

**Measuring Bioavailable Copper in Estuarine Waters  
using an Ion Exchange Resin**

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(ABSTRACT)

Anion exchange methods were used to measure the percent of weakly bound and strongly bound copper in estuarine waters and agricultural drainage. Copper bound to an ion exchange resin has been operationally defined as bioavailable copper, or copper that is available for uptake by aquatic organisms.

Initial laboratory testing indicated that the percent of copper bound to the resin did not differ between a freshwater solution (pH 6.5 buffered with 30 mg/L as calcium carbonate) and a saline solution (25 ppt salinity at pH 8). Adding 20 mg/L natural organic matter (NOM) to each solution lowered the amount of copper sorbed to the resin by the same amount in saline and fresh water. Varying salinity from 16 to 30 ppt did not effect % bioavailability This indicates that the ions typically found in seawater do not compete with copper for ion exchange onto the resin. Varying initial copper concentration from 195 to 495 ppb for the given NOM content of 12.5 mg/L did not effect % bioavailability, indicating that as the copper concentration of this water is increased over the range studied, the fraction of potentially toxic copper is constant.

Decreasing the pH of the system from 8.5 to 4 and the NOM from 12.5 to 0 mg/L statistically increased % bioavailability in both cases. However, copper retention onto the resin at pH values near 4 was hindered, possibly because resin functional groups become inefficient at low pH values. MINEQL+ predicted that the resin was not retaining approximately 30%  $\text{Cu}^{+2}$  at a pH of 4. Copper retention to the resin was also not as high as expected at NOM contents between 0 and 3 mg/L at pH 8.1. MINEQL+

indicated that the water studied at pH 8.1 was comprised primarily of strongly bound copper-organic ligand species and  $\text{Cu}(\text{OH})_2$ . Experimental results suggested that the resin retained only 50%  $\text{Cu}(\text{OH})_2$ , which is a potentially bioavailable copper complex. However, MINEQL+ computer modeling indicated that the resin was accurately retaining potentially bioavailable copper in waters with a pH from 5.5 to 8.5, containing 3 to 12.5 mg/L NOM.

After assessing the characteristics of copper retention by the resin, it was applied to an estuarine system and agricultural drainage to measure bioavailable copper. The results indicated that in copper-containing agricultural runoff and groundwater the percent bioavailable copper was approximately 15% of the dissolved copper. This percentage was relatively constant over the range of copper concentrations observed. In estuarine systems receiving agricultural runoff, the percent bioavailable copper was about 11%.

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## Chapter 1: Project Overview

During the 1980's, the United States Environmental Protection Agency (USEPA) set metal discharge criteria for industry and waste water treatment plants. Although the USEPA criteria for metals, including copper, are not enforceable; they do provide for the implementation of state water quality regulations. These criteria were initially established in terms of total copper concentration, because there was insufficient knowledge about copper toxicity. In the early 1990's, regulatory standards for metals were generally changed to dissolved metal concentrations because some research showed if a metal was strongly bound in the particulate phase, it was not readily toxic (Renner, 1997). Recent research has demonstrated that particulate copper can be mobilized by organisms and can become bioavailable (Labreche, 1998; Baerselman *et al.*, 1998).

Even within the dissolved phase, metals may be bound to dissolved organic matter, which impacts the toxicity. Therefore, copper regulations could be set in terms of species specific bioavailable copper. Conceptually, bioavailable metal is considered that portion of the total metal concentration which interacts with a given organism. If a metal is or is made to be bioavailable, it can exert toxicity, and therefore should be regulated. Unfortunately, even in the 1990s, metal bioavailability and toxicity are still not well understood.

Bioavailability changes with factors such as organism type, organism stage, water characteristics, and the specific metal type. Because of these intricacies, the measurement of potentially harmful, or bioavailable copper is a difficult task. Thus, bioavailable metal concentrations are typically operationally defined based on the measurement technique and usually do not relate to a target organism.



Furthermore, some organisms, including filter feeders such as larval clams and oligochaete worms, not only take up soluble metals, but also ingest particulate-bound metals through the food chain (LaBreche, 1998, Baerselman *et al.* 1998). Once inside the stomach, the acidity of the system can reduce the particulate copper to a form that is readily bioavailable, thus causing a toxic effect that would not be directly noted from a water sample. Although current measurement methods do not consider every possible form of copper in the environment, they are still valuable for understanding how the dissolved fraction of bioavailable copper changes in natural water given characteristics such as pH, NOM, and hardness.

As of 1999, there are no standard methods to perform dissolved bioavailable metal analyses. A Chelex 100<sup>®</sup> ion exchange resin technique can be used to measure bioavailable dissolved copper. Chelex 100<sup>®</sup> resin is a styrene-divinylbenzene copolymer that has imminodiacetate functional groups that are able to form complex compounds with free metals through ion exchange. Furthermore, the copper affinity of the resin may be great enough to dissociate and retain copper ions that were previously weakly complexed. Species such as copper hydroxides may dissociate in response to the resin, depending on the characteristics of the resin. These weakly bound copper complexes are generally considered bioavailable, whereas copper strongly bound to organic ligands that pass through the resin is not. One major advantage of the Chelex 100<sup>®</sup> resin technique is that samples may be processed through the resin in the field, and the actual metal analysis can occur later using standard techniques.

Because the amount of copper that is bioavailable in the natural environment is subject to changes in water characteristics such as pH and NOM, the resin should respond

in a similar manner in order to be considered useful for measuring bioavailable copper. Furthermore, the resin should not respond to changes in water characteristics that do not effect bioavailable copper concentrations in the natural environment.

Bioavailable copper measurements would be useful in any natural aquatic environment, and are especially relevant to the Eastern Shore of Virginia, where farmers and aquaculturists are located very close to one another. Farmers routinely apply copper-based pesticides to tomato plants and other plants during the growing season. Many tomato growers also cover the tomato mounds with impermeable plastic to increase yields and quality. Up to 55% of the field can be covered with plastic, leading to increased copper runoff to clam hatcheries. Studies have shown that 16-300  $\mu\text{g/L}$  of dissolved copper are present in the runoff from the plasticulture tomato fields, and concentrations of dissolved copper in the receiving stream can be as high as 126  $\mu\text{g/L}$  (Klawiter, 1998; Brady, 1999). This runoff has been implicated in clam toxicity in downstream aquaculture facilities.

To better study the potential for copper runoff and resulting toxicity, greenhouse-scale mesocosms of agricultural fields and agriculture-impacted estuaries were designed and constructed (Stall, 1999; Cheadle, 1999). A rain simulator delivered water to 2.13M  $\times$  1.22M  $\times$  .91M tomato beds that were routinely sprayed with copper-based pesticides. Each bed contained Bojac Sandy Loam indigenous to the Eastern Shore of Virginia. The resulting copper runoff and ground water was collected and delivered to simulated estuaries that cycled between high and low tides through the use of a pump. These estuaries contained plant and animal life indigenous to a tidal estuary. The bioavailability

of copper in the runoff, groundwater, and estuarine water was monitored on approximately a weekly basis.

The objectives of this paper were to:

- Determine if the Chelex 100<sup>®</sup> resin responds to changes in water characteristics in a logical and consistent manner when measuring bioavailable copper. Characteristics studied were:
  - ◆ Salinity
  - ◆ Copper concentration
  - ◆ pH
  - ◆ Natural Organic Matter (NOM)
- Apply the researched Chelex 100<sup>®</sup> resin technique to measure bioavailable copper in agricultural runoff, groundwater, and estuarine water

## Chapter 2: Literature Review

### 2.1: Copper Speciation in the Natural Environment

Copper as cupric ion ( $\text{Cu}^{+2}$ ) is potentially toxic to aquatic organisms. However, copper strongly bound to organic ligands is usually not considered toxic. For this reason, it is beneficial to know what species of copper will form in a natural aquatic system.

Dissolved elements make up the background electrolyte in seawater, and other saline waters. This background electrolyte not only sets the ionic strength of the water, but also provides inorganic ligands which may complex trace copper (Tessier *et al.*, 1995). Copper can form strongly bound species with dissolved organic carbon content (DOC) and suspended particulate matter (SPM). These complexes are generally considered not as toxic as copper in the ionic form. Therefore, increasing the amount of DOC or SPM in a natural water may reduce the amount of toxic copper. However, as the pH of a natural water is lowered, copper has a greater tendency to form inorganic complexes that are generally considered toxic. Table 1 depicts typical water characteristics that effect copper speciation and therefore toxicity in natural waters.

As seen, seawater shows less variation in composition. This is because freshwater is generally poorly buffered, and can vary widely in nature (Tessier *et al.*, 1995).

Table 1: Water quality characteristics that effect copper speciation and typical values for natural waters

Characteristic	Seawater	High Alkalinity River Water <sup>a</sup>	Low Alkalinity River Water <sup>a</sup>
Ionic Strength	0.7 M	0.004 M	0.001 M
Salinity	35 ppt	0.144 ppt	0.041 ppt
pH	8.1	8.4	<7.5
DOC <sup>b</sup>	0.4 - 2.5 mg/L	2 - 100 mg/L	2 - 100 mg/L
SPM <sup>c</sup>	0.020 - 0.050 g/L	0.08 - 38 g/L	0.08 - 38 g/L

<sup>a</sup>As defined by Dyrssen and Wedborg (1980)

<sup>b</sup>Dissolved organic carbon; data from Buffle (1988)

<sup>c</sup>Suspended particulate matter; seawater data from Whitfield and Turner (1987), river water from Martin and Whitfield (1983)

In seawater, the concentrations of the major ions are usually constant, so that the interaction between trace metals and inorganic ligands can be predicted with confidence.

In general, these inorganic complexes form rapidly. If a given trace metal does not interact with organic ligands, the percent of metal that is in the free form is fixed given water characteristics such as pH, alkalinity, total suspended solids, and hardness. Metals such as manganese and cadmium only form inorganic complexes (Mackey *et al.*, 1989).

However, copper metal ions not only can form well-quantified complexes with inorganic ligands such as OH<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, NH<sub>3</sub>, and Cl<sup>-</sup>, but also can form stable complexes with organic ligands such as EDTA (Raspor *et al.*, 1980). Natural organic ligands, also referred to as natural organic matter (NOM), usually consist mainly of humic material, which can be further operationally classified into three categories. Fulvic acids are soluble at any pH, humic acids are soluble above pH 1.0, and humin is insoluble at any pH. Dissolved organic carbon consists of both humic and fulvic acids, where fulvic acids are of a lesser molecular weight (<1000). Humics come from the degradation of plant debris, and also from the excretion of organic matter from aquatic organisms (Tessier *et al.*, 1995). In general, copper complexes with organic ligands more than any other

divalent metal (Irving *et al.*, 1953). Recent experiments suggest that greater than 99% of the total amount of copper in the sea is complexed by organic ligands (Sunda *et al.*, 1987).

Copper can also adsorb onto the surface of particles. These particles usually contain hydroxyl groups on their surface, which can gain or lose protons, thus resulting in a net positive or negative charge. This surface complexation is highly pH dependent. A pH change of a few units can change sorption from 0 to 100%. For example, a solution containing  $5 \times 10^{-7}$  M copper does not surface complex in the presence of  $10^{-3}$  M total ferric oxide at pH 4.0, but when the pH was increased to 6.0 over 90% of the copper was surface bound (Dzombak and Morel, 1990). In addition, laboratory experiments have shown that clay and mineral oxide particles can have organic coatings (Hunder *et al.*, 1979). The NOM on the particle surface enhances the adsorption of copper, and is pH dependent with the greatest NOM sorption occurring at pH 4 to 6 (Davis *et al.*, 1982 and 1984).

