PREDICTING TOXIC EFFECTS OF COPPER ON AQUATIC BIOTA IN MINERALIZED AREAS BY USING THE BIOTIC LIGAND MODEL[1]

Kathleen S. Smith[2], James F. Ranville, Marti K. Adams, LaDonna M. Choate, Stanley E. Church, David L. Fey, Richard B. Wanty, and James G. Crock

Abstract. The chemical speciation of metals influences their biological effects. The Biotic Ligand Model (BLM) is a computational approach to predict chemical speciation and acute toxicological effects of metals on aquatic biota. Recently, the U.S. Environmental Protection Agency incorporated the BLM into their regulatory water-quality criteria for copper. Results from three different laboratory copper toxicity tests were compared with BLM predictions for simulated test-waters. This was done to evaluate the ability of the BLM to accurately predict the effects of hardness and concentrations of dissolved organic carbon (DOC) and iron on aquatic toxicity. In addition, we evaluated whether the BLM and the three toxicity tests provide consistent results. Comparison of BLM predictions with two types of C*eriodaphnia dubia* toxicity tests shows that there is fairly good agreement between predicted LC_{50} values computed by the BLM and LC_{50} values determined from the two toxicity tests. Specifically, the effect of increasing calcium concentration (and hardness) on copper toxicity appears to be minimal. Also, there is fairly good agreement between the BLM and the two toxicity tests for test solutions containing elevated DOC, for which the LC_{50} is 3-to-5 times greater (less toxic) than the LC_{50} for the lower-DOC test water. This illustrates the protective effects of DOC on copper toxicity and demonstrates the ability of the BLM to predict these protective effects. In contrast, for test solutions with added iron there is a decrease in LC_{50} values (increase in toxicity) in results from the two *C. dubia* toxicity tests, and the agreement between BLM LC_{50} predictions and results from these toxicity tests is poor. The inability of the BLM to account for competitive iron binding to DOC or DOC fractionation may be a significant shortcoming of the BLM for predicting site-specific water-quality criteria in streams affected by iron-rich acidic drainage in mined and mineralized areas.

Additional Key Words: bioavailability, toxicological testing, C*eriodaphnia dubia*, ecological risk assessment, water chemistry, dissolved organic carbon, DOC, calcium, iron, metals, water-quality criteria, hardness

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[2] Kathleen S. Smith, Research Geologist, U.S. Geological Survey, Box 25046, M.S. 964, Denver Federal Center, Denver, CO 80225, James F. Ranville, Associate Professor, Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO 80401, Marti K. Adams is a graduate student at the Colorado School of Mines; LaDonna M. Choate, Stanley E. Church, David L. Fey, Richard B. Wanty, and James G. Crock are all employed by the U.S. Geological Survey in Denver, CO.

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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. Also, there is a general relationship between the chemical composition of water and the mineralogical composition and weathering characteristics of the rocks with which the water is in contact (i.e., the underlying geology). For example, water collected from watersheds underlain by sedimentary rock tends to have higher mean pH, alkalinity, and Ca concentrations than water collected from watersheds underlain by igneous rock. In addition, the chemical composition of water originating from mineralized areas can be quite different from that of water originating from non-mineralized areas. Water originating from areas underlain by mineralized rock commonly has lower pH and alkalinity values, and has higher SO_4^{-2} concentrations resulting from the chemical breakdown and oxidation of sulfide minerals (Smith, 2005). Several of these water constituents and parameters can affect metal toxicity to aquatic biota. For example, Ca and Mg concentrations (hardness) are known to mediate metal toxicity to aquatic biota (Pagenkopf, 1983), which is the principle behind the currently used hardness-based water-quality criteria for metals.

The Biotic Ligand Model (BLM) is a computer model that mathematically estimates the effects of water chemistry on the speciation of metals and on their acute toxicity to aquatic biota (Di Toro et al., 2001; Santore et al., 2001; Gorsuch et al., 2002; Niyogi and Wood, 2004; Slaveykova and Wilkinson, 2005; Villavicencio et al., 2005). It is being used to develop site-specific water-quality criteria and to assess aquatic risk for metal exposure. The BLM has been incorporated into the 2003 draft update of ambient water-quality criteria for Cu (U.S. Environmental Protection Agency, 2003), and is being used to determine regulatory site-specific concentration criteria for Cu. Given 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 results in the death of 50% of a group of test organisms. 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 speciates 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 concentrations of Ca, Mg, Na, K, SO_4^{-2}, Cl^-, alkalinity, dissolved organic carbon (DOC), pH, and the metal of interest. The BLM does not take into account competition between trace metals. Because DOC can strongly bind many metals, higher DOC concentrations tend to reduce metal toxicity. The BLM uses the Windermere Humic Aqueous Model (WHAM) of Tipping (1994) to determine metal binding with DOC.

In this study, we compared results from laboratory toxicity tests with BLM predictions for different water types. We prepared two test-water compositions based on stream-water samples associated with specific types of underlying geology that are being collected in Colorado by the U.S. Geological Survey (USGS) as a part of the Central Colorado Assessment Project. One simulated test-water type is based upon stream-water samples collected from Colorado stream-drainage basins underlain by plutonic rock (intrusive igneous rocks), and the second is based upon samples collected from basins underlain by various sedimentary rocks associated with the Maroon and Morrison formations. In order to examine toxicity in water influenced by mineralization, we also simulated a Colorado water collected from a mineralized area. These test-water solutions were used in three different laboratory toxicity tests to examine Cu toxicity to a fresh-water invertebrate (*Ceriodaphnia dubia*, a water flea) and a bacterium (*Escherichia coli*). We varied concentrations of some water constituents, including Ca, DOC, and Fe. The Ca was used to examine the effects of water hardness on Cu toxicity, DOC to examine its ability to bind Cu and thereby reduce Cu toxicity, and Fe because of its strong affinity for DOC, which can decrease Cu binding with DOC. Predicted LC_{50} values computed by the BLM are compared with measured LC_{50} values from the three laboratory toxicity tests. Therefore, we evaluate the ability of the BLM to accurately predict the effects of hardness and concentrations of DOC and Fe on aquatic toxicity. Finally, we determine whether the BLM and the three toxicity tests provide consistent results.

