

Consideration of Iron-Organic Matter Interactions when Predicting Aquatic Toxicity of Copper in Mineralized Areas¹

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ABSTRACT

Chemical speciation of dissolved metals influences their potential toxicity to aquatic biota. The biotic ligand model (BLM) is a computational approach to predict metal speciation and acute metal toxicity to aquatic biota. In predicting the toxicity of copper, the BLM takes into account the concentration of dissolved organic matter (DOM), which is known to bind copper and hence reduce toxicity in aquatic systems. We investigated the ability of DOM isolated from an iron- and aluminum-rich source to reduce acute copper toxicity to *Ceriodaphnia dubia* (water flea). We conducted acute toxicity tests in water amended with DOM that was isolated from (1) a stream receiving acidic iron- and aluminum-rich drainage, (2) an adjacent stream not impacted by iron- and aluminum-rich drainage, and (3) IHSS Suwannee River fulvic acid. We found that the DOM isolated from the stream water receiving acidic iron- and aluminum-rich drainage was 3 times less effective at reducing copper toxicity than was DOM isolated from the two non-impacted sources. In contrast, organic matter isolated from suspended sediment in the iron- and aluminum-rich stream was 1.5 times more effective at reducing copper toxicity than was DOM isolated from the two non-impacted sources. These findings demonstrate that fractionation of organic matter between the dissolved and sediment phases in iron- and aluminum-rich mineralized areas can result in more bioavailable dissolved copper and greater potential for copper toxicity to aquatic biota.

Additional Key Words: biotic ligand model, dissolved organic matter, DOM, dissolved organic carbon, DOC, fulvic acid, metal toxicity, bioavailability

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INTRODUCTION

Dissolved metals in surface water occur in many different chemical forms, including free ions and inorganic and organic complexes. This chemical speciation of metals influences their toxicological effects on aquatic organisms, with free-metal ions typically being the most bioavailable form. The chemical composition of water originating from mineralized areas can be quite different from that of water originating from non-mineralized areas (Smith, 2005; Smith et al., 2006). For example, water originating from areas underlain by mineralized rock commonly has lower pH and alkalinity values, higher sulfate concentrations, and higher dissolved iron and aluminum concentrations than water originating from non-mineralized areas. Several of these water constituents and parameters can affect metal toxicity to aquatic biota. For example, calcium and magnesium concentrations (principal components of a water's hardness) are known to mediate metal toxicity to aquatic biota (Pagenkopf, 1983), which is the principle behind hardness-based water-quality criteria for metals. Commonly the dissolved iron and aluminum present in mineralized water precipitate as hydrous oxides as pH increases at confluences with less acidic water.

The presence of dissolved organic matter (DOM) in stream water tends to reduce metal toxicity to aquatic biota (Playle et al., 1993). DOM consists of a mixture of biomolecules that have a broad spectrum of metal-binding sites and metal-binding intensities. Models of metal-organic-matter binding attempt to address this continuous distribution of metal-organic-matter interactions by taking into account factors such as site heterogeneity, electrostatic effects, and ion-competition effects (Tipping, 1998).

McKnight et al. (1992, 2002) demonstrated that DOM fractionates in the presence of precipitating iron and aluminum oxides. They found that some DOM is removed from solution by sorption onto precipitating hydrous iron and aluminum oxides, and this DOM sorption results in chemical fractionation of the organic matter. Fractionation occurs because molecules with greater contents of aromatic moieties, carboxylic acid groups, and amino acid residues (which have the greatest metal-binding capacity) are preferentially sorbed onto the hydrous iron and aluminum oxides. As a result, the remaining DOM in the water is depleted in the constituents that were preferentially sorbed onto the sediment.

