Title
Bioavailability and Toxicity of Heavy Metals in Urban Runoff in Simulated Natural Conditions

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Bioavailability and Toxicity of Heavy Metals in Urban Runoff in Simulated Natural Conditions

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ABSTRACT:

Copper is so ubiquitous in urban streams that it has been labeled a priority pollutant by the Nationwide Urban Runoff Program. Regulations and monitoring programs that have been developed in response to this problem concentrate on only total or total and dissolved copper concentrations. Our research has focused on measuring the partitioning of copper into its labile and non-labile fractions in urban storm water from the San Francisco Bay Area and its implications to toxicity. We found dissolved copper levels in runoff varied from 5 to 40 μg/L, but labile copper comprised only 10% to 25 % of the total dissolved copper levels. In experiments with runoff spiked with additional copper, we found that labile copper concentrations predicted toxicity better than dissolved copper concentration.

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PROBLEM AND RESEARCH OBJECTIVES

Copper at trace levels is a vital component of biological molecules, including a variety of enzymes and an intermediate in photosynthesis (Stryer, 1988). At higher concentrations, however, copper has been shown to be toxic to algae and bacteria (Tubbing, et al., 1993), zooplankton (Meador, et al., 1993), (Suedel, et. al., 1996), and fish (Borgman, et al., 1983), (Erickson, et al., 1996). Copper is of particular concern in urban environments where industrial processes, metal roofs, and highway runoff can add substantial amounts of copper into urban creeks (Flemming, et al., 1989), (Morrison, et al., 1988). Copper concentrations exceeded the freshwater acute toxicity criteria in 50% of the streams sampled for the Nationwide Urban Runoff Program (Cole, 1984), (Heany, 1986).

In the San Francisco Bay Region, non-point sources are estimated to account for 64 % of the copper entering the San Francisco Bay from Alameda County. Furthermore, copper exceeded water quality objectives at least once for all the streams monitored in Alameda County (Alameda County-Summary Report, 1991).

Measurements of total copper concentration give neither a complete, nor even an accurate assessment of ecological. Copper in natural water can be found in both soluble forms, which includes ionic free copper, inorganic and organic complexes, colloidal forms, and insoluble or particulate copper (Turner, 1990). Ionic (free) copper, Cu^{++}, seems to be the most toxic, with $CuOH^+$, $Cu(OH)_2$, and $Cu_2(OH)_2^{+2}$ also displaying toxicity (Meador, et al., 1993). The acute toxicity range for these inorganic species is between 5.8 and 600 μg/L, depending on the water chemistry (Allen, et al., 1996). Copper-carbonate complexes and copper-organic complexes generally seem to make the copper less available to organisms (Winner, 1985), (Azenha, et al., 1995), though there has been some evidence that some weakly bound copper-organic complexes can also exhibit a toxic response (Borgmann, et al., 1983).

In Alameda County streams, Woodward-Clyde measured total and dissolved, defined as copper species that are able to pass through a 0.45 μm filter, copper. They did not however attempt to further partition the dissolved fraction into biologically available and unavailable fractions.

This parameter measures the amount of ligands available in the system to bind up heavy metals, thereby making the metal unavailable to organisms. Knowing complexing capacity will give us, or a regulator, a better insight into the concentrations of metal that should be toxic.

METHODOLOGY

Study site and sampling

Storm water samples were collected at various sites in the East Bay during several storms during the 1995-1996 and 1996-1997 rainy seasons using acid washed HDPE bottles. Sampling sites were chosen such that they seemed likely to have typical urban stream copper concentrations.

The storm water samples were frozen until they could be filtered. After thawing, an aliquot was taken for alkalinity measurements, then the rest was immediately filtered through 0.45μm nylon filter, which had first been cleaned with methanol and 0.1 M nitric acid. The filtered water was stored at 4 °C until analyzed.

Copper speciation

The particulate copper fraction has been measured two different ways. The first method involves drying and digesting the 0.45 μm filters with warm nitric acid. This digestate was then analyzed using a Perkin Elmer 3100 graphite furnace atomic absorption spectrophotometer (GF-AAS). A second method that has been used involved finding a total copper concentration by digesting an aliquot of the unfiltered storm water sample with concentrated nitric acid, then analyzing with the GF-AAS. The particulate fraction can be then be defined by subtracting the dissolved from the total concentration.

The total dissolved fraction was measured by digesting an aliquot of the 0.45 μm filtered water with concentrated nitric acid and analyzing the digestate with GF-AAS. For any samples that also had a toxicity test, the dissolved fractions were measured using the actual water from the toxicity test.

