterized by anoxic waters. Since the supply of oxygen has been exhausted, microorganisms in these ecosystems utilize sulfate as an electron acceptor in the oxidation of organic matter, converting this species into sulfide. Location 130 is likely to be affected by marsh chemistry and thus to have a significant presence of sulfide species. It seems that sulfides cause a fraction of Cu present in the system to become inert Cu. Sediments with sulfide fluxes are likely to effect inert Cu in surface waters.

4.5 Future Work

This field study demonstrates that some Cu in Boston Harbor is present in a form that is irreversibly bound to ligands or sequestered within colloidal matrices. These inert Cu compounds are not present as constant fractions of total dissolved Cu, although the inert Cu concentration typically bears values that maintain relatively constant seasonal concentrations of exchangeable Cu at each particular site.

Whether inert Cu compounds typically enter coastal systems directly as inert Cu or indirectly by reacting with certain constituents (e.g. sulfides) in the water is a subject for future work. Inert Cu is correlated with high levels of TSS, implying that resuspension of contaminated sediments due to tidal or wind shear currents may serve as a source of inert Cu. A close analysis of inert Cu compounds in resuspended sediments could help determine how inert Cu is formed and transported to surface waters from sediments. Other sources of inert Cu seem to exist and may reveal different mechanisms for the formation of inert Cu. For example, as sulfides seem to be responsible for forming inert Cu compounds, a study of marsh waters would likely reveal information about another mechanism.

Since inert Cu is likely to be present in many coastal waters, the effect of inert Cu on Cu toxicity is another subject for future work. Cu toxicity studies should be developed to determine how inert Cu fits into speciation models. Such a study could reveal that the formation of inert Cu is vital to the survival of some aquatic species. Alternatively, it could reveal that some organisms have a mechanism for directly taking up inert Cu to nutritive or even toxic levels.

References

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Appendix A

This appendix includes the concentrations of exchangeable, inert, and total Cu at each sampling location over the duration of this field study. Italics indicate that two replicate measurements were averaged to derive the given value. For some samples, total dissolved Cu (and thus inert Cu) was not determined.

Sampling Location	Julian Day	Exchangeable Cu [nM]	Uncertainty [nM]	Total Dissolved Cu [nM]	Uncertainty [nM]	Inert Cu [nM]	Uncertainty [nM]	% Inert [%]
024	211	33.7	5.5	*25.7*	*6.8*	-7.9	8.7	-30.9
024	225	42.8	10.7					
024	248	14.6	2.1	29.2	2.3	14.7	3.1	50.2
024	267	17.0	2.0	25.8	7.1	8.8	7.3	34.0
024	295	4.8	2.5	13.9	2.3	9.1	3.3	65.1
024	318	4.6	3.4	*14.2*	*1.3*	9.6	3.6	67.5
077	155	5.2	2.8	8.5	1.2	3.3	3.1	39.2
077	169	13.7	2.1	16.4	2.8	2.7	3.5	16.3
077	190	7.4	2.5	14.7	1.3	7.3	2.8	50.0
077	211	8.5	4.0	40.6	10.1	32.1	10.8	79.0
077	225	-8.2	4.1	17.5	3.0	25.7	5.0	147.2
077	248	13.6	3.0	12.1	2.4	-1.5	3.9	-12.2
077	267	-2.3	1.8	12.2	2.6	14.5	3.2	119.0
077	295	6.5	3.7	11.6	2.0	5.1	4.2	44.0
077	318	5.3	4.0	7.4	7.6	2.1	8.6	28.2
106	155	5.6	3.3	17.0	3.3	11.3	4.7	66.8
106	169	12.5	3.0					
106	190	1.6	5.4	37.7	5.8	36.2	7.9	95.8
106	211	15.9	2.2	25.7	0.7	9.8	2.3	38.2
106	225	*16.8*	*1.3*	22.8	1.6	6.0	2.1	26.4
106	248	8.8	1.7	11.2	1.9	2.4	2.5	21.7
106	267	*8.3*	*4.6*	*6.3*	*0.1*	-2.0	4.6	-31.3
106	295	*13.3*	*0.8*	13.8	1.7	0.5	1.9	3.6
106	318	8.8	0.2	24.4	2.1	15.6	2.1	63.8
124	155	4.3	1.5	21.0	3.8	16.8	4.1	79.6
124	169	7.6	2.3	13.4	0.8	5.8	2.4	43.3
124	190	8.5	1.6	11.2	3.5	2.7	3.8	23.8