The biotic ligand model (BLM) is a computer model that mathematically estimates the effects of water chemistry on the speciation of metals and their acute toxicity to aquatic biota (Di Toro et al., 2001; Santore et al., 2001; Niyogi and Wood, 2004; Slaveykova and Wilkinson, 2005). It is being used to develop site-specific water-quality criteria (WQC) and to assess aquatic risk for metal exposure. The BLM has been incorporated into the aquatic life ambient freshwater quality criteria for copper (U.S. Environmental Protection Agency, 2007), and is being used to determine regulatory site-specific WQC for copper. Given select parameters of site-specific water chemistry, a chosen metal, and a chosen organism, the BLM predicts the LC_{50} for the chosen metal and organism. The LC_{50} is the metal concentration that is lethal to 50% of a group of test organisms within a given timeframe (48 to 96 hours). The accuracy of BLM toxicity predictions generally are within a factor of two of measured LC_{50} values (Allen, 2002). The BLM can perform calculations for several combinations of metals and aquatic organisms, one pair at a time. The BLM applies to dissolved systems only, assumes

equilibrium, and computes speciation of the chosen metal between its various inorganic and organic complexes, and with a “biotic ligand” (e.g., gill surface) that is specific to the chosen organism. The required BLM input includes pH, temperature, and concentrations of calcium, magnesium, sodium, potassium, sulfate, chloride, alkalinity, dissolved organic carbon (DOC), and the metal of interest. The BLM does not currently take into account competition between trace metals, nor does it allow input of multiple metals. The BLM uses the Windermere Humic Aqueous Model (WHAM) of Tipping (1994) to determine metal binding with DOC.

In this study we investigated the ability of fulvic acid (FA) isolated from different sources to decrease acute copper toxicity to *Ceriodaphnia dubia* (water flea). We conducted toxicity tests in water amended with FA that was isolated from (1) a stream receiving acidic iron- and aluminum-rich drainage, (2) an adjacent stream not impacted by iron- and aluminum-rich drainage, and (3) IHSS Suwannee River fulvic acid. This study builds on previous studies in which we observed that the presence of dissolved iron decreased the protective ability of DOM on metal toxicity to aquatic biota (Smith et al., 2006).

MATERIALS AND METHODS

Fulvic acid sources and isolation

In this study, we used samples of FA isolated by McKnight et al. (1992). The FA samples were isolated from filtered stream water below the confluence of an acidic, metal-enriched stream (Snake River, Colorado) with a pristine stream, and from the pristine stream (Deer Creek, Colorado) (Fig. 1). McKnight et al. (1992) followed a FA-isolation procedure using XAD-8 resin columns (Thurman and Malcolm, 1981; Aiken 1985). More details of the isolation procedure can be found in McKnight et al. (1992).

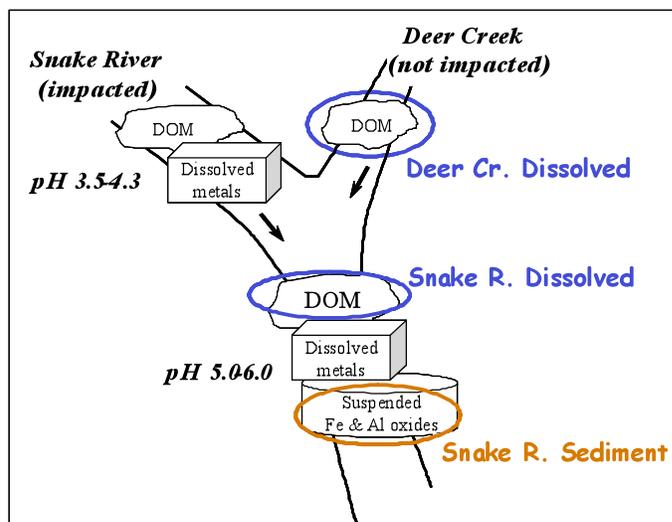


Figure 1. Schematic of the organic matter sampling locations above and in the mixing zone at the confluence of the Snake River with Deer Creek near Montezuma in Summit County, Colorado (modified from McKnight et al., 1992).