The labile copper fraction was separated from the sample using BioRad Chelex-100 ion exchange resins in columns. 50 mL aliquots of the filtered sample were run through the chelex columns, and the labile copper was eluted off the column using either 5 mL or 10 mL of 1 N nitric acid. The volume of eluent is determined by how much concentration was desired. The labile copper concentration was quantified with GF-AAS.

Chelating capacity

The chelating capacity was measured on several of the storm water samples. 50 mL aliquots of the filtered samples were spiked in duplicate with increasing amounts of $CuSO_4$. Chelex-100 columns and GF-AAS were used to measure the amount of labile copper. A graph of [labile Cu] vs. [Cu added] was plotted with this data, and the complexation capacity was measured at the point where the data points become a 1:1 slope. The data can also be linearized following Donat's method with [labile Cu]/[Complexed Cu] vs. [labile Cu] (Donat, et al, 1994). [Complexed Cu] is measured by subtracting [labile Cu] from [Total dissolved Cu]. The inverse of the slope is the complexation capacity

Absorbance at 254 nm has been determined to have a rough correlation to DOC concentration (Morrison, et al., 1988). Aliquots of the filtered storm water were analyzed at 254 nm with a Hewlett Packard 8453 UV-Visible spectrophotometer to give relative values of DOC between samples.

Ceriodaphnia dubia toxicity test

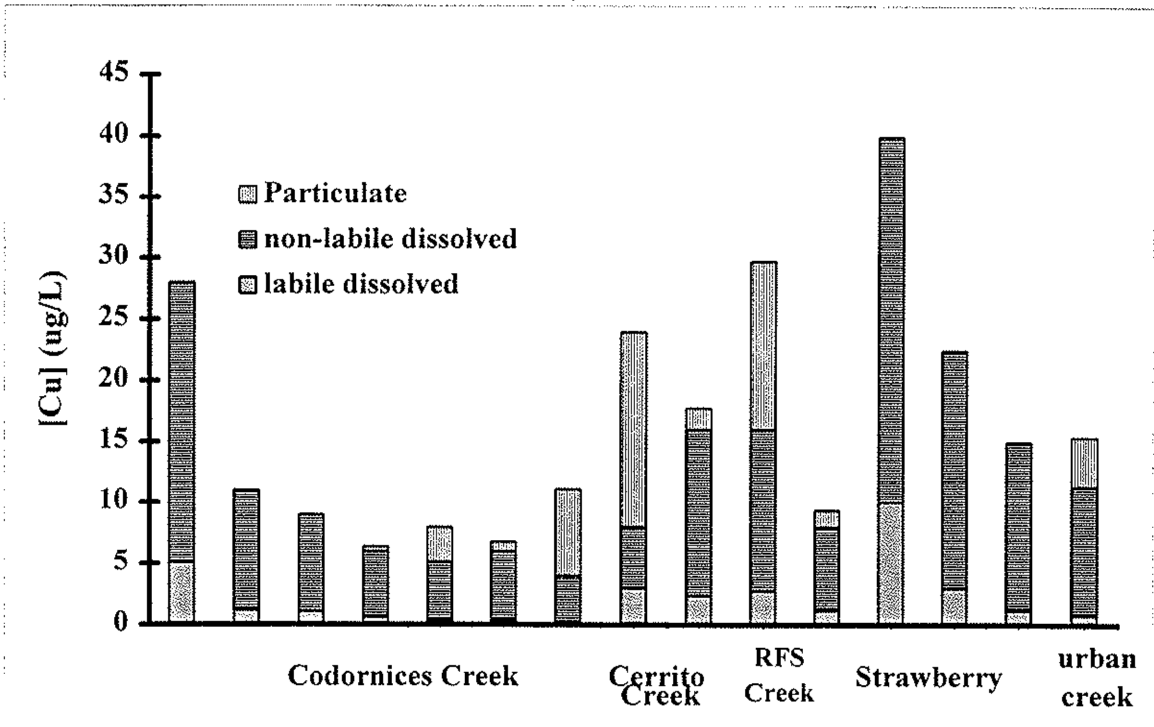
Toxicity tests were performed using the standard 7-day chronic *Ceriodaphnia dubia* toxicity test using the EPA methods (Weber, 1993). The *Ceriodaphnia* were purchased from Aquatic Biosystems. The zooplankton were fed a diet of 100 μL of yeast, trout chow, and cerophyll (YTC) and 100 μL of *Selenastratum capricornutum* per 15 mL of sample. Both the YTC and the *Selenastratum capricornutum* were purchased from Aquatic Biosystems.