Methods

Preparation of Simulated Test Water

In order to test the influence of hardness, DOC, and Fe on the toxicity of Cu to aquatic organisms, a series of simulated test-water compositions was prepared by diluting a high-DOC (approximately 7 mg C/L) stream-water sample and adding laboratory reagents to obtain the desired water compositions for toxicity tests. Two levels of dilution with deionized water were computed in order to prepare two solutions containing approximately 2 and 5 mg C/L, respectively. These DOC concentrations represent the range of DOC measured in several Colorado stream waters (USGS, unpublished results). Using these solutions, two water types were prepared based on constituents measured in stream-water samples collected in Colorado. One water type is from streams in drainage basins underlain by plutonic rock, and the other water type is from streams in drainage basins underlain by various sedimentary rock types, including the Maroon and Morrison Formations. The composition of a Colorado water sample collected from a mineralized area was used to determine additional Ca and SO_4^{-2} concentrations necessary to simulate the influence of mineralized water; these simulated mineralized waters are labeled “hard.” Table 1 lists the simulated test-water compositions used in laboratory toxicity tests. Due to laboratory toxicity test requirements, it was necessary to prepare some of the waters more than once. These multiple preparations are labeled “A” and “B” in Table 1.

A concentration of approximately 2 mg/L Fe was added to the simulated pluton water types that were to contain Fe, and a concentration of approximately 0.5 mg/L Fe was added to the simulated sedimentary water types that were to contain Fe. Addition of Fe resulted in precipitates, so all of the test-water solutions to which Fe was added (except for the sedimentary water-hard plus Fe (A)) were filtered through a 0.45 μm filter prior to conducting the toxicity tests. The sedimentary water-hard plus Fe (A) test water also contained precipitates, but this particular water was not filtered prior to the toxicity tests due to an oversight. The precipitates were orange in the pluton test water, and orange and white in the sedimentary test water.

Table 1. Composition of simulated test waters used in laboratory toxicity tests. Waters labeled "(A)" and "(B)" denote separate laboratory preparations.

Simulated Test Water	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	Fe mg/L	SO_4^{-2} mg/L	DOC mg/L	Alkalinity mg/L	pH
Pluton water	5.0	1.3	0.93	0.41	0.16	16.0	1.7	3.2	6.7
Pluton water-hard	57	1.3	0.91	0.38	0.15	147	1.6	3.6	7.0
Pluton water-hard plus iron	57	1.4	3.9	0.86	0.01	150	0.8	4.4	7.4
Pluton water-elevated DOC	5.7	1.3	1.8	0.45	0.82	11.9	4.3	4.8	7.5
Pluton water-elevated DOC plus iron	5.4	1.3	1.7	0.64	0.69	11.5	2.8	9.2	7.7
Sedimentary water (A)	6.2	6.3	1.5	0.70	0.13	24.4	2.4	24	7.6
Sedimentary water (B)	8.4	7.0	1.9	0.95	0.28	26.8	2.4	28	8.4
Sedimentary water-hard (A)	41	6.3	1.5	0.74	0.17	113	1.3	35	7.8
Sedimentary water-hard (B)	41	7.0	1.9	0.79	0.27	115	2.0	14	7.8
Sedimentary water-hard plus Fe (A)-unfiltered	41	6.3	1.6	0.96	1.5	113	--	15	7.7
Sedimentary water-hard plus Fe (B)-filtered	42	7.0	1.9	0.76	0.01	120	2.0	15	7.8
Sedimentary water-elevated DOC	7.4	6.7	1.8	0.93	0.80	25.4	4.8	29	8.0
Sedimentary water-elevated DOC plus iron	9.3	6.7	1.9	0.90	0.10	26.7	3.8	29	7.6

Analytical Methods

Simulated test-water samples used for laboratory toxicity tests were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using a Perkin Elmer Optima 3000 at the Colorado School of Mines (CSM). An internal standard of scandium was used to correct for variations in sample uptake and plasma conditions. Water samples collected from Colorado streams were analyzed at the USGS by inductively coupled plasma-mass spectrometry (ICP-MS) (Lamothe et al., 2002). Dissolved organic carbon (DOC) in simulated test-water samples was analyzed by a UV-catalyzed persulfate oxidation method on samples acidified to pH <4 with H_3PO_4 acid using a Sievers TOC 800 Turbo instrument at CSM.

The Biotic Ligand Model

The BLM is available for download 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 Cu 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 are based on measured values of constituents in the simulated test-water solutions.

Toxicity Tests

Three types of toxicity tests were performed and their results compared. Two types of laboratory toxicity tests were performed using *C. dubia* (water flea), which is a sensitive fresh-water invertebrate commonly used in toxicity testing of contaminants. One of these tests was a traditional aquatic toxicity test and one used commercially available *C. dubia* ephippia (dormant eggs, which were reconstituted prior to the test). The third type of toxicity test was the commercially available MetPLATE™ enzymatic toxicity assay test kit, which uses the bacterium *E. coli* as the test organism. Test kits have the advantages of being less time-consuming and requiring less laboratory infrastructure than traditional laboratory methods.

<u>Traditional *C. dubia* tests.</u> The U.S. Environmental Protection Agency standard operating procedure was used for the traditional *C. dubia* toxicity tests (U.S. Environmental Protection Agency, 2002). This procedure involves a 48-hour static test during which time the samples are held at 20°C ± 1°C. Each day of the test consists of 16 hours of light and 8 hours of dark to simulate a diurnal cycle. Our tests were performed at the U.S. Environmental Protection Agency Region 8 Toxicology Laboratory. In our tests, each individual test chamber consisted of a 30-mL plastic cup containing 15 mL of test solution (Fig. 1). 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 at which 50% of the test organisms died (LC$_{50}$). The results of the test were considered valid if 90% of the organisms in the control survived. In this study, Cu was added to each simulated test-water composition to obtain a range of five Cu concentrations and a control (pure simulated test water with no added Cu) (Fig. 1). The range of desired Cu concentrations (1 to 200 µg/L) was determined by running the BLM to obtain a predicted LC$_{50}$ for Cu in the various simulated test-water types and then bracketing that predicted LC$_{50}$ with a range of Cu concentrations. Figure 1 illustrates the setup of the *C. dubia* tests. Values for LC$_{50}$ were estimated by linear interpolation and by the Trimmed Spearman-Karber Method (available online at http://www.epa.gov/ceampubl/fchain/lc50/).