In this study, we also used FA isolated by McKnight et al. (1992) from Snake River suspended sediment collected below the confluence with Deer Creek (fig. 1). McKnight et al. (1992) isolated the sediment organic matter by (1) filtering stream water through 1 μm and 0.3 μm Balston filters in sequence (filtrate used for isolation of dissolved FA), (2) removing the flocculent hydrous metal oxide from the filters by dissolution in 0.1 N HCl, and (3) following the previously described XAD-8 resin column DOM-isolation procedure (Thurman and Malcolm, 1981; Aiken 1985). All of the isolated FA was saturated with H^+ by cation exchange and freeze-dried. Suwannee River reference FA (SRFA; International Humic Substances Society (IHSS), St. Paul, MN) also was used in this study.

Analytical methods

Water samples were analyzed for inorganic elements using inductively coupled plasma-mass spectrometry (ICP-MS) (Lamothe et al., 2002). DOC was analyzed by a UV-catalyzed persulfate oxidation method using a Sievers TOC 800 Turbo instrument on samples acidified to $\text{pH} < 4$ with phosphoric acid.

The biotic ligand model

The BLM is available for download at <http://www.epa.gov/waterscience/criteria/copper/2007/index.htm> and at http://www.hydroqual.com/wr_blm.html. In this study, we used the BLM version 2.1.2 to obtain predicted LC_{50} values for copper and *C. dubia*. The recommended (default) value of 10% humic acid content of the DOC was used. The predicted LC_{50} values in this report were computed using measured values of constituents in the toxicity test-water solutions.

Toxicity tests

The U.S. Environmental Protection Agency standard operating procedure was used for toxicity tests with *C. dubia* (water flea), a sensitive fresh-water invertebrate commonly used in toxicity testing of contaminants (U.S. Environmental Protection Agency, 2002). This procedure involves a 48-hour static test during which time the samples are held at $20^\circ\text{C} \pm 1^\circ\text{C}$. Each day of the test consists of 16 hours of light and 8 hours of dark to simulate a diurnal cycle. To prepare test waters for toxicity tests, the various sources of organic matter were added to laboratory-prepared moderately hard reconstituted water (see U.S. Environmental Protection Agency, 2002; table 1). For each set of FA samples, copper was added to obtain a range of five copper concentrations. The organic matter/copper test waters were allowed to equilibrate for at least 24 hours before test organisms were added. This equilibration was done to allow time for copper to bind to the organic matter. Multiple tests were performed with variable copper concentrations in order to bracket the LC_{50} . Five test organisms were used per test chamber, and each of the organisms was less than 24 hours old, cultured in moderately hard reconstituted water, and fed for two hours prior to transfer into the test chambers. After 48 hours, each test chamber was examined to determine the mortality of the test organisms. The reported value is the lethal concentration for 50% of the test organisms (LC_{50}), calculated by Probit Analysis version 1.5 (available for download at

<http://www.epa.gov/nerleerd/stat2.htm>). The results of the test were considered valid if 90% of the organisms in the negative control (no added copper) survived.

Table 1. Composition of test waters used in laboratory toxicity tests. [Alk=alkalinity as mg/L CaCO₃, which was determined for only one sample]

Test Water	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	Cl mg/L	SO ₄ mg/L	DOC mg/L	Alk mg/L	pH
No added DOC	12.9	10.4	23.2	2.1	1.9	78.0	0.3*	45	8.0
Deer Cr dissolved	15.0	13.4	29.4	2.4	2.1	93.4	3.1	45	7.9
Suwannee R fulvic acid	14.9	13.5	29.8	2.3	2.1	94.1	3.2	45	8.0
Snake R dissolved	14.6	13.5	29.9	2.4	2.2	93.4	3.0	45	8.0
Snake R sediment	15.0	14.6	33.3	2.9	2.7	100.8	3.0	45	8.0

* Background DOC is near the instrument detection limit and is unlikely to behave like FA with respect to metal binding.