## **2.2: Methods for Quantifying Copper Ligand Binding**

Since some copper complexes are toxic to aquatic organisms whereas others are not, it is beneficial to know what copper species will be present in a natural water. For example,  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$  are considered highly toxic, whereas  $[\text{Cu}_2(\text{OH})_2]^{2+}$  is sometimes considered toxic. Copper-carbonate species are generally not considered toxic (Meador, 1991). Increases in carbonate species are usually associated with increases in alkalinity. Therefore, alkaline waters have more of a potential to form copper-carbonate species, thus reducing copper toxicity (Snoeyink and Jenkins, 1980). Inorganic calcium and magnesium complexes associated with water hardness may compete with copper for

binding sites in an organism, also reducing toxicity (Brezonik *et al.*, 1991). Although organic copper species are generally considered non-toxic, they do have the potential to bioaccumulate (Stumm and Morgan, 1996).

Much effort has been spent on modeling natural waters in order to determine which forms of copper will be present in a natural aquatic system. With inputs of water characteristics such as pH, ionic strength, alkalinity, and hardness, computer models can predict copper species. Copper forms well-defined inorganic hydroxide and carbonate complexes, and a water that contains these species exclusively can be modeled with a high degree of accuracy. However, copper-organic complexes are more difficult to model. As binding sites on the organic matter are filled, the energetic properties of the complex change. The value of the first acid dissociation constant ( $K_{A1}$ ) will depend on which binding site is involved, and will also effect the second acid dissociation constant ( $K_{A2}$ ). Therefore subsequent dissociation constants are not only a function of the binding sites involved, but also depend on the previous dissociation constant. Considering that an organic molecule may have over a hundred binding sites, modeling is extremely difficult (Schecher *et al.*, 1998). Furthermore, natural waters may contain multiple organic ligands which may compete for metals, resulting in mixed complexes, which are strongly favored over complexes involving only one type of ligand (Buffle, 1988).

Although the exact nature of copper-organic ligand complexation is not well quantified, copper-organic ligand species can still be predicted through empirical modeling. Models can be established through mathematical calculations given reasonable chemical assumptions. A model can also be established and calibrated directly from an experimental data set. For example, the nature of copper binding to

fulvic acid was investigated by titrating a known solution with copper and measuring the amount of free copper (Cabaniss and Shuman, 1987a and 1987b). These data were then used to derive and calibrate a model that predicted copper speciation in natural waters given NOM content, pH, ionic strength, and total copper concentration with a high degree of accuracy.

Much research has been performed in order to quantify copper's ability to complex a given organic ligand through modeling. A simple 1:1 complexation model has been shown reasonable when predicting copper-organic ligand complexes (Ruzic, 1982). A titration method was developed which directly models copper-ligand binding as:  $\text{Cu} + \text{L} \leftrightarrow \text{CuL}$  yielding a stability constant defined as  $K_L = [\text{CuL}]/[\text{Cu}][\text{L}]$ . The total ligand concentration is also determined, and is defined as:  $[\text{L}_T] = [\text{CuL}] + [\text{L}]$ . The method plots inorganic copper vs. the amount of inorganic copper divided by the amount of ligand-bound copper. If 1:1 complex formation is expected, the plot should form a straight line yielding  $K_L$  and  $[\text{L}_T]$  through the slope and intercept. A linear trend suggests that only complexes involving one trace metal and one ligand form over the concentration range of interest.

Several authors have demonstrated Ruzic's method to be accurate for measuring 1:1 copper complexation in saline waters (Coale *et al.*, 1988), (Van Den Berg *et al.*, 1989), and (Labuda *et al.*, 1994). Using differential pulse anodic stripping voltammetry (DPSV) to measure inorganic copper, Coale *et al.*, 1988 was able to titrate a copper-EDTA system. Titration results were compared to calculated results with a good degree of accuracy. The added EDTA concentration was 10.0 nM, whereas the titration-derived concentration was 10.4 +/- 1.6 nM. The conditional stability constant derived from the



titration was  $8.6 \pm 0.1$ . By taking into account inorganic side reactions ( $\alpha_{\text{Cu}}$ ) and also ionic strength corrections ( $\gamma_{\text{Cu}}$ ) for the saline system, the calculated stability constant was determined to be 9.2. This value can vary, based on the numbers chosen for  $\alpha_{\text{Cu}}$  and  $\gamma_{\text{Cu}}$ .

Once the total organic ligand concentration ( $[L_T]$ ) is well quantified, along with its affinity for copper complexation ( $K_L$ ), computer programs such as MINEQL+ version 4.0, by Schecher and McAvoy (1998) can predict both organic and inorganic metal complexation. In this paper the fraction of inorganic copper measured in the Ruzic method described above was operationally defined as the amount of copper exchanged onto the Chelex 100<sup>®</sup> resin surface; the derived  $K_L$  and  $L_T$  values were strictly conditional. These values were dependent on resin characteristics such as sample retention time in the resin and the amount of resin used. Therefore, applying the conditionally measured Ruzic  $K_L$  and  $L_T$  values to a mathematical model to predict resin results would be illogical. However, the values determined by the Ruzic method would be compared to other natural estuarine  $K_L$  and  $L_T$  values in the literature, in order to indicate if the resin is complexing the correct fraction of copper.

### ***2.3: Copper Toxicity in Natural Waters***

When a toxic metal in the dissolved form first reaches an aquatic organism, it usually first comes in contact with a protective layer. In microorganisms and some plants, this is usually the cell wall; in animals it is the mucus layer. After passing through this protective layer, the dissolved metal then encounters the plasma membrane. The plasma membrane varies considerably from one organism to another, but all have similar characteristics. It is hydrophobic in nature, and contains transport proteins an/or ion

channels which transport ions across the membrane. Thus, the metal has the potential to bind in many different ways. If the metal ion binds at physiologically inert sites it may accumulate without adversely affecting the organism. However, if the metal ion binds at a physiologically active site, it may affect cell metabolism directly. Furthermore, metal ions can interact with a large number of intracellular sites, thus adversely effecting metabolic activity even more (Williams, 1981).

In regards to aquatic organisms, the cell membranes discussed above are usually the gills or body tissue. However, some organisms, including filter feeders such as larval clams and oligochaete worms, not only take up soluble metals, but also ingest particulate-bound metals through the food chain (LaBreche, 1998, Baerselman *et al.* 1998). Marine phytoplankton may accumulate metals from the water (Tessier *et al.*, 1995). Once these plants are inside the stomach, the acidity of the system can reduce the particulate copper to a form that will readily pass through the cell membrane, potentially causing a toxic effect.

Copper is an essential element to most aquatic organisms at very low concentrations. However, concentrations as low as 8 µg/L have been known to have negative effects on larval shellfish (LaBreche, 1998). The toxicity of copper depends on many water characteristics, as described in Table 1. Increased amounts of natural organic matter, carbonate, and higher pH levels may reduce the toxic effect of copper.

Copper toxicity also depends on the organism considered. Some aquatic organisms are more susceptible to copper than others. The concentration required to kill 50 percent (LC<sub>50</sub>) of the marine mussel *Mytilus edulis* in 4, 10, 14, and 30 days is 200 to 300, 90, 15, and 2 µg/L total copper respectively (Luoma and Carter, 1991).

Furthermore, ionic copper concentrations as low as 5.8 µg/L stunts the growth of the bay scallop *Argopecten irradians*. The 42 day LC<sub>50</sub> for this organism is 9.3 µg/L ionic copper (Pesch *et al.*, 1979). The soft shell clam *Mya arenaria* experiences a 7 day LC<sub>50</sub> of 35 µg/L added copper during the summer. However, the organism's tolerance to copper increases as the weather becomes colder. The 21 day LC<sub>50</sub> at 17°C during the fall is 86 µg/L added copper. The 14 day LC<sub>50</sub> at 4°C is greater than 3000 µg/L added copper during the winter (Eisler *et al.*, 1994). The larval form of the hardshell clam *M. mercenaria* experiences an 8 to 10 day LC<sub>50</sub> of 16.4 µg/L added copper (Calabrese *et al.*, 1977).

The toxic effect of copper also depends on the organism's life stage. The larval stage of the marine mussel *Mytilus edulis* cannot survive total copper concentrations exceeding 400 µg/L. This organism is even more susceptible to copper in the embryonic form, where the organism cannot survive in waters containing 5 to 6 µg/L total copper (Luoma and Carter, 1991). The embryonic form of the american oyster *C. virginica* experiences a 12 day LC<sub>50</sub> of 103 µg/L added copper, whereas the larvae form 12 day LC<sub>50</sub> is only 32.8 µg/L added copper (Calabrese *et al.*, 1973). Aquatic organisms can also bioaccumulate copper over time, leading to toxic responses and even death (Luoma and Carter, 1991).

## **2.4: Copper Regulations**

Because aquatic organisms have been shown to exhibit toxicological responses to copper in the µg/L range, criteria have been established to help regulate copper discharge to natural waters. In 1972, the Clean Water Act was established, and in 1977 the United

States Environmental Protection Agency (USEPA) was required to set criteria for toxic pollutant discharge for industry and waste water treatment plants. Although the USEPA criteria are not enforceable, they do provide for the implementation of state water quality regulations. These criteria were initially established in terms of total metal concentrations. The established total copper criteria are shown below, where hardness was set in terms of mg/L as calcium carbonate (USEPA, 1985).

- Saltwater 24 hour average: 4.0 µg/L total copper
- Saltwater average not to exceed 23 µg/L total copper at any time
- Freshwater 24 hour average: 5.6 µg/L total copper
- Freshwater average not to exceed  $e^{(.94(\ln \text{hardness})-1.23)}$  total copper at any time

Research has shown that if a metal was strongly bound in the particulate phase, it was not readily toxic (Renner, 1997). Therefore, soluble copper was deemed the better parameter in copper toxicity. In the early 1990's criteria for metals were generally changed to dissolved metal concentrations. In 1995 the USEPA published a saltwater draft in terms of dissolved copper (USEPA, 1995). The dissolved copper criteria are shown below, and are not to be exceeded more than once every three years on average.

- Acute (24hr average): 4.8 µg/L dissolved copper
- Chronic: 3.1 µg/L dissolved copper

Virginia regulations have also moved to dissolved copper concentrations, as shown below:

- Saltwater
  - Acute (1 hr average): 2.9 µg/L dissolved copper
  - Chronic (4 day average): 2.9 µg/L dissolved copper
- Freshwater
  - Acute (1 hr average):  $< e^{(.9422(\ln \text{hardness})-1.464)}$
  - Chronic (4 day average):  $< e^{(.8545(\ln \text{hardness})-1.465)}$

These values are not to be exceeded more than once every three years on average. Again, hardness values were set in terms of mg/L as calcium carbonate.

Even within the dissolved phase, metals may be bound to dissolved organic or inorganic components, which impacts toxicity. Copper regulations should consider bioavailable copper. Conceptually, bioavailable metal is considered that portion of the total metal concentration that is available to aquatic organisms. If a metal is or is made to be bioavailable, it can exert toxicity, and therefore should be regulated.

## **2.5: Methods to Measure Bioavailability**

In order to determine bioavailable copper, measurement methods that determine specific copper species are necessary. Electrochemical methods, such as voltammetry and potentiometry, use electrodes to determine metal species (Ruzic, 1982), (Coale *et al.*, 1988), (Van Den Berg *et al.*, 1984), and (Buffle *et al.*, 1979). Non-electrochemical methods such as dialysis, ion exchange resins, ligand competition methods, and size-based separations are also used to determine metal species (Mazidji *et al.*, 1992) and (Tubbing *et al.*, 1994). Furthermore, mass spectrometry and high-performance liquid chromatography have been applied to metal speciation studies (Marshall, 1988).