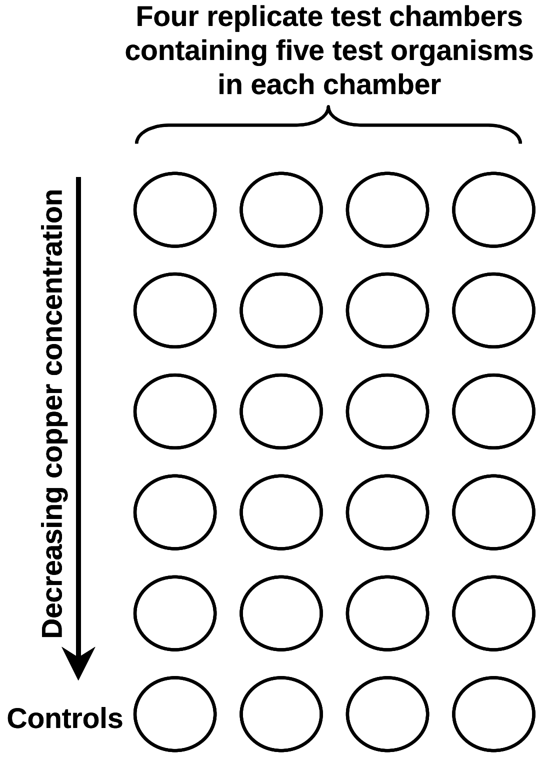

Figure 1. Setup for *C. dubia* toxicity tests for each simulated test-water composition.

<u>*C. dubia* ephippia.</u> *C. dubia* ephippia (dormant eggs) were obtained through Strategic Diagnostics Inc. (Newark, Delaware). Use of ephippia eliminates the necessity of maintaining a laboratory culture of *C. dubia*. The ephippia were hatched by placing them in 50 mL of control water under 6000 lux for 100 hours. The rest of the test followed the traditional procedure described above, with the exception that each individual test chamber contained 15-20 mL of test solution.

<u>MetPLATE™ enzymatic toxicity assay.</u> The MetPLATE™ enzymatic toxicity assay test kit (University of Florida, Gainesville, Florida) uses *E. coli* as the test organism, and defines toxicity by the activity of β-galactosidase enzyme, which is monitored colorimetrically. In this test,

organisms are not directly counted. Instead, their activity is measured colorimetrically through the enzyme activity. The kit includes freeze-dried *E. coli*, moderately hard water, phosphate buffered enzyme (β-galactosidase) substrate, and a 96-well microplate. The freeze-dried bacteria were rehydrated in the moderately hard water and mixed thoroughly. Then, 0.1 mL of the bacteria suspension was added to 0.9 mL of test solution, and the mixture was mixed and incubated at 35°C for 60 minutes. After 60 minutes, 0.2 mL of the bacteria/test solution suspension was dispensed into wells of the microplate, and 0.1 mL of the enzyme substrate was added to each well. Each of the wells was mixed, and the microplate was incubated an additional 60-120 minutes at 35°C for color development. Absorbance was measured at 575 nanometers using a PowerwaveX 340 96-well microplate reader. The intensity of the color indicates the amount of enzyme activity (related to test-organism activity), and is inversely proportional to sample toxicity. MetPLATE™ test results are reported as an EC_{50} rather than an LC_{50}, where the EC_{50} is the effective concentration at which 50% of the bacterial suspension is adversely affected. Values for EC_{50} were graphically estimated. This type of enzymatic bioassay provides a relatively simple screening procedure for metal toxicity (Blumenstein et al., 2005).

Results and Discussion

Chemical Characteristics of Water Originating from Different Rock Types

Two simulated test-water types were prepared in the laboratory to simulate two stream-water types (see the Methods Section for preparation details of simulated test-water types). In Fig. 2 and 3 are box plots showing ranges in pH, DOC, and major-cation concentrations of stream-water samples collected in drainage basins underlain by plutonic rock and sedimentary rock, respectively. The red crosses in Fig. 2 and 3 are measured values of simulated test-water compositions prepared for use in toxicity tests. Note the different y-axis scales in Fig. 2 and 3.

Table 2 lists concentration ranges of several constituents in the stream-water samples. The stream-water samples collected from drainage basins underlain by sedimentary rock have slightly higher pH, K, and Na, and noticeably higher Ca and Mg concentrations, than stream-water samples collected from drainage basins underlain by plutonic rock. Hardness, alkalinity, and SO_4^{-2} concentrations also were noticeably higher in the stream-water samples collected in sedimentary basins. Dissolved organic carbon concentration ranges were similar for both stream-water types, but DOC tends to be slightly higher in stream-water samples collected in sedimentary basins. The composition of the simulated test-water types is comparable with the composition of the stream-water samples (Fig. 2 and 3). By design, both simulated test-water types contain approximately 2 mg C/L DOC (see the Methods Section for preparation details of simulated test-water types).