RESULTS AND DISCUSSION

Copper toxicity tests were conducted using *C. dubia* in water amended with FAs from different sources. The DOM used in this study was isolated from (1) the dissolved fraction of a stream receiving acidic iron- and aluminum-rich drainage (Snake R. dissolved), (2) the sediment fraction of a stream receiving acidic iron- and aluminum-rich drainage (Snake R. sediment), (3) an adjacent stream not impacted by iron- and aluminum-rich drainage (Deer Cr. dissolved), and (4) IHSS Suwannee River fulvic acid reference material.

Table 2 lists the LC₅₀ values measured in the laboratory toxicity tests and predicted LC₅₀ values computed using the BLM. Although the test waters had nearly identical DOC values (3 mg/L) and pH values (pH 8), there are significant differences in measured LC₅₀ values for the different sources of organic matter. The measured LC₅₀ value for tests waters with no added organic matter is low (4.5 µg Cu/L), which means that an estimated concentration of 4.5 µg Cu/L would be lethal to 50% of the test organisms. Measured LC₅₀ values for test waters with added FA are higher (14 to 62 µg Cu/L), which demonstrates the expected protective effect of DOM on copper toxicity. Measured LC₅₀ values for the two waters containing FA from non-impacted sources (Deer Cr. dissolved and Suwannee R. fulvic acid) are the same (40 µg Cu/L). However, measured LC₅₀ values for the two different sources of FA from the Snake R. are significantly different. For the dissolved fraction the LC₅₀ is 14 µg Cu/L, whereas the LC₅₀ value for the sediment-derived fraction is 62 µg Cu/L. Figure 2 shows the toxicity results for the Snake R. dissolved and sediment fractions.

Table 2. Results from copper toxicity tests using *C. dubia*, biotic ligand model computation of LC₅₀ values (BLM predictions), and selected chemical parameters for test-water compositions.

Test Water	Predicted (BLM) LC ₅₀ (µg Cu/L)	Measured LC ₅₀ (µg Cu/L)	Measured DOC (mg C/L)	Measured pH
No added DOC	7.1*	4.5	0.3**	8.0
Suwannee R fulvic acid	70	40	3.2	8.0
Deer Cr dissolved	66	40	3.1	7.9
Snake R dissolved	65	14	3.0	8.0
Snake R sediment	68	62	3.0	8.0

* When BLM predictions are performed for 0.1 mg C/L, the predicted LC₅₀ is 2.8.

** Background DOC is near the instrument detection limit and is unlikely to behave like FA with respect to metal binding.