Although a wide range of analytical methods are available for measuring trace metal species, voltammetric techniques with low detection limits have been extensively used. Furthermore, ion exchange resins have often been implemented in speciation studies.

### **2.5a: Application of Voltammetry**

Voltammetry uses an electrode in order to distinguish between various metal species. Solutes come in contact with the working electrode, which is usually a mercury drop. The solute then undergoes either oxidation or reduction reactions on the electrode which produce a current that is measured while keeping the electrode potential constant.

Voltammetry can also be accomplished by keeping the current constant and varying the electrode potential. The reactions at the surface of the working electrode are a function of the solution's thermodynamic equilibria, the mass transfer rate of the solute from the bulk solution to the electrode, and the adsorption of surface active species at the electrode. Solution composition is related to the difference in potential (or current) between the working electrode and a reference electrode, where the reference electrode potential is assumed to remain constant during the measurement process.

Voltammetric methods, such as anodic stripping voltammetry (ASV) and adsorptive cathodic stripping voltammetry (ACSV) can determine free ions in the presence of ligands, and differentiate between the various valency states of a specific element. Voltammetry can be directly applied to determine trace metal speciation in saline waters. However, because the reactions at the working electrodes are a function of many parameters as discussed above, the information obtained from voltammetric methods is usually highly complex, making it very hard to interpret. Furthermore, voltammetry requires that samples be transported to the laboratory prior to analysis. During this time crucial water characteristics such as pH, may change in the sample, thus changing metal speciation.

### **2.5b: Application of the Chelex 100<sup>®</sup> Resin**

A field portable approach uses a Chelex 100<sup>®</sup> ion exchange resin to bind ionic and weakly bound copper. Samples may be processed in the field and the actual metal analysis can occur later using standard atomic absorption equipment. Chelex 100<sup>®</sup> resin is a styrene-divinylbenzene copolymer with imminodiacetate functional groups that are able to form complex compounds with free metal species and weakly bound metal ion

complexes through ion exchange. Complex formation is referred to as chelation. Functional groups in the resin, referred to as ligands, form coordination compounds which bind the central metal ion using multiple sites. The resin is usually pretreated, in order to convert the resin to the hydrogen, calcium, or ammonium form. Figure 1 depicts the mechanics of ion exchange in a calcium-based resin, as used in this research.

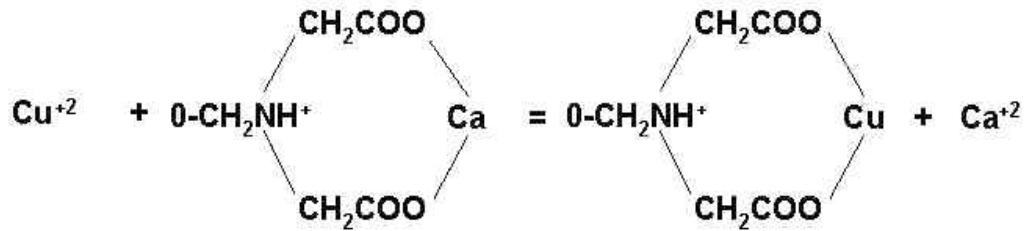


Figure 1: Chelex 100<sup>®</sup> Ion Exchange Mechanism shown for Sorbing Copper

Frequently the resin is used in the hydrogen form, although the effluent pH may be as low as 2.8. Research showed that the hydrogen form did not completely remove labile zinc, cadmium, lead, or copper from sea water until the passage of 500 mL of sample had increased the pH of the effluent to 6.5. Converting the resin to the calcium or ammonium form increases copper retention onto the resin, thus helping to alleviate this problem (Florence and Batley, 1976).

The Chelex 100<sup>®</sup> ion exchange resin has been put to use in many different ways. The resin has been used to determine trace metal concentrations in natural waters, when they are too low for detection. A large volume of water is passed through the resin; this allows metals to concentrate onto the resin. The metal ions are then recovered from the resin using a small volume of acid. By using a pre-concentration factor equal to the ratio of the volume of sample to the volume of eluent, the concentration of metal in the initial water can be determined using flame or graphite atomic-absorption spectrometry.

However, the presence of fulvic and humic acids can inhibit metal ion complexation (Florence *et al.*, 1976; Figura *et al.*, 1977). This can be overcome by pre-treating the water sample through irradiation with ultraviolet light (Leyden, 1982).

Metals that are strongly bound to sediments or organics are not chelated by Chelex 100<sup>®</sup> (Figura *et al.*, 1979). Hence the resin will retain only free metal ions and some weakly bound metal species. The species retained are potentially bioavailable. By measuring the amount of copper in the water sample both before and after contact with the resin, the amount of bioavailable copper can be determined by difference.

If the rate of metal uptake by a biological system is defined by the kinetics of dissociation of metals across the cell membrane, the chelex system can be used to measure bioavailable metals. This assumes the resin mimics the key binding sites on the biological membrane. However, these dissociation kinetics will only be important when the metal binding reactions at the cell surface are slow in comparison to the transport of metal across the membrane. Current thinking is that this is usually not the case (Turner, 1984). For this reason, bioavailable metals are operationally defined as the ionic and weakly bound metal species retained on the Chelex 100<sup>®</sup> resin.

Chelex 100<sup>®</sup> measurements of bioavailable metal concentrations are affected by various parameters. The resin is available in various particle sizes (50-100, 100-200, and 200-400 mesh). Chakrabarti and Lu (1994) reported that copper uptake rates decrease with increasing mesh sizes. While it is generally known that exchange is more efficient with smaller beads, it has been shown that a larger size is preferred if the exchanged metals are to be recovered from the resin. Elution of metals with acid is particularly inefficient with the 100-200 mesh size (Pakalns, 1978).



The contact time between the resin and the water may determine which species chelate. Tubbing *et al.* (1994) found that contact times ranging from 0.3 to 6 seconds showed no increased retention of copper. However, very long contact times, i.e. 60 hours, showed an increase in copper retention of 10-20%. This is further complicated when the water contains organic matter. Copper uptake rate by the Chelex 100<sup>®</sup> resin slowed with increasing ratios of [EDTA]/[Cu] or in the presence of Cu-fulvic complexes (Chakrabarti and Lu, 1994). Table 2 summarizes past research with the Chelex 100<sup>®</sup> resin, and shows how the detention time may effect copper sorbtion onto the resin.

Table 2: Past research Chelex 100<sup>®</sup> resin: detention time, water type studied, and copper retention to the resin

<b>Paper</b>	<b>Column Setup</b>	<b>Water Characterisitcs</b>	<b>Cu binding affinity</b>
(Rasmussen, 1981)	Ammonium Form	Seawater	Retained 100 to 107% of 1.87 ppb Cu
	Flowrate: 0.1 ml/min until resin had shrunk. Flow was then increased to 1 ml/min	pH adjusted to 5.0	
	Detention time not given		

<b>Paper</b>	<b>Column Setup</b>	<b>Water Characterisitcs</b>	<b>Cu binding affinity</b>
(Chakrabarti <i>et al.</i> , 1994)	Resin was used in a batch setup	Rideau river water	Cu(II) total= 133.5 ppb
		pH was adjusted to 5.0	[EDTA]/[Cu(II)] = 0; retained 99% Cu after 1000 seconds
	1% resin on a wieght basis used		[EDTA]/[Cu(II)] = .38; retained 63% Cu after 2000 seconds
		DOC: 6.6 mg/L	[EDTA]/[Cu(II)] = .77; retained 45% Cu after 2000 seconds
			[EDTA]/[Cu(II)] = 2.3; retained 7% Cu after 2000 seconds

<b>Paper</b>	<b>Column Setup</b>	<b>Water Characterisitcs</b>	<b>Cu binding affinity</b>
(Kuhn <i>et al.</i> , 1998)	Sodium form	Artificial Brine	retained a mean of 84.4%Cu, given 10 ppm Cu
	0.9g resin	Total Dissolved Solids (TDS): 35 to 250 mg/L	

	flow= 1.8 ml/min	(NaCl, CaCl <sub>2</sub> , MgCl <sub>2</sub> , Na <sub>2</sub> SO <sub>4</sub> )	
	detention time: 3.7 seconds		
		pH: 4-6	

Paper	Column Setup	Water Characterisitcs	Cu binding affinity
(Chakrabarti <i>et al.</i> , 1993)	detention time: 6-9 seconds	Rain Water, pH 4.7	Rain: retained 82.9% of 24.7 ppb Cu
	5 g resin	Snow Samples, pH 5.3	Snow: retained 83.3% of 4.0 ppb Cu

Paper	Column Setup	Water Characterisitcs	Cu binding affinity
(Tubbing <i>et al.</i> , 1994)	1.5 mg resin	River Rhine	50 to 60% Cu retained over the range of 15.9 to 99.8 ppb Cu
	Calcium form		
	flow: 1.7 ml/min		
	contact time: 33 seconds		

Paper	Column Setup	Water Characterisitcs	Cu binding affinity
(Ryan <i>et al.</i> , 1985)	calcium form		
		pH 6.0	75% of 100 ppb Cu retained in setup 1
	Bore: 7mm		
		25 mg/L soil-derived fulvic acid	61.9% of 27.8 ppb Cu retained in setup 2
	setup 1: 10cm length, Flowrate 3 ml/min detention time: 77 seconds		
	setup 2: 30cm length, Flowrate 2 ml/min detention time: 346.5 seconds		

Paper	Column Setup	Water Characterisitcs	Cu binding affinity
(Miwa <i>et al.</i> , 1989)	30mm length; 3 mm id	Laboratory reagent water	no DOC: retained 100% of 10 ppb Cu
	detention time: 5.1 seconds		0.5 mg/L Humic Acid: retained 48% of 10 ppb Cu
	calcium form	pH 7.0	
	2.5 ml/min		

Divalent cations such as Ca<sup>2+</sup> and Mg<sup>2+</sup> are common in water and may compete with heavy metals for active sites on chelating resins. Chelating resins were able to complex 3.5 ppm copper up to a hardness of 195 ppm as CaCO<sub>3</sub>. As hardness was increased to 254 ppm as CaCO<sub>3</sub>, elevated effluent copper concentrations were observed,

indicating some breakthrough of the resin (Mazidji *et al.*, 1992). Furthermore, pH may affect the Chelex 100<sup>®</sup> resin performance. Mazidji *et al.* (1992) and Kuhn *et al.* (1998) both found that pH values near 4 result in decreased resin performance compared to higher pHs.

Little to no chelating changes were seen in brines ranging from 35 to 250 g/L in total dissolved salts (Kuhn *et al.*, 1998). This suggested that chelating resins may be used in estuarine waters, although typical salinity ranges in estuaries are generally lower than those tested by Kuhn *et al.* (1998).

Domestic and industrial discharges of ionic and non-ionic detergents, formulated detergents, and detergent additives may effect also chelex performance. Pakalns and Batley (1978) reported better than 95% recovery of metals, but this recovery was diminished to less than 50% in the presence of 20 mg/L soap and 10 mg/L nitrotriactic acid (NTA) for metals such as 0.5 mg/L zinc, 1.0 mg/L nickel, 1.0 mg/L cobalt, and 3.0 mg/L lead. Furthermore, 1.0 mg/L copper was not effected by NTA, but cationic surfactants were totally retained by the resin, thus taking up 13% of the total resin capacity. It must be noted that Pakalns and Batley (1978) worked with a cationic surfactant concentration of 100 mg/L in order to note possible interferences, which is much higher than would be seen in polluted waters.