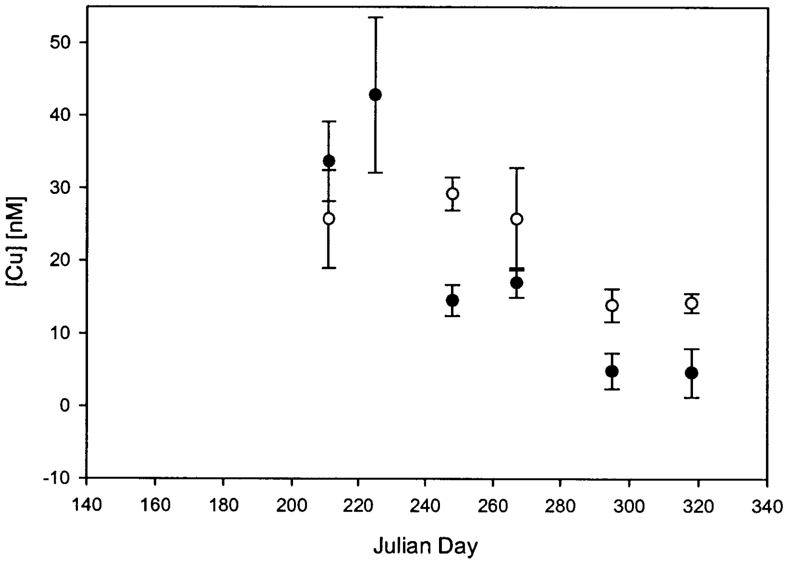
Sampling Location	Julian Day	Exchangeable Cu [nM]	Uncertainty [nM]	Total Dissolved Cu [nM]	Uncertainty [nM]	Inert Cu [nM]	Uncertainty [nM]	% Inert [%]
124	211	10.6	4.2	16.8	2.3	6.2	4.8	36.9
124	225	-2.2	7.3	22.3	3.2	24.6	7.9	109.9
124	248	*8.6*	*3.4*	*15.6*	*6.6*	7.0	7.4	45.0
124	267	5.3	4.6	8.0	1.4	2.7	4.8	33.5
124	295	7.9	1.5	15.4	3.1	7.5	3.4	48.5
124	318	-0.1	3.9	13.4	2.3	13.5	4.5	100.8
130	155	8.6	4.7	18.5	2.9	9.9	5.5	53.7
130	169	2.3	3.1	15.3	4.3	13.0	5.3	85.3
130	190	-2.0	3.5	*10.6*	*0.6*	12.5	3.6	118.8
130	211	*10.4*	*2.5*	19.7	0.7	9.3	2.6	47.0
130	225	14.1	4.4	29.1	2.8	15.0	5.2	51.5
130	248	10.6	4.6	20.1	3.1	9.5	5.5	47.5
130	267	22.8	0.5					
130	318	4.8	2.9	10.1	1.7	5.3	3.3	52.5
137	155	8.5	2.6	14.1	3.2	5.6	4.2	39.6
137	169	14.5	5.2	40.7	3.5	26.2	6.3	64.4
137	190	14.2	1.7	24.1	7.1	9.9	7.3	41.2
137	211	4.4	3.7					
137	225	14.5	2.3	23.5	2.3	9.0	3.3	38.3
137	267	7.7	2.9	18.4	2.6	10.8	3.9	58.4
137	295	14.8	7.3	29.9	5.0	15.1	8.9	50.5
137	318	16.8	5.7	15.0	4.8	-1.8	7.4	-12.3
138	155	14.5	0.2	19.3	2.3	4.8	2.3	24.8
138	211	*19.0*	*4.2*	34.3	8.9	15.3	9.8	44.6
138	225	40.0	5.6	58.0	38.2	17.9	38.6	31.0
138	248	12.5	1.6	39.7	6.0	27.2	6.2	68.5
138	295	12.2	2.9	27.5	2.8	15.4	4.1	55.8