PRINCIPAL FINDINGS AND SIGNIFICANCE

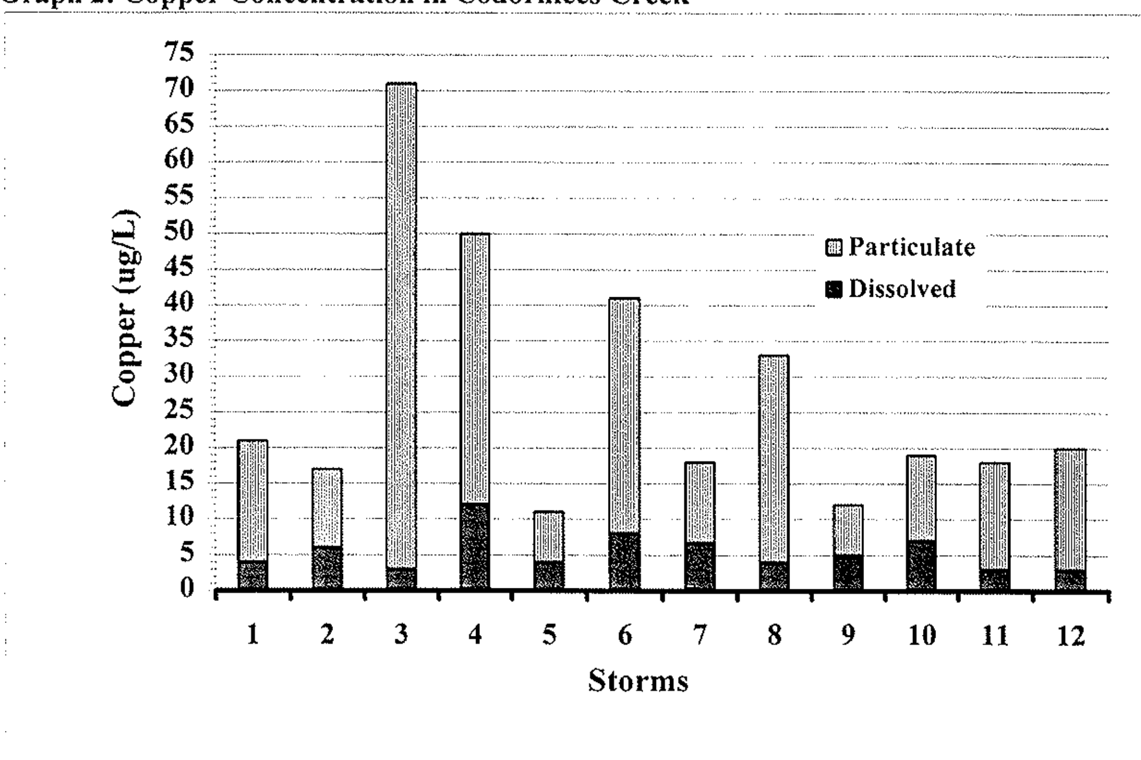
Copper concentrations

Graph 1 shows typical copper levels that we have found in several East Bay creeks. Total dissolved copper concentrations ranged from about 5 μg/L to 40 μg/L. Particulate copper concentrations were measured for several of the runoff samples, and showed no trend with the dissolved copper concentrations. Dissolved copper concentrations measured by our lab are on the whole higher than the dissolved copper concentrations found in Codornices Creek by