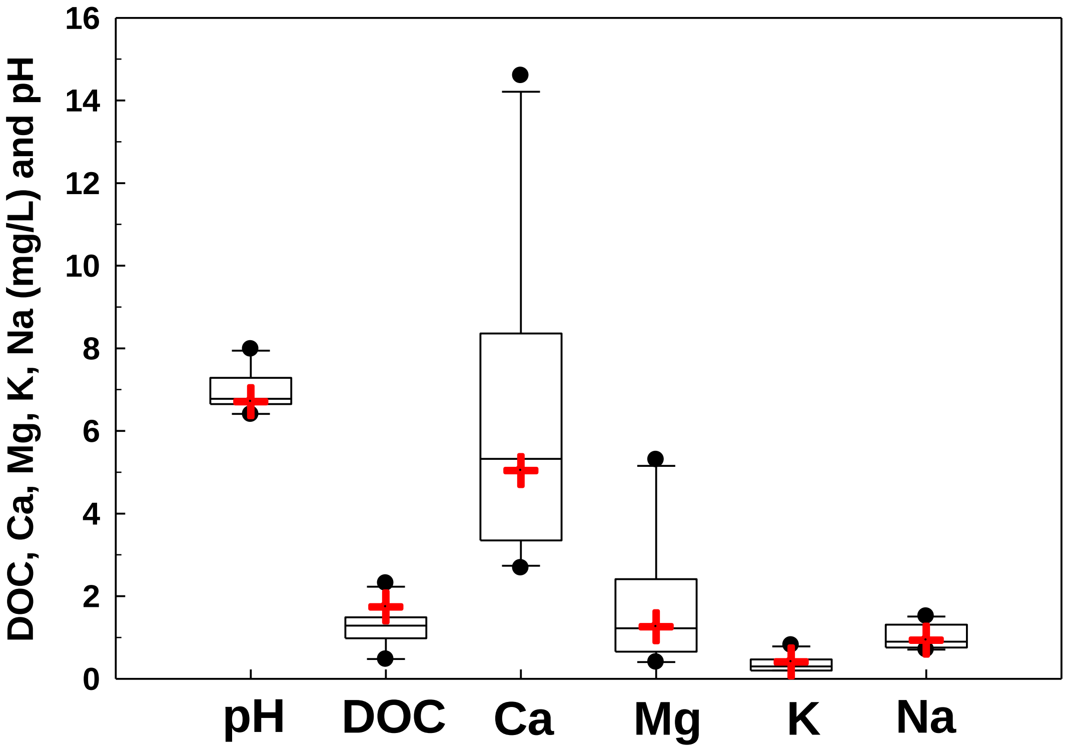

Figure 2. Box plots showing ranges in pH, dissolved organic carbon (DOC), and major-cation concentrations for 10 Colorado stream-water samples from drainage basins underlain by plutonic rock. The red + symbols are measured values in simulated test water used in toxicity tests.

Table 2. Range of values and concentrations in 10 Colorado stream-water samples collected from drainage basins underlain by plutonic rock, and in 13 Colorado stream-water samples collected from drainage basins underlain by sedimentary rock. Hardness was calculated from measured Ca and Mg concentrations.

Constituent	Pluton Stream Water	Sedimentary Stream Water
pH	6.4 – 8.0	6.4 – 8.5
Hardness (mg/L $CaCO_3$)	8.6 - 58	20 - 175
Alkalinity (mg/L $CaCO_3$)	9.1 - 48	18 - 141
DOC (mg C/L)	0.5 – 2.3	0.6 – 3.9
Ca (mg/L)	2.7 - 15	6.5 - 48
Mg (mg/L)	0.40 – 5.3	0.96 - 16
K (mg/L)	0.20 – 0.81	0.32 – 0.95
Na (mg/L)	0.70 – 1.5	0.43 – 2.1
SO_4 (mg/L)	0.66 - 12	1.5 - 26

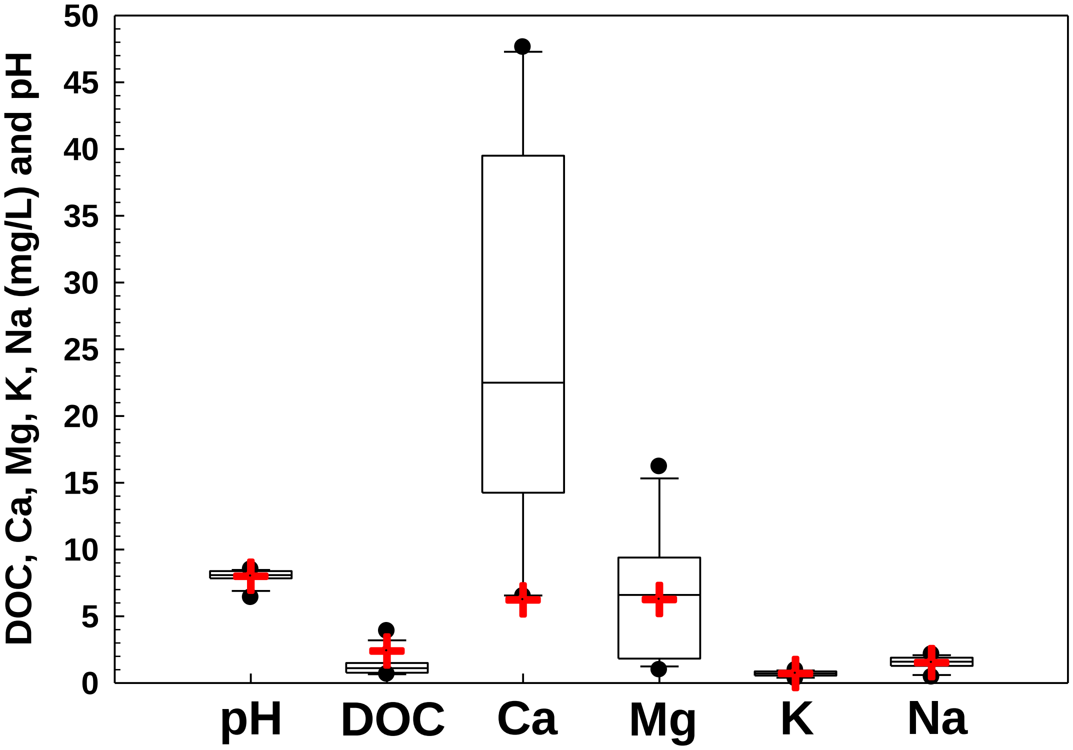

Figure 3. Box plots showing ranges in pH, dissolved organic carbon (DOC), and major-cation concentrations for 13 Colorado stream-water samples from drainage basins underlain by various sedimentary rock types associated with the Maroon and Morrison Formations. The red + symbols are measured values in simulated test water used in toxicity tests.

<u>Stream water in mineralized areas.</u> Water originating from mineralized areas commonly has lower pH and alkalinity, and has higher SO_4^{-2} and Fe concentrations than water in unmineralized areas. Calcium and Mg concentrations also tend to be elevated due to acid dissolution of rock-forming minerals. Calcium and Mg concentrations in water are an important consideration in toxicological studies because both Ca and Mg have been shown to protect aquatic biota from metal toxicity. In order to examine toxicity in water influenced by mineralization, we incorporated water constituents (Ca and SO_4^{-2}) based on a Colorado water sample collected from a mineralized area into the preparation of our simulated test-water types, as discussed in the Methods section.

Figure 4 shows the relationship between Ca and alkalinity for three Colorado stream-water types. For the non-mineralized stream-water samples, there is a linear trend between Ca and alkalinity. However, the mineralized stream-water samples deviate from this linear trend in that they have increased Ca concentrations (and increased hardness) with little or no alkalinity. In preparation of our simulated test-water types for toxicity studies, we added $CaSO_4$ to some solutions to examine the effect of increasing Ca concentration (and hardness) without increasing alkalinity (test waters labeled “hard”).