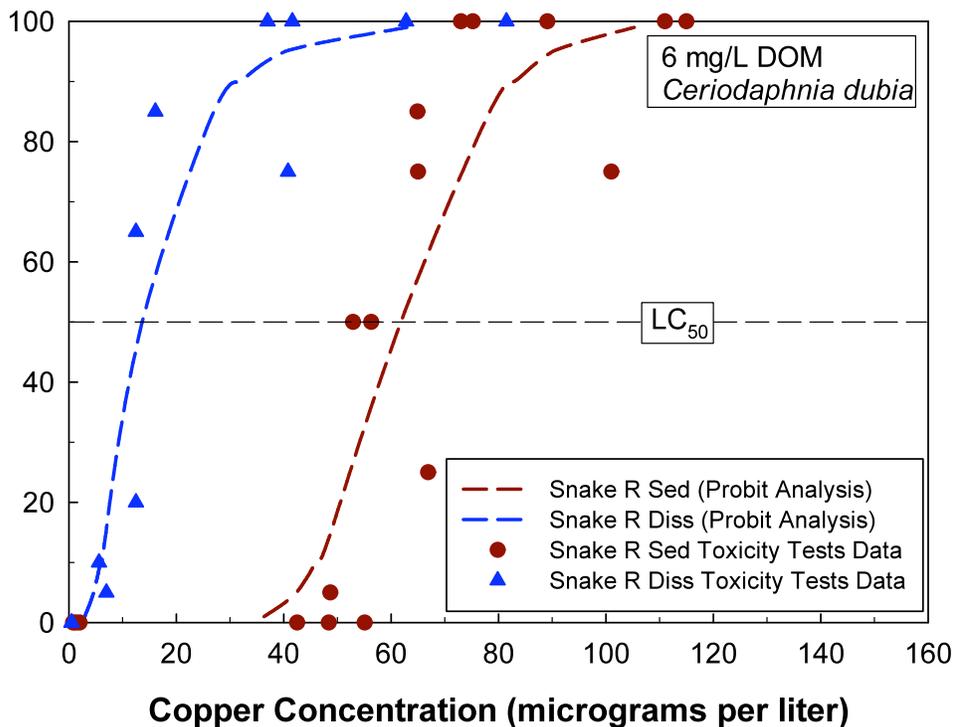


Figure 2. Dose-response results for copper acute toxicity tests using *C. dubia* in water amended with 6 mg/L of different fractions of fulvic acid isolated from water (blue) and suspended sediment (red) collected from the Snake River, Colorado. Note that there is more than four times greater toxicity measured in the test solution containing the DOM fraction compared with the test solution containing the sediment organic matter fraction.

Based on measured LC₅₀ values (Table 2), our results show that FA isolated from the stream water receiving acidic iron- and aluminum-rich drainage (Snake R. dissolved) is 3 times less effective at reducing copper toxicity than was FA isolated from non-impacted sources (Deer Cr. and Suwannee R.). In contrast, FA isolated from suspended sediment in the iron- and aluminum-rich stream (Snake R. sediment) is 1.5 times more effective at reducing copper toxicity than was FA isolated from the two non-impacted sources. These results imply that FA with greater affinity for metal binding tends to be preferentially sorbed to the sediment phases in iron- and aluminum-rich systems. This is consistent with the finding of McKnight et al. (1992, 2002) that the Snake River sediment-derived fraction contains organic molecules with greater contents of aromatic moieties, carboxylic acid groups, and amino acid residues, whereas the DOM remaining in the stream water is depleted in these constituents. These findings demonstrate that chemical fractionation of organic matter in iron- and aluminum-rich systems can result in DOM with reduced metal-binding capacity, which results in more bioavailable dissolved copper and greater potential for copper toxicity to aquatic organisms. Our results highlight the importance of considering the potential effects of iron and aluminum when attempting to anticipate potential toxic effects of metals in mineralized areas.

Some fairly simple measurements may be useful to determine if DOM from a particular location is chemically fractionated. Specific UV absorbance (SUVA) has been used to determine the degree of fractionation of organic matter in stream water. Weishaar et al. (2003) report that SUVA determined at 254 nm is strongly correlated with percent aromaticity in organic matter. Schwartz et al. (2004) report that optically darker, more allochthonous organic matter binds copper and lead better than optically lighter, more autochthonous-like organic matter. De Schampelaere et al. (2004) found that UV absorbance at 350 nm is a good measure of biologically relevant differences in the copper complexing properties of DOM, and provide guidance about how this information can be incorporated into the BLM.

Also listed in Table 2 is predicted LC₅₀ values computed using the BLM. Default values for FA complexation constants and binding site densities were used in our BLM computations. Comparison of the BLM-predicted LC₅₀ values with the measured values shows that the BLM-predicted values do not vary significantly for the different toxicity test-water solutions. This is because the BLM does not currently take the effects of iron and aluminum into account in its computations. The recent revision to the aquatic life ambient freshwater quality criteria for copper (U.S. Environmental Protection Agency, 2007) discusses this issue, but does not require users to monitor iron or aluminum or to include iron and aluminum effects in BLM simulations. It is likely that a modified version of the BLM that incorporates the effects of iron and aluminum will need to be used to compute site-specific WQC and potential metal toxicity in many mineralized areas.

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