Graph 1: Copper Concentrations in East Bay Creeks

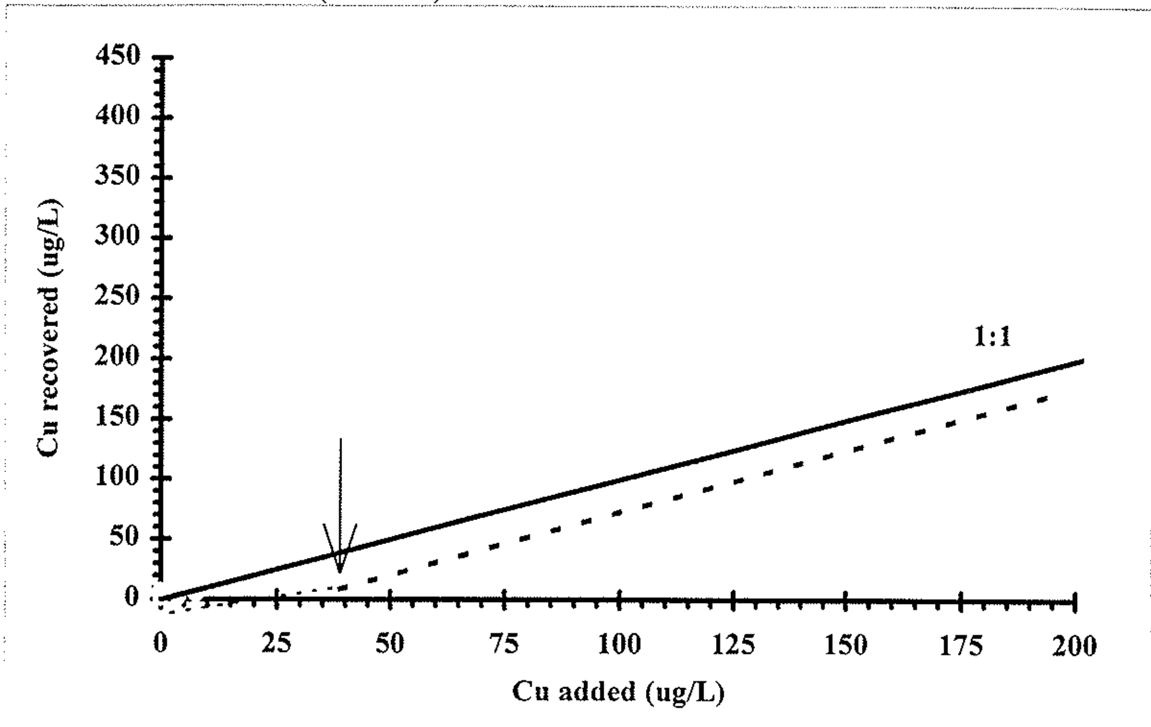


Graph 2: Copper Concentration in Codornices Creek

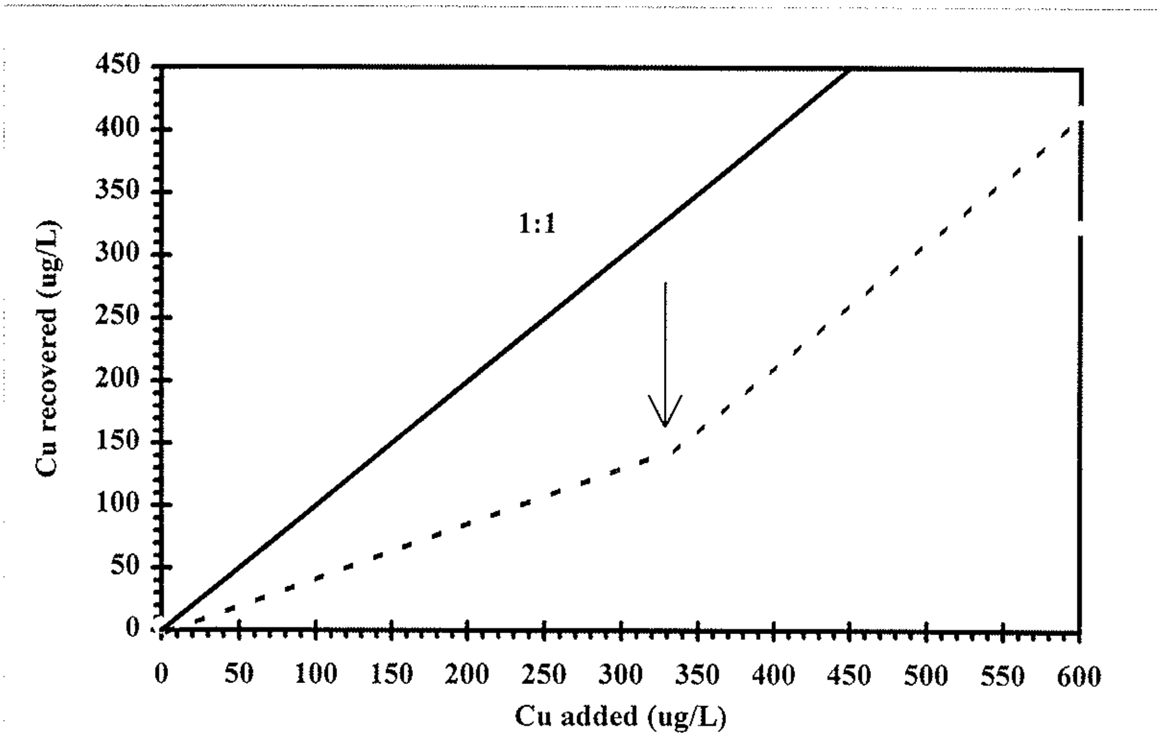


Source: WCC, 1991

Graph 3: Data from complexing capacity experiment on dry weather sample taken from Codornices Creek (10/31/95)



Graph 4: Data from complexing capacity experiment on stormwater sample taken from Codornices Creek 10/31/95.