Sampling Location	Julian Day	Exchangeable Cu [nM]	Uncertainty [nM]	Total Dissolved Cu [nM]	Uncertainty [nM]	Inert Cu [nM]	Uncertainty [nM]	% Inert [%]
138	318	32.9	4.8	22.4	0.8	-10.5	4.9	-46.7
139	155	11.2	2.7	15.9	2.4	4.7	3.6	29.8
139	169	14.7	2.8	24.1	4.2	9.4	5.0	38.8
139	190	13.1	2.5	13.2	0.8	0.1	2.7	0.6
139	211	30.7	0.5					
139	225	*7.5*	*0.1*	25.8	12.9	18.3	12.9	70.9
139	267	11.9	4.6	17.6	6.5	5.8	8.0	32.6
139	318	-1.3	1.7	16.2	4.3	17.5	4.7	108.3
140	169	7.9	3.1	22.1	4.4	14.2	5.4	64.4
140	211	-4.7	11.2	53.4	5.2	58.2	12.4	108.9
140	225	*1.7*	*0.7*	28.5	6.1	26.9	6.2	94.1
140	267	14.7	4.6	10.5	2.3	-4.2	5.1	-40.2
140	295	9.7	2.3	13.3	1.2	3.6	2.6	27.3
140	318	6.7	4.2	8.8	2.6	2.0	4.9	23.2
141	155	3.6	1.3	14.9	4.8	11.2	5.0	75.6
141	169	3.4	4.4	17.3	4.4	13.9	6.2	80.1
141	190	4.0	3.9	2.6	1.0	-1.4	4.1	-51.9
141	211	6.1	5.5	17.7	3.0	11.6	6.3	65.4
141	225	14.8	6.7					
141	248	9.7	5.7	11.2	3.5	1.6	6.7	13.8
141	267	5.8	2.1	*22.3*	*3.5*	16.5	4.1	74.0
141	295	12.2	5.2	12.7	5.9	0.5	7.9	3.7
141	318	5.5	3.2	4.8	3.5	-0.7	4.7	-15.0
142	155	1.7	2.0	24.1	9.1	22.3	9.3	92.8
142	169	10.7	0.1	23.4	3.1	12.7	3.1	54.2
142	190	-1.9	3.5	27.1	4.8	29.1	5.9	107.1

Sampling Location	Julian Day	Exchangeable Cu [nM]	Uncertainty [nM]	Total Dissolved Cu [nM]	Uncertainty [nM]	Inert Cu [nM]	Uncertainty [nM]	% Inert [%]
142	211	10.6	2.5	24.5	4.9	13.9	5.5	56.8
142	225	*13.3*	*0.5*	22.3	2.7	9.0	2.7	40.5
142	248	-1.7	4.9	*21.1*	*4.0*	22.7	6.4	108.0
142	267	8.3	1.6	14.1	2.2	5.9	2.7	41.4
142	295	23.8	2.0	23.3	4.9	-0.5	5.3	-1.9
142	318	21.5	3.2	44.6	9.8	23.2	10.3	51.9

Appendix B

This appendix includes figures showing the measured concentrations of exchangeable (dark circles) and total dissolved Cu (hollow circles) at each sampling location over the duration of this field study. For some samples, total dissolved Cu was not measured.



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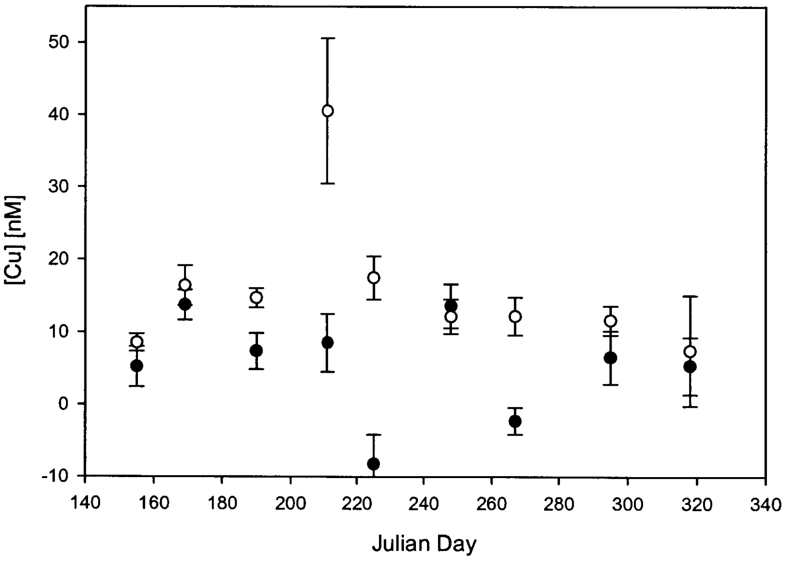