As discussed above, the chelex resin has been implemented to measure bioavailable copper. Current regulations are in terms of dissolved copper, but researchers are striving to understand bioavailable copper. The Chelex 100<sup>®</sup> resin technique measures the amount of free copper and weakly bound copper in a natural water. However, as shown above, the metal complexing ability of the ion exchange resin

depends many different factors. Resin characteristics such as flowrate and resin form can effect metal complexation. Water characteristics such as pH, alkalinity, and NOM can effect metal complexation. Furthermore, the chelex resin technique must be calibrated for a specific species type and maturity. Because of the many factors listed above, the copper that binds to the Chelex 100<sup>®</sup> resin is operationally defined as bioavailable copper, and does not relate to a specific water or aquatic organism.

In the past, some researchers have made attempts to relate Chelex 100<sup>®</sup> performance to toxicity for a given aquatic organism. In a study using hatchery-reared juvenihle coho salmon (*Oncorhynchus kisutch*) in river water, there was a significant correlation between truncated mortality (partial kills) and the fraction of Chelex 100<sup>®</sup> bound cadmium ( $r = .681$ ) (Buckley, 1985). However, in a similar study no correlation was seen between the fraction of copper bound to the Chelex 100<sup>®</sup> resin and the fraction of copper inhibiting the growth of the marine diatom *Nitzschia closterium*. Furthermore, some researchers concluded that the copper-chelex fraction overestimated toxicity (Florence *et al.*, 1983). Tubbing (1992) found a significant correlation between Chelex 100<sup>®</sup> copper and the percent reduction in photosynthesis for the algal *S. capricornutum*. This correlation was actually better than that seen with free copper.

## **2.6: Application of the Chelex 100<sup>®</sup> Resin to an Estuarine System**

After preliminary testing, the Chelex 100<sup>®</sup> resin was applied to a scaled model of the eastern shore of Virginia, where agricultural runoff and groundwater has been implicated with aquatic toxicity. Both tomato farmers and aquaculturists use the water resources; and due to geographical constraints the two industries are located fairly close to one another.

Tomato farmers apply copper-based crop protectants to the surface of the crops, which act as a bactericide and fungicide. Specifically, the protectants hinder bacterial spot and bacterial speck (Blancard, 1994). In addition to pesticide use, plasticulture is implemented. Plasticulture is an impermeable plastic placed between the soil and the crop. Plasticulture increases vegetable yield, reduces soil erosion, provides moisture and temperature control, and minimizes herbicide use. Up to 55% of a tomato field can be covered with impermeable plastic, with the rest being compacted by heavy farm equipment (Gayle, 1996). During rain events these copper-based pesticides are washed off the plants, travel across the impermeable soil, and run into nearby tidal creeks to aquaculture facilities.

Clam growing at these aquaculture hatcheries is a very complex and sensitive process. In order to breed clams, natural estuarine water of high quality is needed continuously (Parks, 1996). However, copper concentrations as high as 263  $\mu\text{g/L}$  total copper and 127  $\mu\text{g/L}$  dissolved copper have been noted at the Eastern Shore of Virginia during rain events (Klawiter, 1997). These copper concentrations can have disastrous effects on the aquaculture industry. Labreche (1998) has shown that copper concentrations as low as 8  $\mu\text{g/L}$  can have negative effects on the hardshell clam *Mercenaria mercenaria*, which is typically grown on the Eastern Shore of Virginia.

In order to further assess the implications of agricultural runoff and groundwater to tidal estuaries, a greenhouse model of the Eastern Shore of Virginia was constructed. A greenhouse scale model was chosen instead of in situ testing for many reasons. The model allowed simulated rain events to be held on a weekly basis, holding all parameters constant. The model was also located on Virginia State University and Polytechnic

Institute's campus, thus minimizing travel time and expenses. Also, the effects of a simulated sedimentation basin was noted, and copper could be tracked through mass balances.

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### Chapter 3: Introduction

Copper is an essential element to most aquatic organisms at very low concentrations. However, concentrations as low as 8 µg/L have been known to have negative effects on larval shellfish (LaBreche, 1998). The concentration required to kill 50 percent (LC<sub>50</sub>) of the marine mussel *Mytilus edulis* in 30 days is 2 µg/L (Luoma and Carter, 1991). Ionic copper concentrations as low as 5.8 µg/L stunts the growth of the bay scallop *Argopecten irradians*. The 42 day LC<sub>50</sub> for this organism is 9.3 µg/L ionic copper (Pesch *et al.*, 1979). The soft shell clam *Mya arenaria* experiences a 7 day LC<sub>50</sub> of 35 µg/L added copper during the summer (Eisler *et al.*, 1994).

Consequently, criteria have been established to help regulate copper discharge to natural waters. In 1972, Clean Water Act was established, and in 1977 the United States Environmental Protection Agency (USEPA) was required to create a list of toxic pollutants for industry and waste water treatment plants. Although the USEPA criteria are not enforceable, they do provide for the implementation of state water quality regulations. These criteria were initially established in terms of total metal concentrations (USEPA, 1985). Soluble copper was deemed the better parameter in copper toxicity, and in the early 1990's criteria for metals were generally changed to dissolved metal concentrations, and in 1995 the USEPA published a saltwater draft in terms of dissolved copper (USEPA, 1995). Since then, some research has suggested that if a metal was strongly bound in the particulate phase, it was not readily toxic (Renner, 1997). Recent research has demonstrated that particulate copper can be mobilized by organisms and can become bioavailable (Labreche, 1998; Baerselman *et al.*, 1998).

Even within the dissolved phase, metals may be bound to dissolved organic or inorganic components, which impacts toxicity. Copper criteria could consider bioavailable copper. Conceptually, bioavailable metal is considered that portion of the total metal concentration that is available to aquatic organisms. If a metal is or is made to be bioavailable, it can exert toxicity, and therefore should be controlled.

Numerous methods have been established in order to provide insight into bioavailable metal fractions, by differentiating between various metal species. For example,  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$  are considered highly toxic, whereas  $[\text{Cu}_2(\text{OH})_2]^{2+}$  is sometimes considered toxic. Copper-carbonate species and copper-organic ligand species are generally not considered toxic (Meador, 1991). Increases in carbonate species are usually associated with increases in alkalinity. Therefore, alkaline waters have more of a potential to form copper-carbonate species, thus reducing copper toxicity (Snoeyink and Jenkins, 1980). Inorganic calcium and magnesium complexes associated with water hardness may compete with copper for binding sites in an organism, also reducing toxicity (Brezonik *et al.*, 1991). Although organic copper species are generally considered non-toxic, they do have the potential to bioaccumulate (Stumm and Morgan, 1996).

In order to determine bioavailable copper, measurement methods that determine specific copper species are necessary. Electrochemical methods, such as voltammetry and potentiometry, use electrodes to determine metal species (Ruzic, 1982), (Coale *et al.*, 1988), (Van Den Berg *et al.*, 1984), and (Buffle *et al.*, 1979). Non-electrochemical methods such as dialysis, ion exchange resins, ligand competition methods, and size-based separations are also used to determine metal species (Mazidji *et al.*, 1992; Tubbing

*et al.*, 1994). Furthermore, mass spectrometry and high-performance liquid chromatography have been applied to metal speciation studies (Marshall, 1988).

Although a wide range of analytical methods are available for measuring trace metal species, voltammetric techniques with low detection limits have been extensively used. However, the information obtained from voltammetric methods is usually highly complex, making it very hard to interpret. Furthermore, voltammetry requires that samples be transported to the laboratory prior to analysis. During this time crucial water characteristics such as pH, may change in the sample, thus changing metal speciation.

A field portable approach uses a Chelex 100<sup>®</sup> ion exchange resin to bind ionic and weakly bound copper. Samples may be processed in the field and the actual metal analysis can occur later using standard atomic absorption equipment. Chelex 100<sup>®</sup> resin is a styrene-divinylbenzene copolymer with imminodiacetate functional groups that are able to form complex compounds with free metal species and weakly bound metal ion complexes through ion exchange. Complex formation is referred to as chelation. Functional groups in the resin, referred to as ligands, form coordination compounds which bind the central metal ion using multiple sites. The resin is usually pretreated, in order to convert the resin to the hydrogen, calcium, or ammonium form. Frequently the resin is used in the hydrogen form, although the effluent pH may be as low as 2.8. Research showed that the hydrogen form did not completely remove labile zinc, cadmium, lead, or copper from sea water until the passage of 500 mL of sample had increased the pH of the effluent to 6.5. Converting the resin to the calcium or ammonium form increases copper retention onto the resin, thus helping to alleviate this problem (Florence and Batley, 1976).

Metals that are strongly bound to sediments or organics are not chelated by Chelex 100<sup>®</sup> (Figura *et al.*, 1979). Hence the resin will retain free metal ions and some weakly bound metal species. The species retained are potentially bioavailable. By measuring the amount of copper in the water sample both before and after contact with the resin, the amount of bioavailable copper can be determined by difference.

Chelex 100<sup>®</sup> measurements of bioavailable metal concentrations are affected by various parameters. The resin is available in various particle sizes (50-100, 100-200, and 200-400 mesh). Chakrabarti and Lu (1994) reported that copper uptake rates decrease with increasing mesh sizes. While it is generally known that exchange is more efficient with smaller beads, it has been shown that a larger size is preferred if the exchanged metals are to be recovered from the resin. Elution of metals with acid is particularly inefficient with the 100-200 mesh size (Pakalns and Batley, 1978).

The contact time between the resin and the water may determine which species chelate. Tubbing *et al.* (1994) found that contact times ranging from 0.3 to 6 seconds showed no increased retention of copper. However, very long contact times, i.e. 60 hours, showed an increase in copper retention of 10-20%. This is further complicated when the water contains organic matter. Copper uptake rate by the Chelex 100<sup>®</sup> resin slowed with increasing ratios of [EDTA]/[Cu] or in the presence of Cu-fulvic complexes (Chakrabarti and Lu, 1994).

Divalent cations such as Ca<sup>2+</sup> and Mg<sup>2+</sup> are common in water and may compete with heavy metals for active sites on chelating resins. Chelating resins were able to complex 3.5 ppm copper up to a hardness of 195 ppm as CaCO<sub>3</sub>. As hardness was increased to 254 ppm as CaCO<sub>3</sub>, elevated effluent copper concentrations were observed,



indicating some breakthrough of the resin (Mazidji *et al.*, 1992). Furthermore, pH may affect the Chelex 100<sup>®</sup> resin performance. Mazidji *et al.* (1992) and Kuhn *et al.* (1998) both found that pH values near 4 resulted in decreased resin performance compared to higher pHs.

Little to no chelating changes were seen in brines ranging from 35 to 250 g/L in total dissolved salts (Kuhn *et al.*, 1998). This suggested that chelating resins may be used in estuarine waters, although typical salinity ranges in estuaries are generally lower than those tested by Kuhn *et al.* (1998).

Domestic and industrial discharges of ionic and non-ionic detergents, formulated detergents, and detergent additives may effect also chelex performance. Pakalns and Batley (1978) reported better than 95% recovery of metals, but this recovery was diminished to less than 50% in the presence of 20 mg/L soap and 10 mg/L nitrotriactic acid (NTA) for metals such as 0.5 mg/L zinc, 1.0 mg/L nickel, 1.0 mg/L cobalt, and 3.0 mg/L lead. Copper at 1.0 mg/L was not effected by NTA, but cationic surfactants were totally retained by the resin, thus taking up 13% of the total resin capacity. It must be noted that Pakalns and Batley (1978) worked with a cationic surfactant concentration of 100 mg/L in order to note possible interferences, which is much higher than would be seen in polluted waters.