Woodward-Clyde Consultants seen in Graph 2 . This difference is not unreasonable since our samples are grab samples, whereas the Woodward-Clyde samples were composite samples throughout a storm. Thus, the average concentration of the metal in the water for the composite sample should be lower than a grab sample.

Labile copper ranged from 10% to 25 % of the total dissolved fraction, indicating that the bioavailavble fraction is at least four times lower than would estimated with just dissolved copper.

Chelating Capacity

One predictive measurement of a streams ability to 'de-tox' metals is chelating capacity. In runoff, much of this chelating ability comes from organic molecules that have eroded from stream banks or have washed off from stream surfaces. During storm events, higher flow levels will result in higher chelating capacities. Graphs 3 and 4 show the difference between the chelating capacity for dry weather and wet weather flow for the Codornices Creek on the same day. The sample taken before the rain has a complexation capacity of about 30 - 40 μg/L. This capacity is most likely due to inorganic ligands in the water. Once the rain started, the chelating capacity increased to approximately 300 - 350 ug/L. Runoff sampled from East Bay creeks had chelating capacities ranging from 150 μg/L to 350 μg/L. Samples taken during the dry season gave a maximum chelating capacity of 90 μg/L.

Since it appears that much of the chelating capacity derives from organic ligands, absorbance at 254 nm, a surrogate for dissolved organic carbon, was compared with chelating capacities for samples taken from Codornices Creek. Results were not very enlightening with a logarithmic regression giving an R^2 of only 0.24. Absorbance at 254 nm is a non-specific measure of DOC, thus will not distinguish between different organic ligands. One might suspect that each type of organic ligand would affect the chelating capacity differently, thus a non-specific DOC measurement would not be the best surrogate measurement of chelating capacity.

Ceriodaphnia dubia toxicity

In measuring toxicity of the runoff samples with the 7 day *Ceriodaphnia dubia* toxicity test, the question arose whether the zooplankton food was influencing our results by chelating the labile copper. Several experiments were done which showed that even though zooplankton food does chelate labile metal, the chelating capacity of the food is much lower than the chelating capacity of the storm water (Refer to Appendix A).

Most of the storm water samples collected by this lab did not exhibit *Ceriodaphnia dubia* toxicity. However, there were samples taken from Strawberry Creek, Cerrito Creek, and the Richmond Field Station creek that were toxic. We then added EDTA to distinguish metal toxicity from non-metal toxicity. EDTA will chelate any free metals present, thus would remove metal toxicity without removing toxicity from organic molecules.

Of the toxic runoff samples, only a Strawberry Creek sample taken on 10/29/96, clearly showed acute metal toxicity. This sample had the highest concentration of both total and labile copper, 40 μg/L and 10 μg/L, respectively, of any runoff sample analyzed.

Since most of the runoff samples did not exhibit toxicity, we spiked copper into the runoff in order to get copper toxicity. An example of such an experiment is one done on a sample taken from the Richmond Field Station creek on 5/15/96. Toxicity and chemistry measurements were made on the raw water and the water spiked at nominal concentrations of 40 μg/L and 80 μg/L. Total dissolved copper concentrations increased from 8 μg/L, to 43 μg/L, to

75 µg/L with each addition of copper. The labile copper concentration increased only from 1 µg/L to 4 µg/L for the spiked samples. A 100% recovery of the copper wasn't achieved due to losses of copper during the toxicity test itself. All three samples exhibited chronic toxicity (measured as number of young per zooplankton) with the raw water having a slightly higher level of reproduction. The two spiked samples had very different total dissolved copper concentrations, yet had similar toxicities and similar labile copper concentrations.

Conclusions

From a monitoring perspective, our results have shown that most East Bay runoff does not exhibit toxicity, however examples of metal toxicity can be found. We have also shown that the labile fraction of copper is 10 - 25 % lower than the dissolved fraction. Relatively high chelating capacities in the creeks during runoff events helps to minimize the amount of labile copper in the creeks.

Differences between creeks and storm events makes toxicity predictions difficult for any given copper concentration, however our spiked samples show that toxicity seems to be driven by the labile dissolved copper concentrations, rather than total concentrations.

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APPENDIX A

Since the *Ceriodaphnia dubia* test was introduced in the mid 1980's, there has been much research done on what food best meets the nutritional needs of the zooplankton (Patteron, et al., 1992), (Winner, 1989). However, there has been little research done on how that food influences the toxicity of the pollutant of interest.