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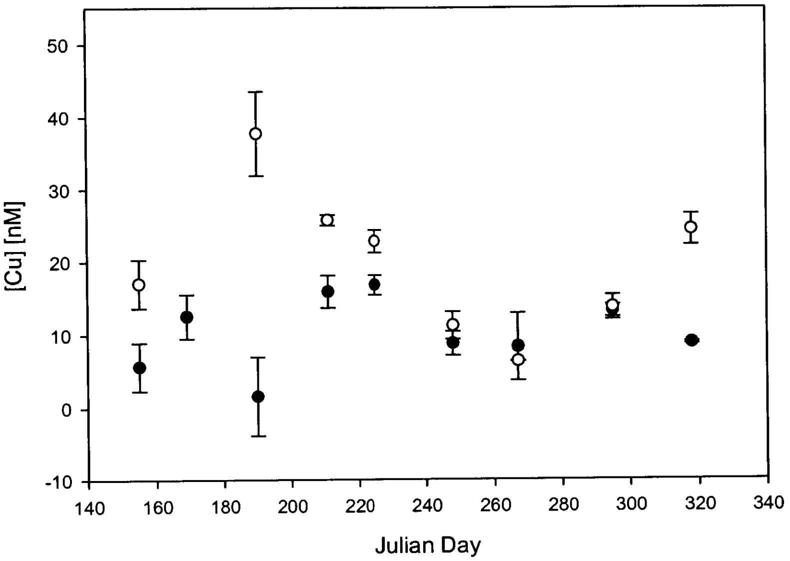

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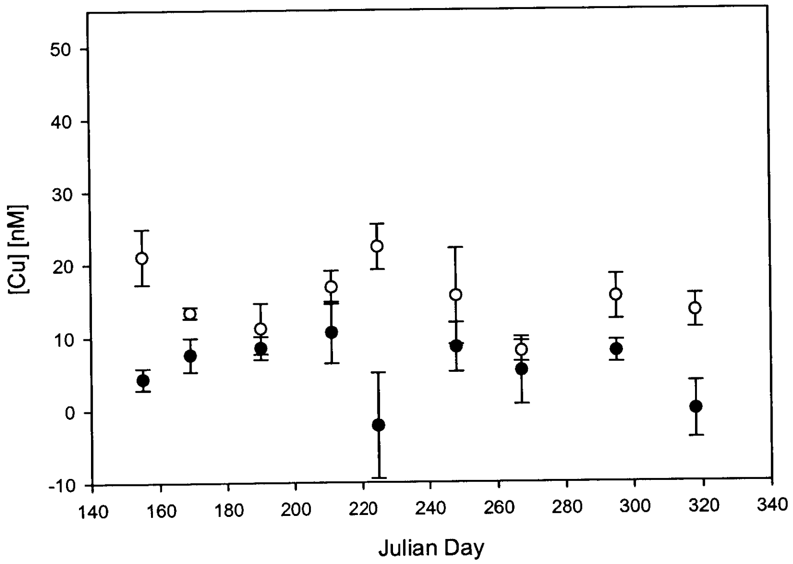