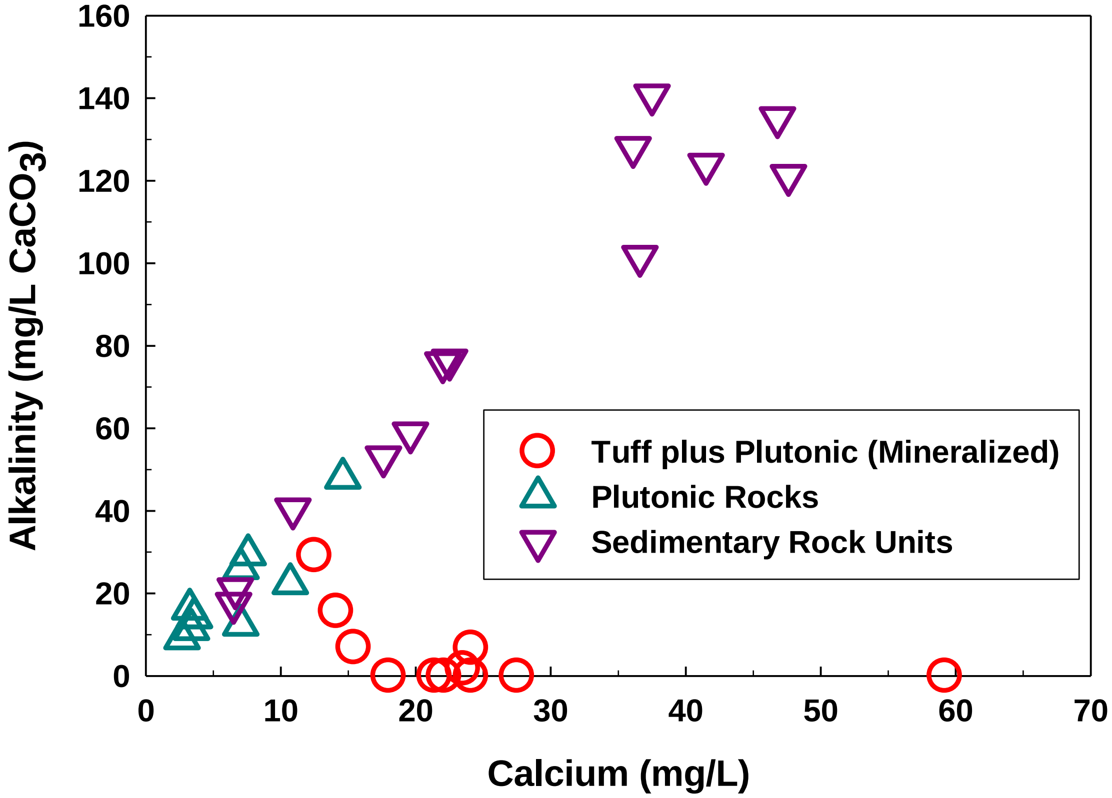

Figure 4. Graph showing the relationship between Ca concentration and alkalinity in Colorado stream-water samples collected from drainage basins underlain by different rock types. Note the deviation of the mineralized water from the trend.

Comparison of *C. dubia* Results with BLM Predictions in Different Simulated Test Waters

Traditional Cu toxicity tests were conducted using *C. dubia* on variations of two simulated test-water types. Table 3 gives details about the different simulated test-water compositions, and lists the LC_{50} values from the traditional Cu toxicity tests using *C. dubia*. Also listed in Table 3 are LC_{50} predictions from the BLM, calculated hardness values, and measured DOC and pH values of the simulated test waters.

The LC_{50} values represent the Cu concentrations at which 50% of the *C. dubia* died. Therefore, water with a lower LC_{50} value is more toxic than water with a higher LC_{50} value. Predictions using the BLM are considered good if they are within a factor of two of results from toxicity tests (Allen, 2002). There is fairly good agreement between predicted LC_{50} values calculated using the BLM and LC_{50} values determined from toxicity tests, except for test solutions to which Fe was added. Another exception is the sedimentary water (B), which may be due to an error in pH measurement. When a pH value of 7.6 instead of 8.4 was used for that water, the BLM predicted an LC_{50} value of 26 μg/L Cu, which is the same LC_{50} value predicted for the sedimentary water (A).

Table 3. Results from traditional Cu toxicity tests using *C. dubia*, selected chemical parameters for simulated test-water compositions, and BLM predictions.

[LC_{50} values are in µg/L Cu. Predicted LC_{50} values were computed using the BLM. Graphical LC_{50} values were estimated by linear interpolation, and TSK LC_{50} values were determined by the Trimmed Spearman-Karber method. All test solutions were unfiltered, except as noted. DOC is reported as mg C/L, and hardness was calculated from measured Ca and Mg concentrations and is reported as mg/L $CaCO_3$. The "A" and "B" notation for some simulated test waters refers to separate preparations of that water.]

Simulated Test Water	Abbreviated Label	Predicted BLM LC_{50}	Measured Graphical LC_{50}	Measured TSK LC_{50}	Measured DOC	Measured pH	Calculated Hardness
Pluton water	PW	6.3	7.5	6.8	1.7	6.7	17.8
Pluton water-hard	PW+Ca	11	9.0	8.7	1.6	7.0	148
Pluton water-hard plus iron[1]	PW+Ca+Fe	10	3.8	3.2	0.8	7.4	149
Pluton water-elevated DOC	PW+DOC	54	50	43	4.3	7.5	19.7
Pluton water-elevated DOC plus iron[1]	PW+DOC+Fe	42	10	10	2.8	7.7	18.8
Sedimentary water (A)	SW(A)	26	32	29	2.4	7.6	41.3
Sedimentary water (B)	SW(B)	66[2]	20	17	2.4	8.4	49.7
Sedimentary water-hard (A)	SW+Ca(A)	24	20	19	1.3	7.8	128
Sedimentary water-hard (B)	SW+Ca(B)	36	25	23	2.0	7.8	132
Sedimentary water-hard plus iron (A)[3]	SW+Ca+Fe(A)	32	85	87	--	7.7	128
Sedimentary water-hard plus iron (B)[1]	SW+Ca+Fe(B)	35	3.7	3.5	2.0	7.8	133
Sedimentary water-elevated DOC	SW+DOC	89	92	86	4.8	8.0	46.0
Sedimentary water-elevated DOC plus iron[1]	SW+DOC+Fe	44	33	35	3.8	7.6	51.1

[1] Filtered with 0.45 µm filter.