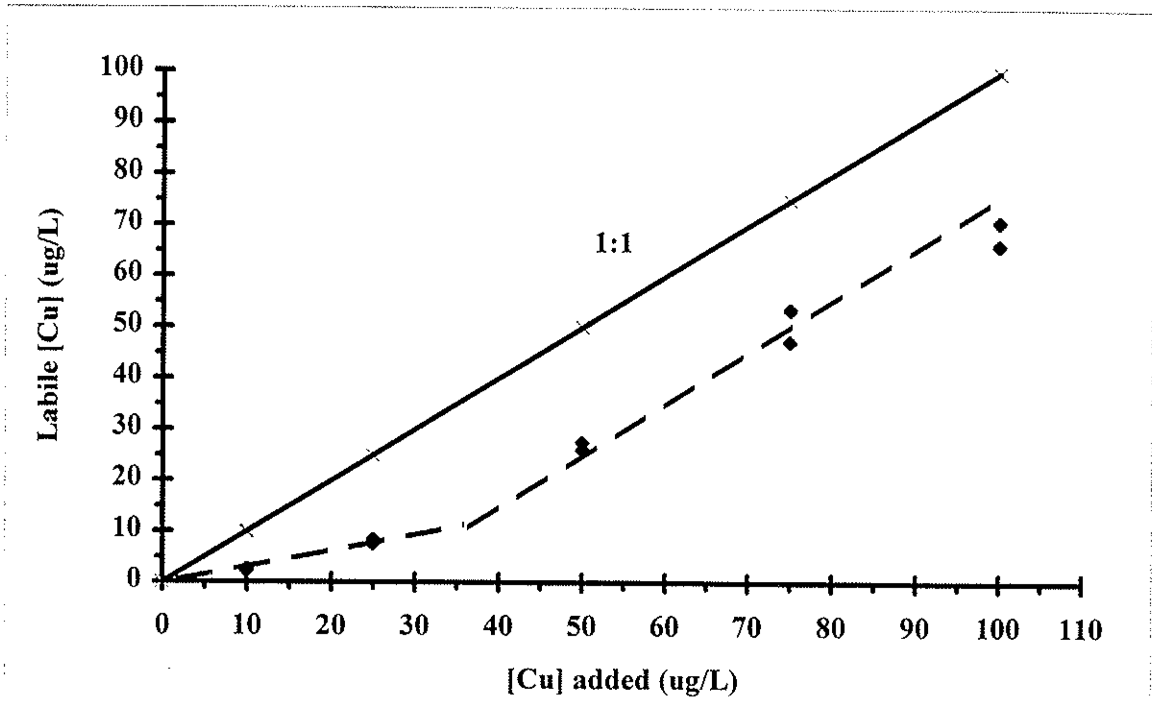
One of the few studies of metal-food interactions that exists is for another zooplankton, *Daphnia* magna. The researchers varied both cadmiuum and food levels for a *Dapnia magna* test (Klüttgan, et al., 1994). These researchers concluded that varying the food concentration influenced the nutritional state of the *Dapnia*, but did not change the toxic fraction of the cadmium. However, the researcher did not do any speciation chemistry on the cadmium, rather they may their conclusions solely on the toxicity data. Further, the *Daphnia* food used in this test was only the algae, *Chlorella saccharaphilia*, while the EPA recommeded food for the *Ceriodaphnia dubia* toxicity test includes both a green algae, *Selenastratum capricornutum*, and an organic mixture of yeast, trout chow, and cerophyll (YTC). It is this organic mixture that particularly raises the concern that the food will bind to the copper redering it less toxic.

Initial experiments done in our lab using the same metal speciation methods used for the stormwater research showed that the *Ceriodaphnia* food has a chelating capacity of around 20 - 30 μg/L (Graph 5). Further experiments were done using culture water spiked with nominal copper concentrations of 50 and 100 μg/L in parallel with toxicity tests. From graphs 6 and 7, one can see that labile copper is less than total copper even before food is added, probably from interactions with inorganic ligands in the culture water. This difference is consistent with what other researchers have found using anodic stripping voltammetry (Deaver, et al, 1996). Adding food further reduces the amount of labile copper. At normal food concentrations, the amount of labile copper is approximately 20% of the nominal total copper concentration. The total copper concentration for each of the food concentrations is essentially constant. There is a difference between the total and nominal copper concentrations. The loss is most likeley from precipitation with inorganic ligands, and interactions with the sample cup walls.