In the past, some researchers have made attempts to relate Chelex 100<sup>®</sup> performance to toxicity for a given aquatic organism. In a study using hatchery-reared juvenihle coho salmon (*Oncorhynchus kisutch*) in river water, there was a significant correlation between truncated mortality (partial kills) and the fraction of Chelex 100<sup>®</sup> bound cadmium ( $r = .681$ ) (Buckley, 1985). However, in a similar study no correlation

was seen between the fraction of copper bound to the Chelex 100<sup>®</sup> resin and the fraction of copper inhibiting the growth of the marine diatom *Nitzschia closterium*. Furthermore, some researchers concluded that the copper-chelex fraction overestimated toxicity (Florence *et al.*, 1983). Tubbing (1992) found a significant correlation between Chelex 100<sup>®</sup> copper and the percent reduction in photosynthesis for the algal *S. capricornutum*. This correlation was actually better than that seen with free copper.

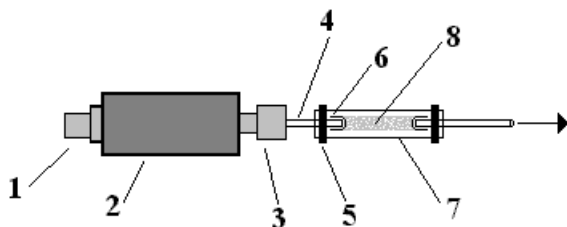
The objectives of this paper were to determine if the Chelex 100<sup>®</sup> resin responded to changes in water characteristics in a logical manner when measuring bioavailable copper. Water characteristics studied were: salinity, copper concentration, pH, and natural organic matter (NOM). The resin was then applied to measure bioavailable copper in agricultural runoff, groundwater, and estuarine systems.

## Chapter 4: Methods

### 4.1: Chelex 100<sup>®</sup> preparation

Fisherbrand<sup>®</sup> Fisher Scientific, (Pittsburgh, Pennsylvania) chemicals, sample containers, and glassware were used exclusively for all laboratory analyses. The sample containers and glassware were cleaned prior to analysis in order to remove any trace chemicals; they were soaked in 10% trace metal grade nitric acid for at least 8 hours, washed 3 times with distilled water, and then washed three times using Nanopure<sup>®</sup> water.

Chelex 100<sup>®</sup> ion exchange resin, Biorad (Hercules, California) #142-2842, was prepared in accordance with Miwa *et al.* (1989). The resin slurry was packed into columns for sampling. Chelex 100<sup>®</sup> columns were constructed using liquid chromatography components and plastic tubing. Figure 2 depicts a typical column.



- Where 1: Luer Lock, Upchurch Scientific (Oak Harbor, Washington) #P 624  
2: Plastic Union, Upchurch Scientific (Oak Harbor, Washington) #P 623  
3: Male Connector, Upchurch Scientific (Oak Harbor, Washington) #P 345  
4: 1/16" id Teflon Tubing, Upchurch Scientific (Oak Harbor, Washington) #1523  
5: Zip Tie, GB brand (Milwaukee, Wisconsin) #45-308  
6: 1/4" \* 1/4" .35  $\mu$ m Polyester Nytex Cloth Frit  
7: 1/8" id clear PVC Tubing Fisher Scientific (Pittsburgh, Pennsylvania) #141697A  
8: 200-400 mesh Chelex 100<sup>®</sup> Resin, Biorad (Hercules, California) #142-2842

Figure 2: Chelex 100<sup>®</sup> Column Apparatus

Items 1 through 7 in Figure 2 were cleaned before resin was added; this was done in order to remove any trace metals. Each column was placed in a 10% trace metal grade

nitric acid bath for 2 hours. The columns were then submersed 3 times in distilled water, and then 30 mL of Nanopure<sup>®</sup> water was passed through each column. The dried columns were tared to 0.0001 grams using an analytical balance. The resin slurry was then drawn into the columns using a syringe that was acid washed in the same manner as items 1 through 7 in Figure 2. Excess water was drawn out using the syringe. The amount of resin in each column was adjusted to 0.085 g on a dry weight basis. A second ¼" × ¼" polyester frit, 1/16" id teflon tubing, and zip tie was then added to the column to retain the resin as shown in Figure 2. Using a KD Scientific (Boston, Massachusetts) 220 screw-driven syringe pump set to 60 mL/min, 4 mL of 2 M calcium chloride was passed through each column, followed by 30 mL of Nanopure<sup>®</sup> water.

#### **4.2: Chelex 100<sup>®</sup> sampling**

In natural waters, some metal ions may bind to particles which may become enmeshed in the resin, giving a false positive bioavailable measurement. In order to guard against these false positive readings, samples were treated to remove organic matter. Samples with a pH of 8.0 or greater were passed through a 3.5 µm Fisherbrand<sup>®</sup> number 09-804-55A glass fiber circle because internal fresh water testing had shown that the 0.45 µm filter sorbed copper at pH values above 8. For the purposes of this paper, samples passed through the 3.5 µm glass filter were operationally deemed as dissolved. Samples were passed through a 0.45 µm Fisherbrand<sup>®</sup> number 09-719-2D filter when if the pH was less than 8.

After filtering, samples were passed through the resin at 60 mL/min, yielding a sample detention time of approximately 0.1 seconds. The first 5 mL of sample was wasted, then a 15 mL sample was taken. Dissolved copper retained by the resin columns

was operationally defined as bioavailable copper. To determine the amount of resin-bound bioavailable copper, dissolved copper was measured both before and after passing through the resin; this was accomplished using either graphite furnace atomic adsorption or flame atomic adsorption in accordance to EPA methods.

#### ***4.3: Instrumental measurement of copper***

Measurement of copper concentrations below 100 µg/L was performed according to EPA Method 7211, using a graphite furnace atomic absorption spectrophotometer (GFAAS) (USEPA, 1996). A 10 µg/L dissolved copper external Centriprep SPEX (Metuchen, New Jersey) #WP-11 check standard was sampled before and after GFAAS copper analysis. If the SPEX standard read between 9 and 11 µg/L dissolved copper, the GFAAS calibration curve was deemed accurate.

For higher concentrations, a Perkin-Elmer 703 flame atomic absorption spectrophotometer (FAAS) was used following the manufacturers' recommendations and EPA Method 7210 (USEPA, 1996). Each solution was sampled in triplicate, using three different ion exchange columns in order to attain a mean and standard deviation.

#### ***4.4: Assessment of Chelex 100<sup>®</sup> performance***

Initial laboratory testing was performed in order to compare the degree of Chelex 100<sup>®</sup> resin copper retention in a fresh water (300 µg/L dissolved copper, pH 6.5 buffered with 30 mg/L as calcium carbonate) to a saline water (300 µg/L dissolved copper, 25 ppt salinity, pH 8). To assess copper-organic ligand binding competitive effects, 20 mg/L natural organic matter (NOM) was also added to each solution. The effects of sample flow rate through the Chelex 100<sup>®</sup> resin was also varied between 60 and 10

mL/min, for a solution consisting of 150 ppb Cu, 21 ppt salinity, pH 4, and 12.5 mg/L NOM.

In order to further assess the Chelex 100<sup>®</sup> resin performance, preliminary laboratory testing was performed using water taken from a simulated estuarine system (Stall, 1999). A sufficient volume of estuarine water was collected, such that every laboratory experiment could be conducted using the same stock solution. The solution's salinity was measured to be 15 ppt using a Fisher model 13-946-27 hand held refractometer. The solution contained 12.5 mg/L natural organic matter (NOM) which was measured using a Sievers 800 TOC analyzer (Boulder, Colorado).

Preliminary laboratory tests varied water characteristics such as copper concentration, salinity, pH, and NOM. A 1000 mg/L copper nitrate Fisher certified copper reference solution was used to adjust the amount of copper. The salinity of the solution was adjusted as needed using Instant Ocean<sup>®</sup> (Mentor, Ohio); the pH of the water was adjusted using either 10 M NaOH or 10 M HCl; the NOM was adjusted by diluting with 21 ppt salinity Instant Ocean<sup>®</sup>. For all testing the variable in question was modified while keeping the remaining sample matrix constant.

#### ***4.5: Mathematical modeling of Chelex 100<sup>®</sup> results***

A copper-organic ligand model was established using MINEQL+ version 4.0, by Schecher and McAvoy (1998), which predicted copper-NOM species in natural waters. A five-ligand model developed by Cabaniss and Shuman (1987a and 1987b) from experimental data was implemented. All of the copper-organic ligand complexes

predicted by the mathematical model were assumed to be strongly complexed and therefore not bioavailable in terms of the ion exchange resin.

The Cabaniss and Shuman (1987a and 1987b) stability constants for copper-ligand (Cu-L) complexation applied in MINEQL+ were as follows:  $\text{CuL}_1$  ( $\log K = 3.90$ ),  $\text{CuL}_2$  ( $\log K = 1.494$ ),  $\text{CuL}_3$  ( $\log K = -0.364$ ),  $\text{CuL}_4$  ( $\log K = -7.483$ ),  $\text{CuL}_5$  ( $\log K = -10.05$ ). MINEQL+ also required the total molar concentration for each the ligand. To convert the NOM concentrations from mg/L to moles/L, Cabaniss and Shuman (1987a and 1987b) multiplied the NOM concentrations by constants of  $5.0 \times 10^{-6}$ ,  $1.9 \times 10^{-7}$ ,  $1.1 \times 10^{-6}$ ,  $1.4 \times 10^{-7}$ , and  $9.6 \times 10^{-6}$  moles/mg respectively.

#### ***4.6: Field application of the Chelex 100<sup>®</sup> to water systems***

The Chelex 100<sup>®</sup> system was applied to an agricultural/estuarine system to better study the potential for copper toxicity. Three greenhouse-scale mesocosms of agricultural fields were designed and constructed, along with three greenhouse-scale estuarine systems. Each agricultural field was associated with an individual estuarine system that received the field's runoff and groundwater (Cheadle, 1999), (Stall, 1999).

The agricultural simulation consisted of three tomato beds, each measuring 2.13M  $\times$  1.22M  $\times$  .91M. One bed depicted a plasticulture field with a sedimentation basin. It was sprayed with Microperse<sup>®</sup> brand crop protectant, manufactured by Microflow, Inc. of Florida. Microperse<sup>®</sup> consisted of 53% metallic copper as copper oxychloride, and was applied to the plants with a commercial hand-held sprayer. During each application, 0.57 to 1.52 grams of copper was applied to each bed. One gram copper per greenhouse tomato bed was equivalent to 3.8 kg copper per hectare. Another bed represented a plasticulture field without a sedimentation basin, and it was also sprayed with the same

copper-based pesticide. A third bed did not implement plasticulture and was used as a copper-free control. Each bed contained Bojac Sandy Loam indigenous to the Eastern Shore of Virginia. On approximately a weekly basis, the two plasticulture beds were sprayed with copper-based pesticides. A rain simulator delivered 163 L of distilled water to each bed. Runoff and ground water were collected from each of the three beds for copper analysis. Typical runoff total and dissolved copper concentrations were  $2102 \pm 433 \mu\text{g/L}$  and  $189 \pm 139 \mu\text{g/L}$  respectively. Likewise, typical ground water total and dissolved copper concentrations were  $312 \pm 198 \mu\text{g/L}$  and  $216 \pm 99 \mu\text{g/L}$  respectively (Stall, 1999). To simulate the sedimentation basin, runoff and groundwater were allowed to settle for at least 24 hours. During this period, the soil settled to the bottom, reducing the total copper concentration by 90%, to a concentration of  $245 \pm 127 \mu\text{g/L}$  (Stall, 1999). The agricultural drainage was delivered to the respective estuary. All agricultural samples were taken before sedimentation was implemented. Each agricultural sample was passed through a  $0.45 \mu\text{m}$  dissolved filter and analyzed for bioavailable copper.