[2] The BLM LC_{50} prediction is 26 when computed at pH 7.6.

[3] This solution was not filtered and likely contained particulate iron. DOC was not measured for this sample. A DOC concentration of 2.0 mg C/L was used in the BLM data file.

Effect of added calcium (hardness). Excess $CaSO_4$ was added to some of the test solutions to determine the effect of increasing Ca concentration (and hardness) on Cu toxicity. Based on the results in Table 3, there is a slight decrease in toxicity in the simulated plutonic test-water solutions, and a slight increase in toxicity in the simulated sedimentary test-water solutions. The simulated sedimentary test-water solutions have somewhat higher alkalinity values (see Table 2). The effect of increasing the Ca concentration (and hardness) on Cu toxicity in these systems appears to be minimal.

Effect of iron-rich particulates. The one Fe-rich water that wasn't filtered (due to an oversight), the sedimentary water-hard plus Fe (A) water, has a predicted LC_{50} of 32 μg/L Cu, but the LC_{50} value determined from the toxicity test is 87 μg/L Cu. This indicates that the BLM predicts this water to be more toxic than it actually is. One possible explanation is that the Cu is bound to the precipitate present in the test system, and hence is not available for uptake by the *C. dubia*. This finding implies that metals bound to suspended particles are not bioavailable to *C. dubia* in the test solutions (in the context of the acute toxicity tests conducted in this study).

Effect of dissolved organic carbon. Dissolved organic carbon is known to have a protective effect against metal toxicity in aquatic organisms. This protective effect is illustrated in results for the simulated test water containing elevated DOC and no Fe (pluton water-elevated DOC and sedimentary water-elevated DOC). For these elevated-DOC test water solutions, the LC_{50} is 3-5 times greater (less toxic) than the LC_{50} for the lower-DOC test-water solutions. For the elevated-DOC test-water solutions, there is good agreement between the predicted LC_{50} values computed using the BLM and the LC_{50} values determined from toxicity tests. This is because the BLM takes the protective effects of DOC into account in its computations by incorporating binding constants for metal-organic matter interactions using the Windermere Humic Aqueous Model (WHAM; Tipping, 1994).

Effect of added iron. In the cases where the test solutions were filtered after the addition of Fe, there is a decrease in laboratory LC_{50} values (increase in toxicity) when compared with results in the test solutions with no Fe added. This shift in LC_{50} values is illustrated in Fig. 5 and 6. An explanation for this shift lies in the interaction between Fe^{+3} iron and DOC. As discussed above, DOC has a protective effect against metal toxicity in aquatic organisms. However, when Fe^{+3} iron is introduced into the system, it can (1) coprecipitate with or sorb DOC, thus removing DOC from solution (McKnight et al., 1992, 2002), (2) preferentially bind to metal-binding sites on DOC, thus leaving more free Cu in solution (Smith, 1991), or (3) coprecipitate with or sorb the more reactive DOC, thus preferentially removing the DOC with the greatest metal-binding capacity from solution (McKnight et al., 1992, 2002). This reduction in the protective effects of DOC on Cu toxicity is most apparent for the elevated-DOC solutions (approximately 5 mg C/L; compare PW+DOC with PW+DOC+Fe, and SW+DOC with SW+DOC+Fe), but also is noticeable for the solutions containing approximately 2 mg C/L (compare PW and PW+Ca with PW+Ca+Fe, and SW(A) and SW+Ca(A) with SW+Ca+Fe(B)).

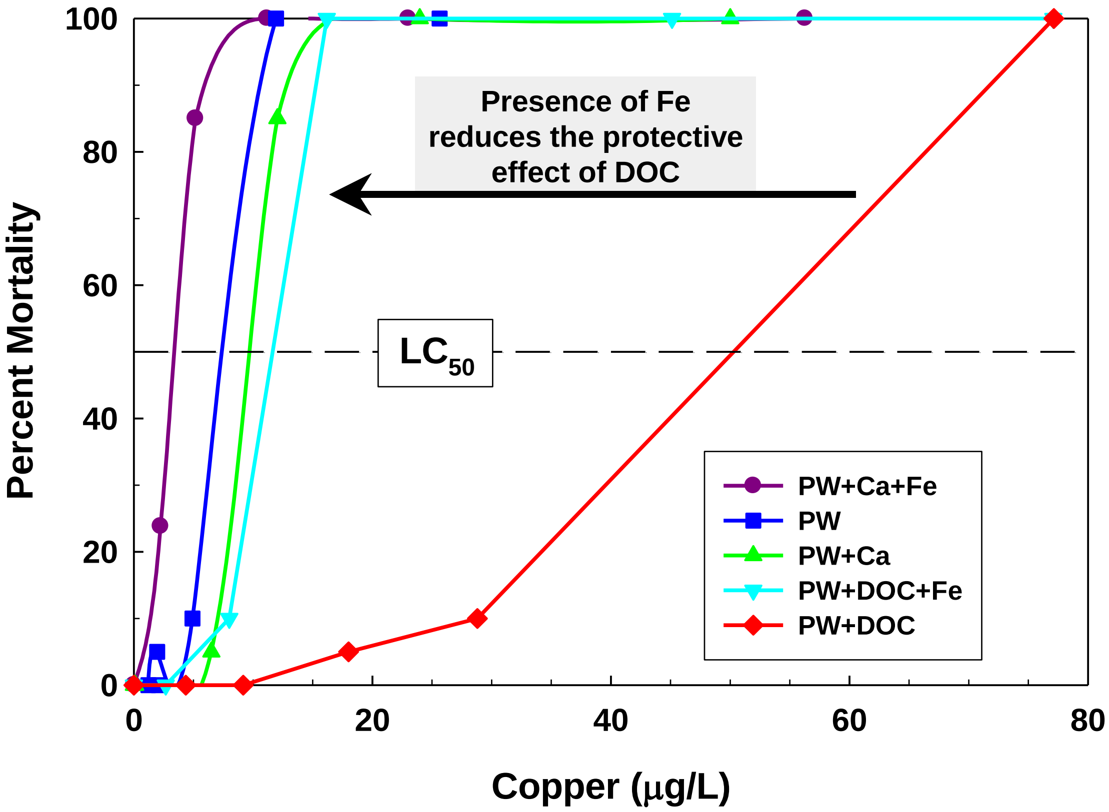

Figure 5. Results from traditional Cu toxicity tests using *C. dubia* in different test solutions based on stream-water samples from drainage basins underlain by plutonic rock. The LC_{50}, shown by the dashed line, shifts as a result of differences in the chemical composition of the test solutions. Table 3 lists some of the details about the test solutions. Note that the presence of Fe in the test solutions results in higher toxicity (lower LC_{50}) by reducing the protective effects of DOC on Cu toxicity.