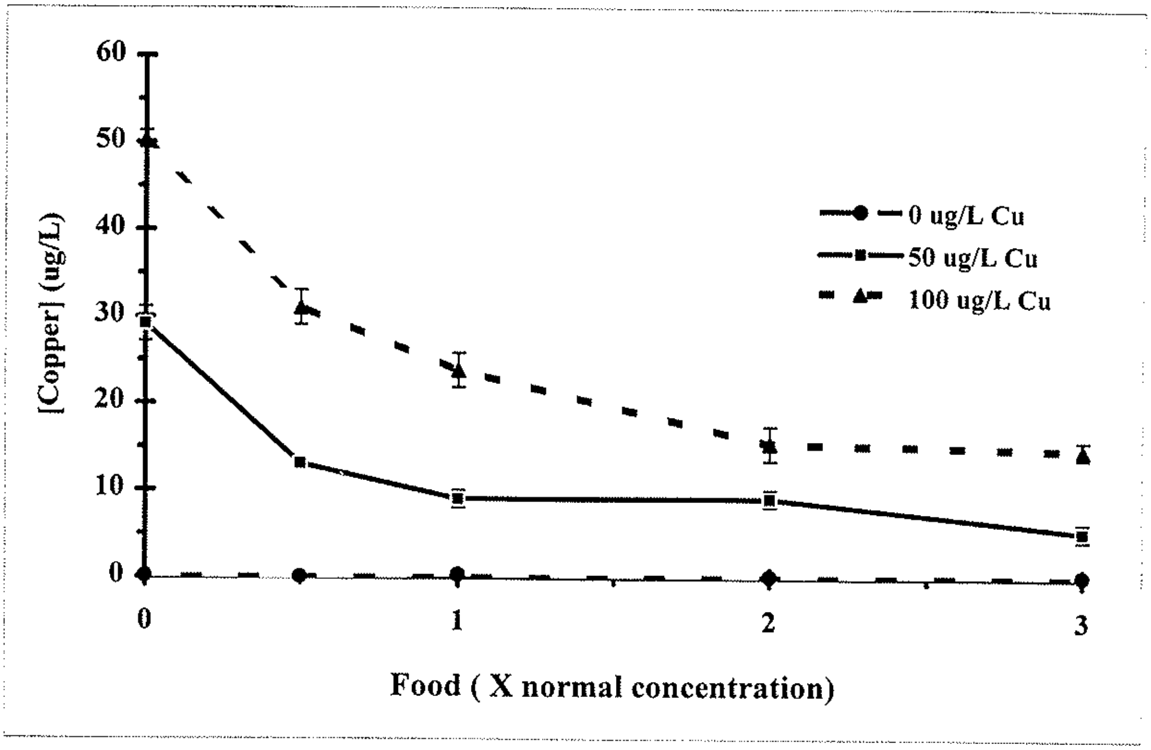
Graph 8 and Graph 9 show the results of one of the toxicity tests done in parallel with with the previous chemistry data. The sensitivity of the *Ceriodpaphnia dubia* to copper varys somewhat between tests, but the general trends seen in this test are true for all of our experiments. Both graphs show that toxicity (acute and chronic) is reduced for 50 and 100 μg/L with increasing concentrations of food.

Our findings then do confirm our suspicions that the *Ceriodaphnia dubia* food does bind with the labile copper. However, the chelating capacity of the food is only approimately 25 - 35 μg/L range, which is about one order of magnitude lower than the chelating capacity of stormwater in this area. Therefore, using the EPA protocols for our stormwater toxicity tests should not affect results.

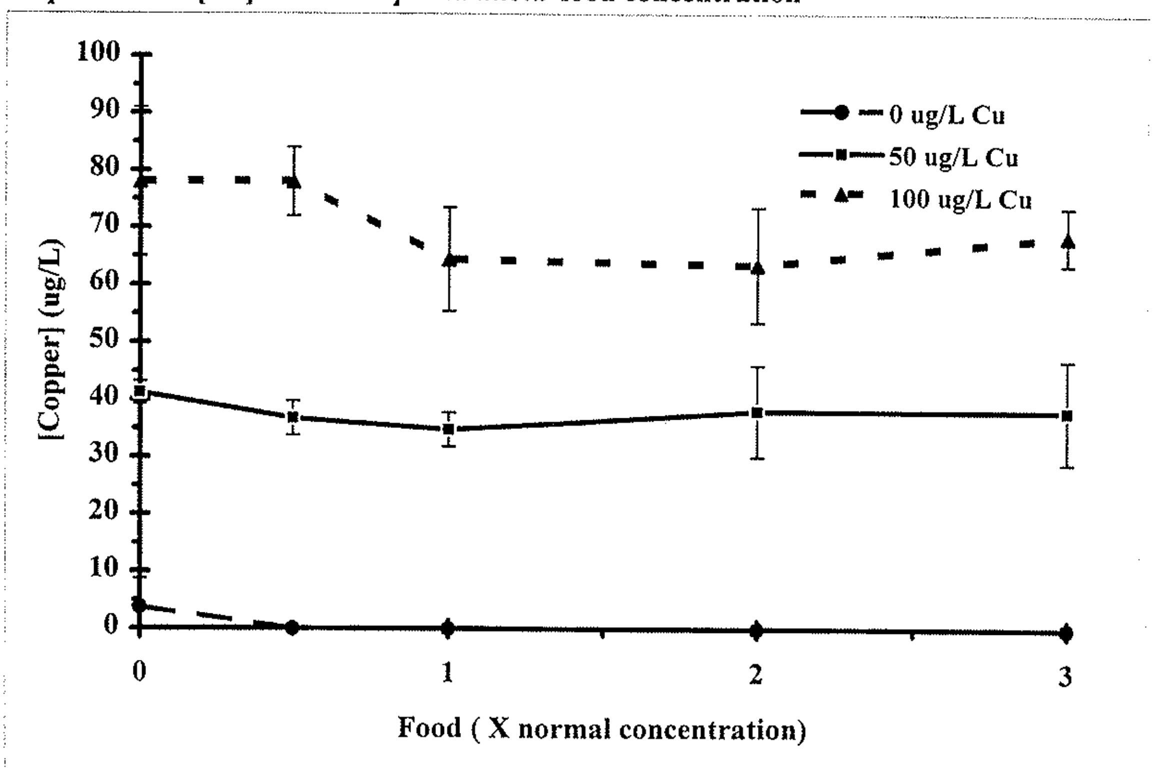
Graph 5: Chelating Capacity of *Ceriodaphnia dubia* food.