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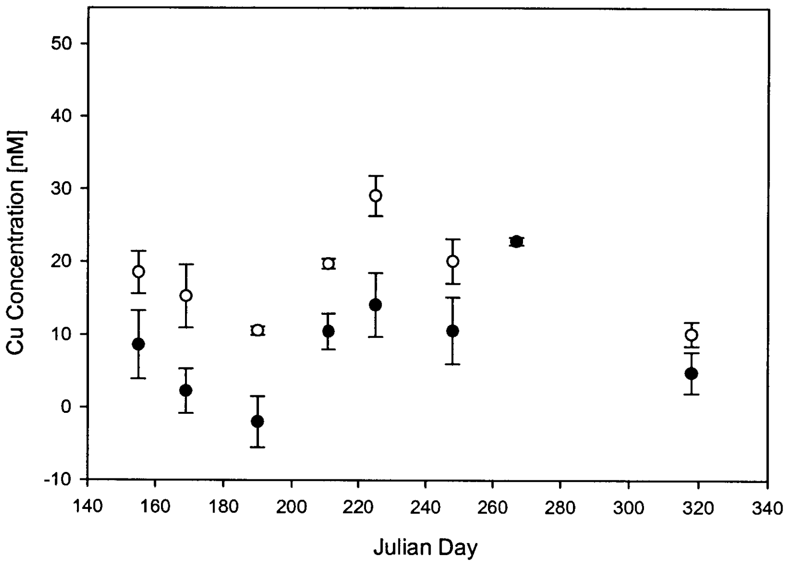

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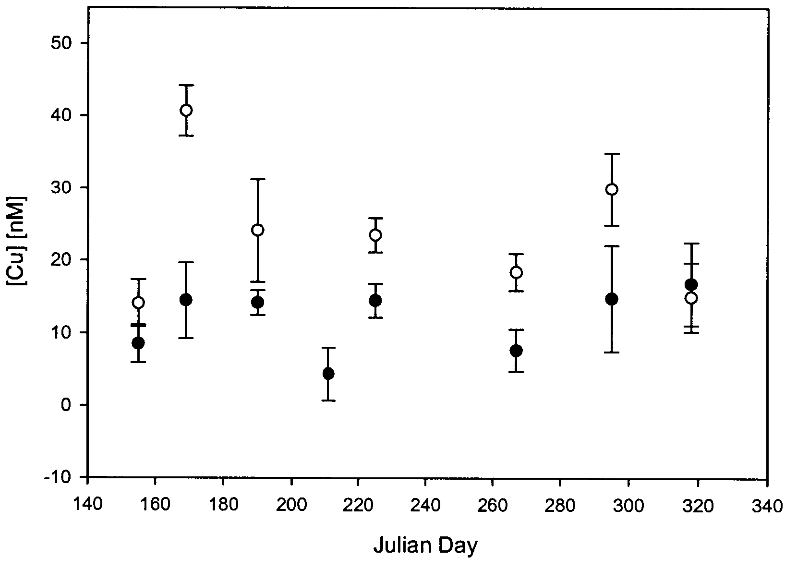

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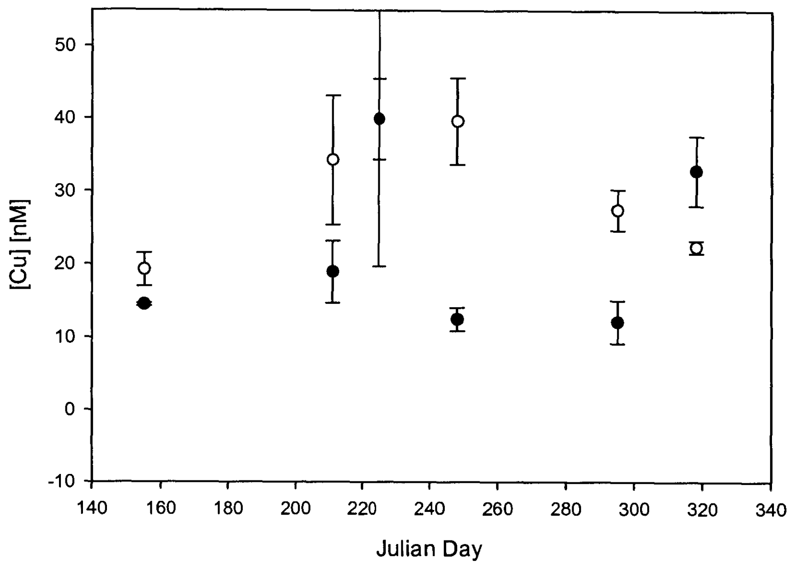