Comparison of Biotic Ligand Model predictions with toxicity tests in iron-rich systems. The agreement between BLM LC_{50} predictions and results from the toxicity studies is poor for test solutions with added Fe. This is because the BLM does not take Fe into account in its computations. Figures 7 and 8 show LC_{50} values as a function of DOC concentration for BLM predictions and toxicity test results. As illustrated in Fig. 7, the influence of added Fe appears to have two effects. First, the added Fe causes some of the DOC to precipitate, which results in loss of some of the DOC from solution. Second, either the added Fe preferentially binds to metal-binding sites on the DOC in solution, or the remaining DOC in solution has less affinity to bind with Cu, both of which would result in less Cu binding to the DOC and increased Cu toxicity. Because the BLM computes predicted LC_{50} values for dissolved constituents, it can account for the DOC loss from solution (see Fig. 7). However, competitive Fe binding to DOC and DOC fractionation are not taken into account in BLM computations. For most stream-water systems, this probably is not an important issue. However, for systems affected by mining, this may be a significant shortcoming of the BLM. Iron speciation is known to be variable in mining impacted systems (e.g., McKnight et al., 1988). Therefore, in Fe-rich mining impacted systems, enough dissolved Fe^{+3} iron may be available to interfere with the protective effects of DOC on Cu toxicity. In the simulated pluton test water (Fig. 7), approximately 2 mg/L Fe was added to the solutions. In the simulated sedimentary test water (Fig. 8), approximately 0.5 mg/L Fe was added to the solutions. Even in these solutions with less Fe added, there is still the effect of DOC coprecipitation with Fe.

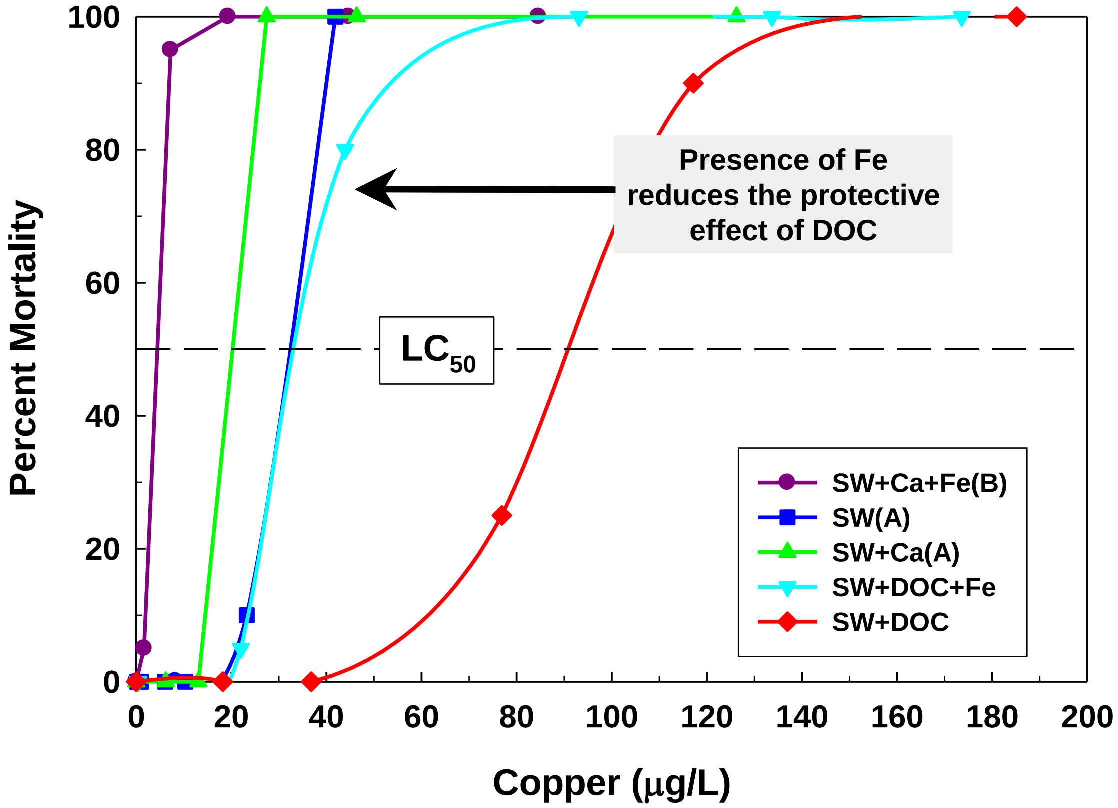

Figure 6. Results from traditional Cu toxicity tests using *C. dubia* in different test solutions based on stream-water samples from drainage basins underlain by sedimentary rock. The LC_{50}, shown by the dashed line, shifts as a result of differences in the chemical composition of the test solutions. Table 3 lists some of the details about the test solutions. Note that the presence of Fe in the test solutions results in higher toxicity (lower LC_{50}) by reducing the protective effects of DOC on Cu toxicity.