Each corresponding estuary was cycled by a pump to simulate high and low tides and contained plant and animal life indigenous to a tidal estuary. Estuary samples were collected before and after the addition of the respective runoff and groundwater. Immediately after the addition of agricultural runoff and groundwater, dissolved copper concentrations of approximately  $18 \mu\text{g/L}$  were noted in the bed receiving settled runoff and groundwater. The concentration of dissolved copper decreased over time, until it reached a background concentration near  $5 \mu\text{g/L}$  after 48 hours. The estuary receiving agricultural runoff and groundwater directly contained approximately  $35 \mu\text{g/L}$  dissolved copper at time zero, and reached a background concentration near  $8 \mu\text{g/L}$  after 48 hours



(Cheadle, 1999). Figure 3 shows the schematic for sampling at the greenhouse scale system.

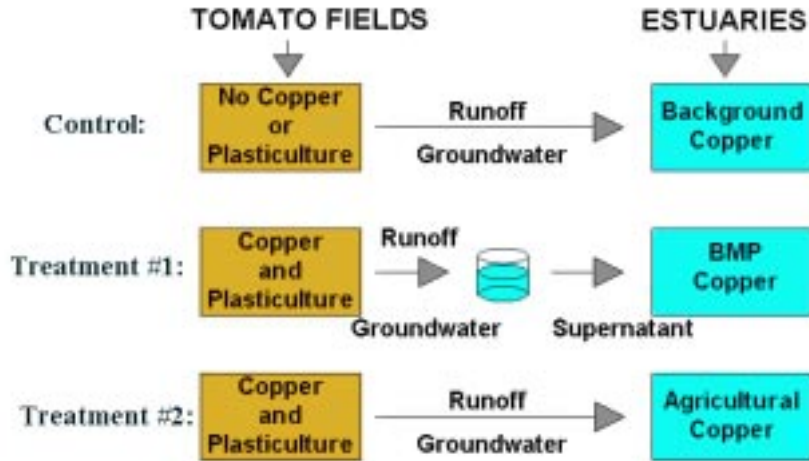


Figure 3: Greenhouse Model Sampling Schematic, where BMP = best management practice

## Chapter 5: Results

### 5.1: Assessment of Chelex 100<sup>®</sup> performance

Initial laboratory testing indicated that the percent of copper bound to the resin did not differ between a freshwater solution (pH 6.5 buffered with 30 mg/L as calcium carbonate) and a saline solution (25 ppt salinity at pH 8), ( $p = .31$ ). Adding 20 mg/L natural organic matter (NOM) to each solution lowered the amount of copper sorbed to the resin in both cases by an equal amount ( $p = .736$ ), (data not shown).

Figures 4 through Figure 8 show the effects of varying copper concentration, pH, and NOM in an estuarine water. Each sample was collected in triplicate, in order to calculate a mean and a standard deviation. In each case, the solution matrix was held constant while the variable in question was modified. Lowering pH and NOM statistically increased percent bioavailability, ( $p = .01$  and  $p = .007$  respectively), whereas

varying the salinity to 16, 21, and 30 ppt (data not shown) and copper concentration from 195 to 579  $\mu\text{g/L}$  had no effect ( $p = .051$  and  $p = .66$  respectively).

Figure 6 and Figure 8 compare MINEQL+ model predictions to the experimental resin pH and NOM titrations respectively. In Figure 6 and Figure 8, the individual model-predicted copper-ligand species are shown (ie-  $\text{CuL}$ ,  $\text{Cu}_2\text{L}$ ,  $\text{CuL}_2$ ), along with the total amount of model-predicted copper-bound ligand, denoted by “Sum of all Cu-Ligands”. The ligand bound copper predicted by MINEQL+ is a calculation of the complexed or “non-bioavailable” copper in the system. Figure 6 shows that at pH values greater than 5, there is a good agreement between the MINEQL+ prediction and the experimentally measured “non-bioavailable” copper as determined by the Chelex 100<sup>®</sup> resin. Figure 8 shows good agreement between the MINEQL+ prediction and the experimentally measured “non-bioavailable” copper at NOM concentrations greater than 3 mg/L.

### ***5.2: Application of the Chelex 100<sup>®</sup> to a natural estuarine system***

Greenhouse agricultural runoff and groundwater samples were taken from each of the three tomato fields. Typical dissolved copper concentrations in the control bed ranged from 1 to 14  $\mu\text{g/L}$ . These low copper concentrations were often close to the detection limits of the GFAAS, and yielded highly variable and inconclusive bioavailable measurements. Table 3 provides the results of the tomato fields with and without sedimentation control. Each measurement was taken in triplicate. The average standard deviation for runoff and groundwater triplicate samples for a typical rain event was 6.26  $\mu\text{g/L}$  dissolved copper, with coefficient of variation values typically below 10%.

Overall, agricultural runoff and groundwater percent bioavailability measurements ranged from approximately 5 to 25 percent dissolved copper, with extremes as high as 45 percent bioavailable copper at acidic pH values between 3 and 4. Agricultural runoff ranged from 50 to 375  $\mu\text{g/L}$  dissolved copper. Agricultural groundwater ranged from 33 to 310  $\mu\text{g/L}$  dissolved copper. Figure 9 indicates that the copper concentration did not impact percent bioavailability in the agriculture samples. A linear regression for runoff showed no trend between dissolved copper and percent bioavailable copper ( $p = 0.79$ ). Groundwater showed a slight increasing trend ( $p = 0.04$ ). A t-test determined no significant difference between runoff and groundwater mean percent bioavailability ( $p = 0.99$ ). These results suggest that, in general, the nature of the copper complexes may not change over the range of copper concentrations observed or the water quality differences between runoff and groundwater.

Both beds did not differ in runoff variance (test value = 2.05, probability level = 0.19) or groundwater variance (test value = 0.01, probability level = 0.93). Because samples were taken before sedimentation was employed, these results indicate expected replication. Data points shown in Figure 9 are for both beds.

Estuary samples were also collected in triplicate, and a typical estuary dosing event yielded a mean standard deviation of 3.29  $\mu\text{g/L}$  dissolved copper with a coefficient of variance typically below 7%. As stated earlier, samples were collected in the estuary before and after crop runoff and ground water was added. Table 3 provides the results of the estuaries after being dosed with runoff and groundwater from the agricultural fields. Each of the three estuaries contained no more than 11  $\mu\text{g/L}$  dissolved copper before the addition of agricultural runoff and groundwater. Typical dissolved copper concentrations

in the control estuary after dosing ranged from 3 to 10  $\mu\text{g/L}$ . Consequently, only “after dose” values for the two estuaries receiving copper-laden agricultural runoff and groundwater are shown in Table 3. Dissolved copper concentrations were typically three times lower in the estuary with sedimentation control in comparison to the estuary without sedimentation control. An increase in bioavailability (both percent and total) over time was not noted in the estuary without sedimentation control, even though the dissolved copper level increased. Similar results were noted in the estuary with sedimentation control.

The use of sedimentation control did not alter the mean percent bioavailable copper in the estuaries ( $p = 0.72$ ) (Figure 10). A Modified-Levene Equal-Variance test indicated that the use of sedimentation control did not alter the variance for percent bioavailability measurements in estuarine water (test value = .208, probability level = .658). Therefore, Figure 10 incorporates data with and without sedimentation control. An increase in percent bioavailability with increasing copper concentration was not noted in the estuarine water ( $p = .26$ ). A Modified-Levene Equal-Variance test determined no difference in variances for bioavailable measurements in the estuaries versus the agricultural fields (test value = .315, probability level = .579). Mean percent bioavailable measurements between the estuarine water and agricultural fields was also similar ( $p = 0.59$ ).

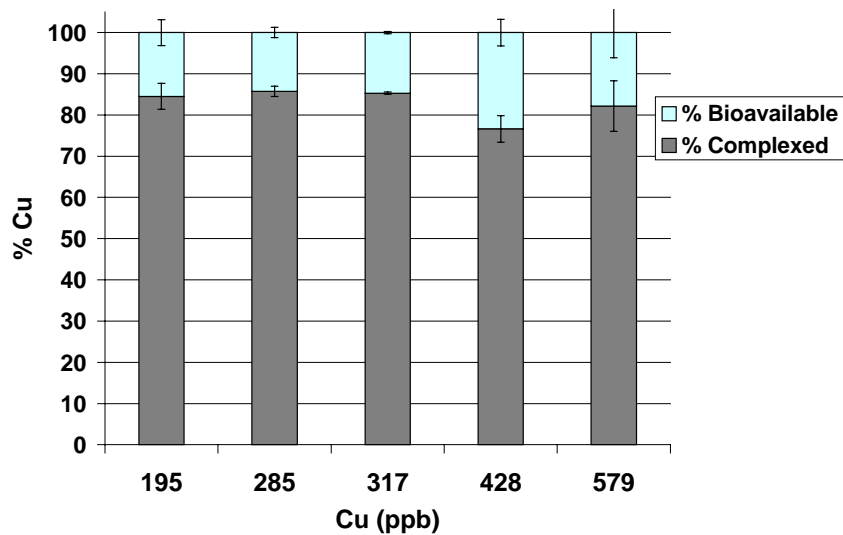


Figure 4: Effect of Copper Concentration on Chelex 100<sup>®</sup> Binding of Copper (pH 7.3, 15 ppt salinity, 12.5 mg/L NOM). Error bars are +/- one standard deviation.

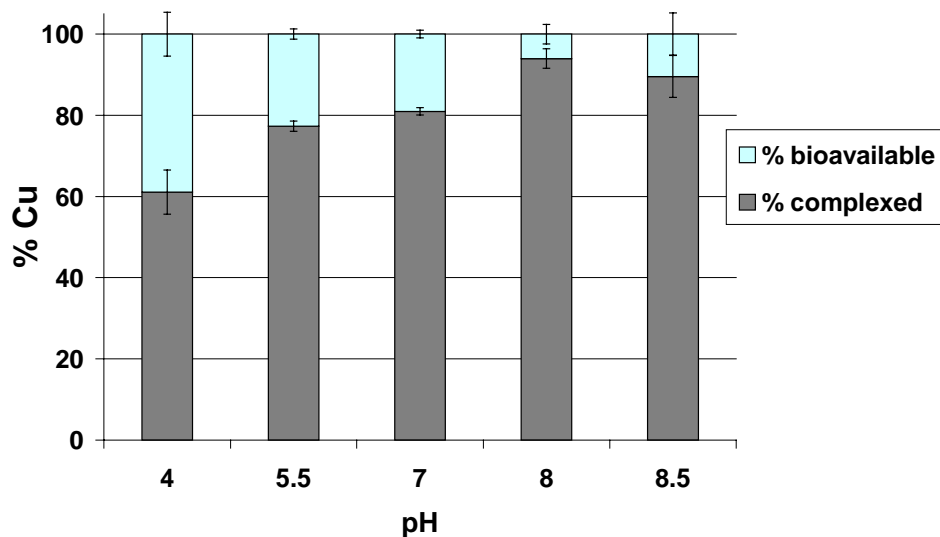


Figure 5: The Effect of pH on Chelex 100<sup>®</sup> Binding of Copper (150ppb Cu, 21ppt salinity, and 12.5 mg/L NOM). Error bars are +/- one standard deviation.