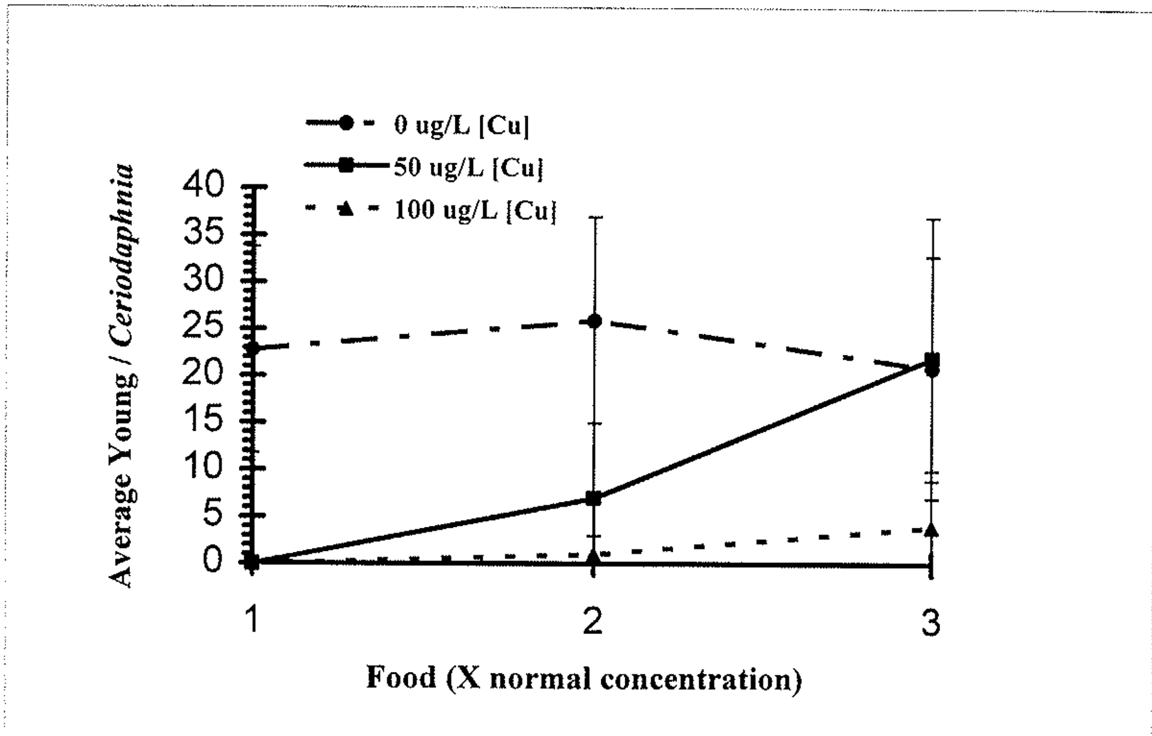
Graph 6: Labile [Cu] vs *Ceriodapnia dubia* food concentration



Graph 7: Total [Cu] vs *Ceriodaphnia dubia* food concentration



Graph 8: Chronic mortality (measured by average young) vs nominal [Cu] and food concentration



Graph 9: Acute mortality vs nominal [Cu] and food concentration