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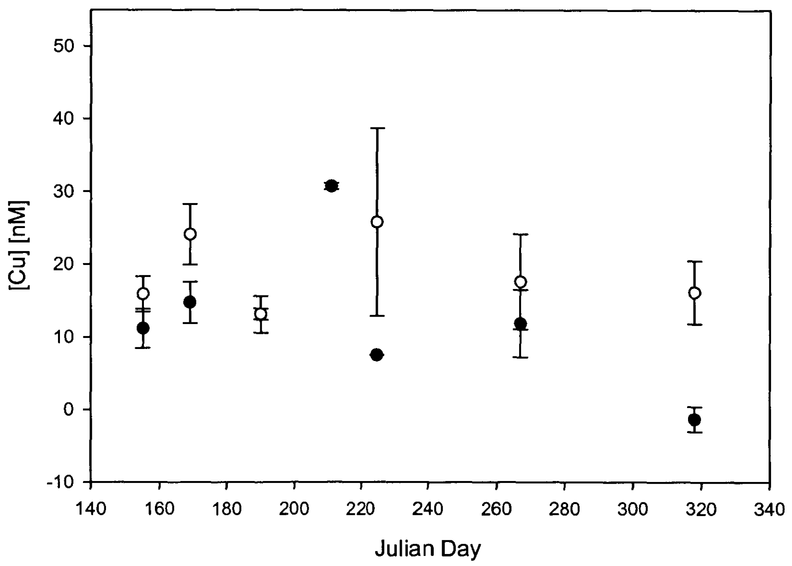

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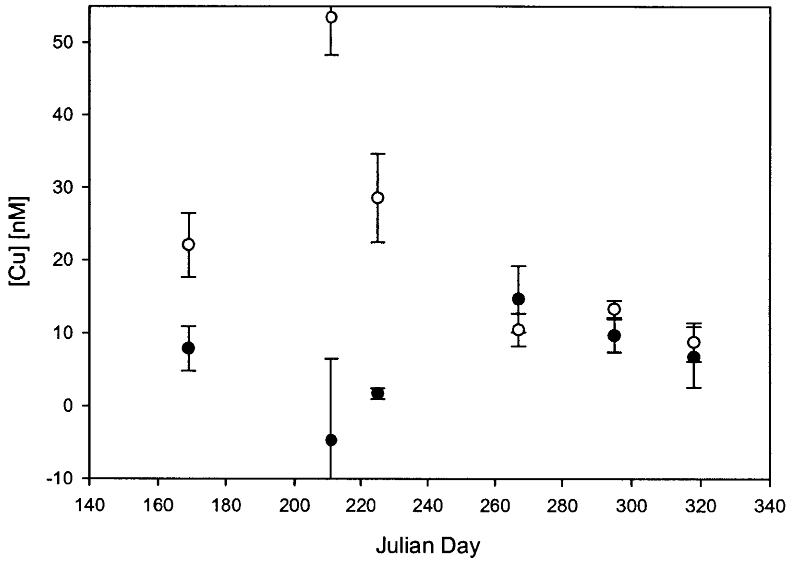

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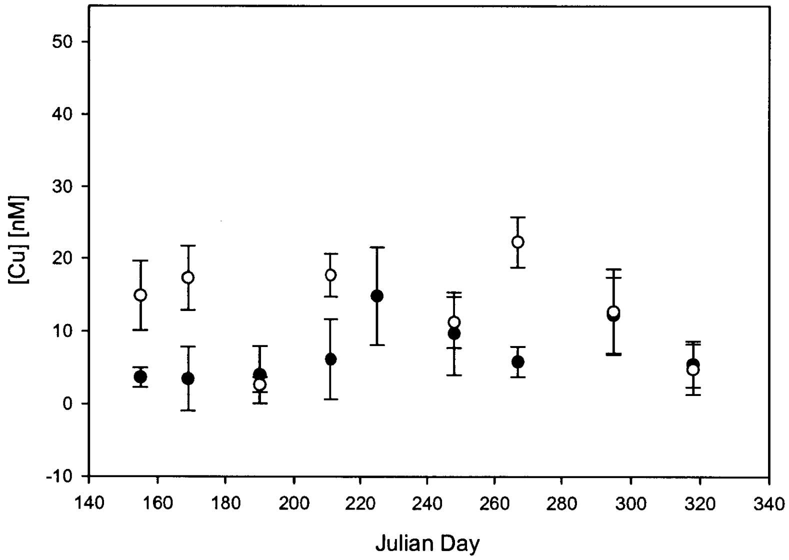

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