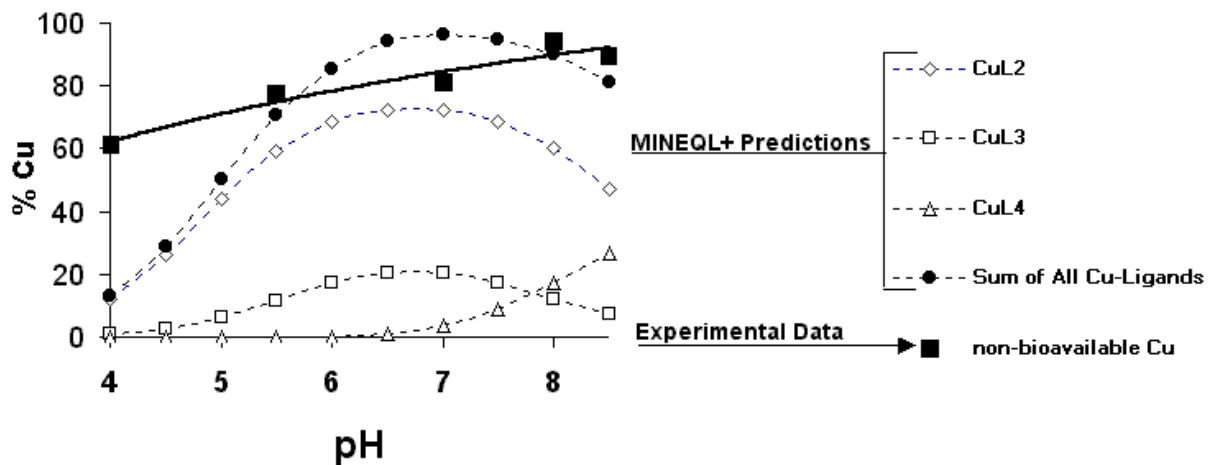


Figure 6: pH Titration: MINEQL+ prediction of non-bioavailable Cu-ligand species (CuL, Cu<sub>2</sub>L, and CuL<sub>2</sub>) vs. Chelex 100<sup>®</sup> Experimental Results (150ppb Cu, 21ppth salinity, and 12.5 mg/L NOM). MINEQL+ complexation constants were calibrated using data from Cabaniss and Shuman (1987a and 1987b).

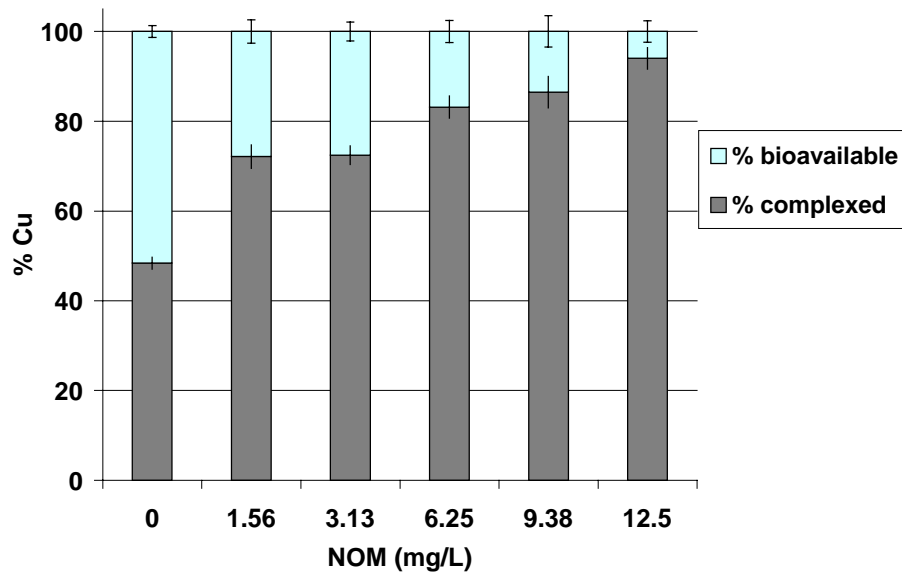


Figure 7: Effect of NOM on Chelex 100<sup>®</sup> Binding (150ppb Cu, 21ppth salinity, pH 8.1). Error bars are +/- one standard deviation.

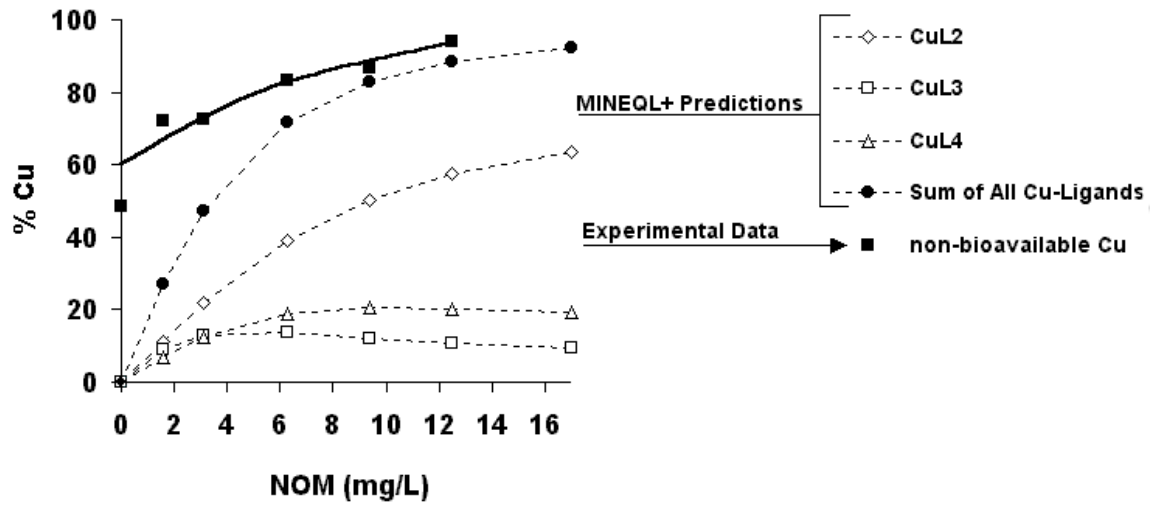


Figure 8: NOM Titration: MINEQL+ prediction of non-bioavailable Cu-ligand species (CuL, Cu<sub>2</sub>L, and CuL<sub>2</sub>) vs. Chelex 100<sup>®</sup> Experimental Results (150ppb Cu, 21ppth salinity, pH 8.1). MINEQL+ complexation constants were calibrated using data from Cabaniss and Shuman (1987a and 1987b).

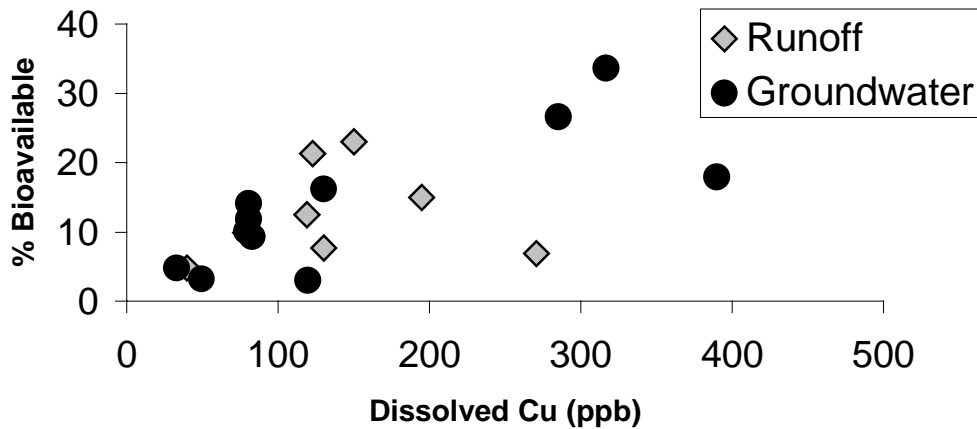


Figure 9: Variation of % Bioavailability with Dissolved Copper Concentrations from Simulated Tomato Beds

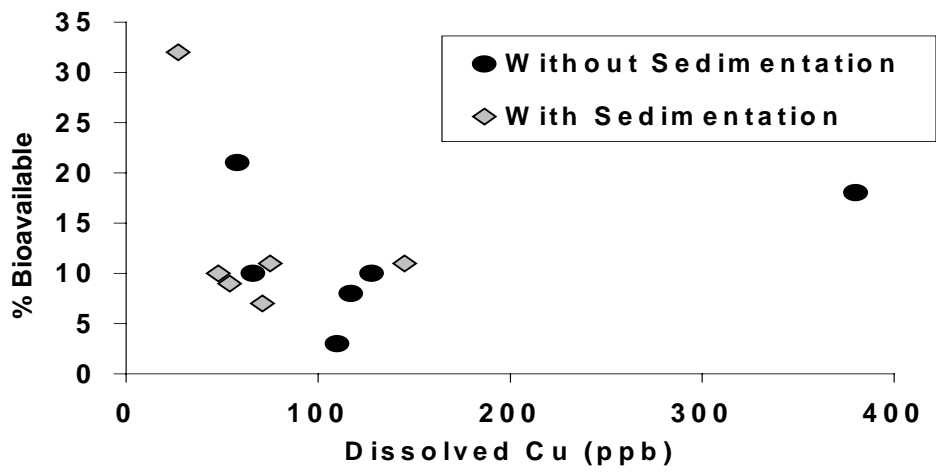


Figure 10: Estuary Event: Cu vs % Bioavailability with and without Sedimentation Control



Table 3: Greenhouse agricultural runoff, groundwater, and receiving estuarine data for all rain events.

(Note that Treatment 1 and 2 are the same for agricultural samples because samples were taken before and after sedimentation.)

Sample type	Date	Dissolved Copper (ppb)	% Bioavailable Copper	pH	NOM (mg/L)
<b>AGRICULTURAL SAMPLES</b>					
Groundwater	2-Sep	49.4	3.2	6.67	
Groundwater	2-Sep	316.67	33.6	6.75	
Groundwater	9-Sep	32.8	4.8	6.78	
Groundwater	9-Sep	80.5	14.1	6.32	
Groundwater	18-Sep	285	26.6	6.56	
Groundwater	18-Sep	390	17.9	6.82	
Groundwater	25-Sep	79.1	10.1	6.35	17.1
Groundwater	25-Sep	130	16.2	6.7	15.5
Groundwater	1-Oct	80.6	11.8	6.6	
Groundwater	1-Oct	82.9	9.3	6.87	
Groundwater	21-Oct	119.7	3	5.35	18.8
Groundwater	21-Oct	260.7	8.9	6.79	
Runoff	2-Sep	57.6	44.2	3.6	
Runoff	2-Sep	380	42.9	3.88	
Runoff	9-Sep	39.8	4.8	7.28	
Runoff	9-Sep	78.5	9.9	6.86	
Runoff	18-Sep	150	23	6.92	
Runoff	18-Sep	130	7.7	7.03	
Runoff	25-Sep	118.9	12.4	6.63	
Runoff	25-Sep	194.7	15	7.05	
Runoff	1-Oct	122.9	21.3	6.53	
Runoff	1-Oct	270.8	6.9	6.7	17.8
Runoff	21-Oct	1130	15.5	6.19	17.6
Runoff	21-Oct	562	7.1	6.77	
<b>ESTUARINE SAMPLES</b>					
Treatment 1	4-Sep	26.6	36	8.29	12.5
Treatment 1	16-Sep	49.3	12.2	8.24	
Treatment 1	23-Sep	52.7	7.8	8.62	
Treatment 1	30-Sep	71.6	6.8	7.12	
Treatment 1	9-Oct	78.3	12.8	---	
Treatment 1	29-Oct	146.5	11.9	8.27	
Treatment 2	4-Sep	59.1	21.7	8.14	
Treatment 2	16-Sep	128.1	12.3	8.06	
Treatment 2	23-Sep	68.3	10	8.42	
Treatment 2	30-Sep	100.5	10	7.12	
Treatment 2	9-Oct	383.3	19.1	---	
Treatment 2	29-Oct	114.4	9.9	8.57	

## Chapter 6: Discussion

### 6.1: Assessment of the Chelex 100<sup>®</sup> resin

Initial testing proved that the Chelex 100<sup>®</sup> resin responds to environmental changes in a logical manner. Salinity did not effect the fraction of bioavailable copper, as defined by the resin. Kuhn *et al.* (1998) also found little to no chelating changes in brines ranging from 35 to 250 g/L in total dissolved salts. This signifies that the ions typically found in an estuarine environment do not compete with copper for active sites on the resin. MINEQL+ modeling of the system did not show any changes in copper speciation with increases in salinity.

Furthermore, the fraction of bioavailable copper exchanged onto the resin remained constant even as the dissolved copper concentration increased (Figure 4). If the system was exclusively comprised of inorganic compounds, this result would be expected considering simple water chemistry theory. For example, the formation of  $\text{CuCO}_3$  is expressed as:  $\text{Cu}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CuCO}_3$ , yielding an acidity constant of:  $K = \frac{[\text{CuCO}_3]}{[\text{Cu}^{2+}][\text{CO}_3^{2-}]}$ . Considering that  $K$  is constant, if the amount of  $\text{Cu}^{2+}$  increases, and the concentration of  $\text{CO}_3^{2-}$  is also held constant, the amount of  $\text{CuCO}_3$  must also increase proportionally, thus not effecting percent bioavailability. However, this scenario is complicated when NOM is introduced. As more copper binds to NOM, the active sites may change, thus effecting Cu-NOM complexation. Although not shown in this paper, the MINEQL+ model indicated that copper speciation in this estuarine water did not change as the copper concentration was increased over the range studied. This is in accordance to the Chelex 100<sup>®</sup> experimental results presented in Figure 1.

By decreasing pH, an increase in % bioavailability was noted (Figure 5). As the pH of a natural water is lowered, copper has a better chance to form inorganic species that are potentially bioavailable. MINEQL+ modeling in Figure 6 also shows that as the pH of a natural water is decreased, the percent of copper bound to NOM decreases. However, the Chelex 100<sup>®</sup> resin did not accurately follow MINEQL+ predictions when the pH was lowered past pH 5.5. However, as the pH is lowered past 5.5, the functional groups on the resin change, and ion exchange becomes less efficient, thus lowering the amount of bioavailable copper measured. Mazidji *et al.* (1992) and Kuhn *et al.* (1998) both found that pH values near 4 result in decreased resin performance compared to higher pHs.

Furthermore, decreasing the amount of natural organic matter increased the percent of bioavailable copper in the resin system (Figure 7). As fewer sites are available for strong copper complexation with organic ligands, copper has a better chance to complex with weaker inorganic species, thus forming potentially bioavailable species. This trend was further noted in MINEQL+ modeling, as shown in Figure 8. However, as the NOM content was lowered below 6 mg/L, the experimental results started to deviate greatly from the model results. The resin did not retain all of the inorganic copper present. Further investigation into copper speciation as a function of NOM content indicated that the concentration of  $\text{Cu}(\text{OH})_2$  increases dramatically as the NOM content is lowered. The results of this model indicate that the resin may not be retaining as much as 55% of this complex, even though it has been deemed bioavailable by researchers in the past (Meador 1991).

The low detention time implemented in this research may explain why the resin system did not retain all of the potentially bioavailable species at low NOM and pH values. Sample detention time through the columns was approximately 0.1 seconds. Decreasing the flow rate from 60 mL/min to 10 mL/min increased the detention time to 0.6 seconds. However, this increase in detention time did not effect the fraction of copper sorbed to the resin (data not shown).

Previous researchers have been able to retain close to 100% copper for various waters. Chakrabarti *et al.*, 1994 was able to retain 99% of 133.5 µg/L copper using a batch process with a 1000 second detention time. Samples were collected from the Rideau river, and adjusted to pH 5.0. Miwa *et al.*, 1989 prepared reagent water at pH 7.0, and did not contain any dissolved organic carbon. Using a detention time of 5.1 seconds, approximately 100% of 10 µg/L copper was retained by the Chelex 100<sup>®</sup> resin. Rasmussen *et al.*, 1981 implemented flow rate of 0.1 mL/min until the resin had swollen. Afterwards the flow rate was increased to 1.0 mL/min. A detention time was not given. However, the researchers were able to retain 100% of 1.87 µg/L copper from sea water, adjusted to pH 5.0.

## **6.2: Application of the Chelex 100<sup>®</sup> resin to Natural Waters**

Precision among the replicates in greenhouse agricultural sampling was generally quite high. The average standard deviation for runoff and groundwater triplicate samples for a typical rain event was 6.26 µg/L dissolved copper, with coefficient of variation values typically below 10%. The bioavailable measurements were somewhat more variable on a relative basis, which is to be expected of values calculated by difference. The control bed showed low dissolved copper concentrations of less than 15 µg/L from

the soil and simulated rainwater. Virtually every tomato bed rain event yielded more copper in runoff than groundwater, due to the presence of the plasticulture and copper-soil sorption. Recall that these samples were collected prior to sedimentation, so the difference between plasticulture and plasticulture with sedimentation are due to the natural variability between rain events. Stall (1999) has reported that sedimentation resulted in an approximately 90% decrease in total copper loading, although the changes in dissolved copper loads were relatively small. This research indicated that approximately 12% of dissolved copper from agricultural runoff and groundwater may be available for aquatic toxicity.

Cheadle (1999) found that the estuary mesocosm receiving settled water showed a much lower initial dissolved copper concentration. The higher bioavailable copper concentrations observed in the plasticulture without sedimentation may indicate that desorption or changes in speciation occurred as the low pH, fresh runoff water mixed with the higher pH, saline water in the estuary. Even though sedimentation control can lower the amount of copper present, a significant percent is still bioavailable to organisms. As an example, in eight to ten days larval hard shell clams (*Mercenaria mercenaria*) experience an LC<sub>50</sub> at only 16.4 µg/L (Calabrese *et al.*, 1977). Percent growth compared to controls at this level is only 51.7% for this species (Calabrese *et al.*, 1977). Figure 10 shows that the bioavailable copper concentrations in the mesocosm receiving direct runoff (without sedimentation) often exceeded 50 µg/L. The amount of bioavailable copper found in the estuaries were approximately the same (13.3% with sedimentation control, 11.6% without sedimentation control) as those measurements in the agricultural fields.

### **6.3: Correlation between laboratory Chelex 100<sup>®</sup> results and natural water results**

Agricultural samples with pH values typically ranged from 5.35 to 7.28, yielding an average of 6.66 with a standard deviation of 0.38. Percent bioavailable copper ranged from 3 to 33 % bioavailable copper, yielded an average of 13% bioavailable copper and a standard deviation of 12% bioavailable copper. Preliminary laboratory testing with the Chelex 100<sup>®</sup> resin indicated that approximately 17% of the total dissolved copper should be bioavailable at this average pH (Figure 5), which correlates well with the greenhouse results.

However, two agricultural samples, shown highlighted in Table 3, had significant higher bioavailability measurements. These bioavailable measurements approached 45% of the total dissolved copper concentration. This increase in percent bioavailability was most likely due to the low pH of the samples. As seen in Table 3, the pH was in the range of 4, which was much lower than the mean agricultural sample range. Figure 5 indicated that approximately 40% of the total dissolved copper concentration should be bioavailable at this low pH, again correlating well with the greenhouse results.

The pH of the estuarine samples were higher than the pH of the agricultural samples, but Figures 9 and 10 indicate that there was not a significant difference in percent bioavailability between the agricultural fields and the estuaries. The estuarine samples ranged from pH 7.12 to 8.62, yielding an average of 8.1 with a standard deviation of 0.54. These estuarine samples ranged from 3 to 32% bioavailable copper, yielding an average of 12.5% bioavailable copper with a standard deviation of 8.1. Although the estuarine pH is higher than the agricultural pH, Figure 5 indicated that this

increase from pH 6.6 to 8.1 should not significantly lower % bioavailability. This result is also noted in MINEQL+ modeling in Figure 6, again correlating well to greenhouse results.

Furthermore, Table 3 shows that there was approximately 17 mg/L NOM in the agricultural samples, which was higher than the 12.5 mg/L NOM found in the estuaries. Again, a difference between percent bioavailability was not expected between the two systems. MINEQL+ modeling in Figure 8 indicated that the amount of copper bound to the NOM at pH 8.1 does not increase past a NOM concentration of 11 mg/L.

## **Chapter 7: Conclusions**

The Chelex 100<sup>®</sup> resin has been shown to complex copper consistently under all of the natural conditions as depicted in this research. Changes in salinity and copper concentration did not effect percent bioavailability, whereas decreases in NOM and pH increased percent bioavailability.

Based on the resin results, approximately 15% of copper-based pesticides from agricultural field runoff may be bioavailable to aquatic organisms, as defined by the Chelex 100<sup>®</sup> procedure. The percent of bioavailable copper in runoff was similar to groundwater. In the saline estuary systems, approximately 11% of all estuarine copper may be bioavailable. Although sedimentation has the potential to lower the total concentration of copper, it does not effect the fraction of bioavailable copper. Even after sedimentation copper concentrations may be high enough to cause significant toxicity to aquatic organisms. Further research is necessary to relate Chelex 100<sup>®</sup> resin results to true bioavailability, but the technique appears promising.

## Chapter 8: References

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## VITA

Jeffrey D. Snyder was born in Kingston, NY on September 10<sup>th</sup>, 1975. He spent his childhood in rural Saugerties NY, where he passed his time playing in the Catskill Mountains. After high school he did not want to spend another four years in school, and grudgingly decided to attain an AAS in Biological Technology from SUNY Cobleskill. Soon after arriving at Cobleskill, he realized that there was a vast world beyond Saugerties, NY. After graduating from SUNY Cobleskill in 1995 with high honors, he decided to continue his education through a four year degree in the area of math and science. He chose to use his scientific skills to help the environment, and started a Bachelor of Science in Civil Engineering with a concentration in Environmental Engineering at Clarkson University in Potsdam, New York. While at Clarkson University he made life-long friends that shared his love for the outdoors, and discovered white water kayaking. In 1998 he graduated from Clarkson University with high honors, and chose to again further his education through a Master of Science degree in Environmental Engineering. Having gone through two winters in Potsdam, he turned his eyes to the south. The schools he considered had excellent scholastic reputation, warm weather, and mountain creeks for kayaking. Consequently, he decided to attend Virginia Polytechnic Institute and State University in Blacksburg, Virginia, and attained a Master of Science in Environmental Engineering in 1999. After discovering the world outside of Saugerties, NY, he is now looking forward to implementing his years of schooling towards the environment, visiting the west coast of America, and eventually even further west.