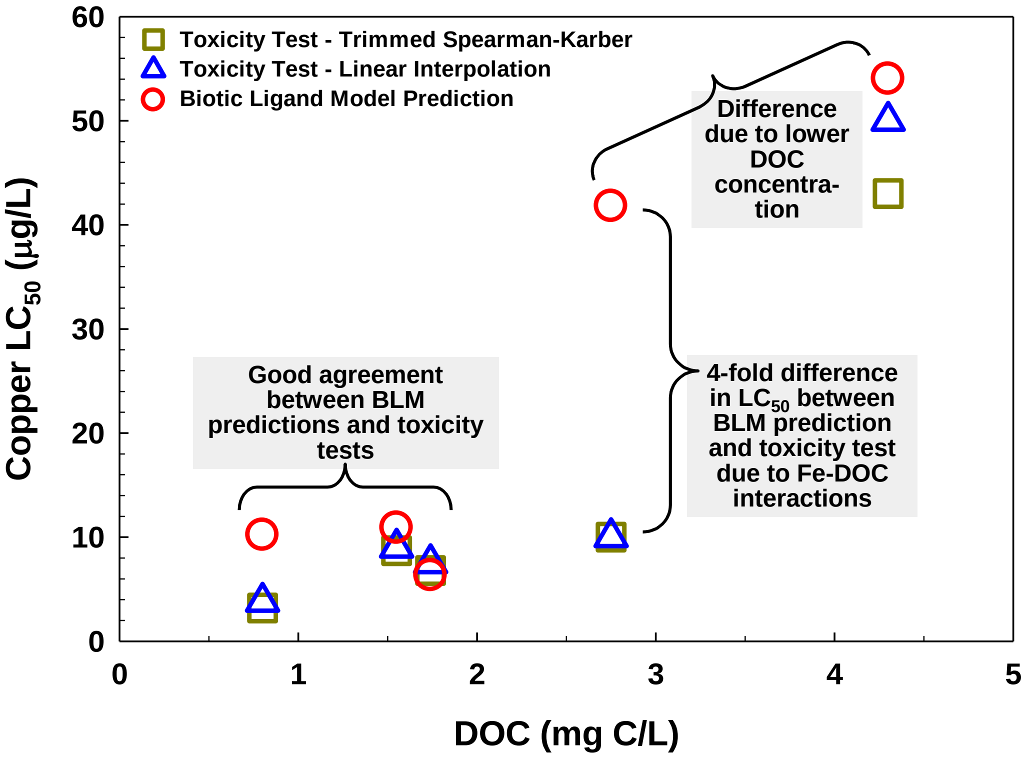

Figure 7. Comparison of Cu LC_{50} values from traditional toxicity tests using *C. dubia* and from predictions computed by the BLM as a function of dissolved organic carbon. Approximately 2 mg/L Fe was added to selected test solutions. Test solutions are based on stream-water samples from drainage basins underlain by plutonic rock.

Comparison of Different Toxicological Test Results

In addition to traditional Cu toxicity tests using *C. dubia*, two other toxicity tests were conducted on the same test solutions as a part of this study. One test involves hatching *C. dubia* from ephippia (dormant eggs). Although this approach removes the necessity of maintaining a *C. dubia* culture, the actual toxicity tests with the hatched *C. dubia* are still fairly cumbersome and time consuming. The other test, the MetPLATE™ assay kit, involves indirectly measuring the response of *E. coli* to test solutions. Conducting tests with this kit is relatively simple and much less time consuming. MetPLATE™ test results are reported as an EC_{50} rather than an LC_{50}, where the EC_{50} is the effective concentration at which 50% of the bacterial suspension is adversely affected. Table 4 gives a comparison of the three toxicity test results; this information is shown graphically in Fig. 9.

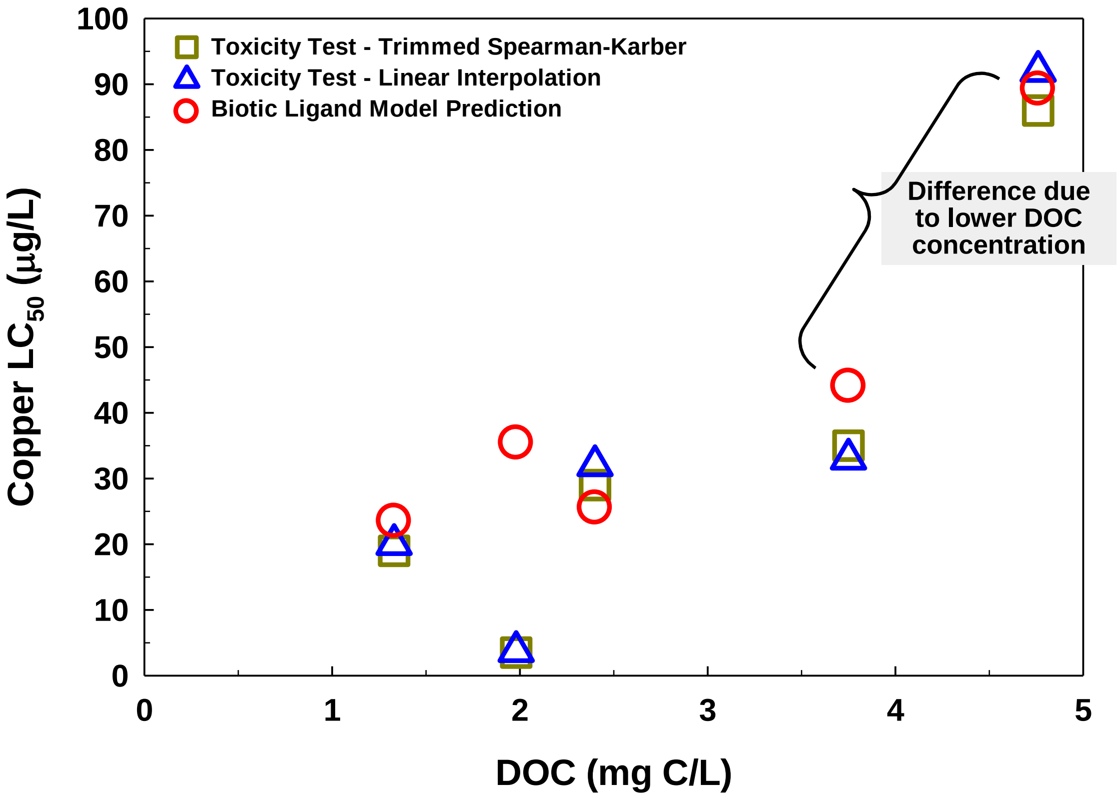

Figure 8. Comparison of Cu LC_{50} values from traditional toxicity tests using *C. dubia* and from predictions computed by the BLM as a function of dissolved organic carbon. Approximately 0.5 mg/L Fe was added to selected test solutions. Test solutions are based on stream-water samples from drainage basins underlain by sedimentary rock.

Table 4. Comparison of results from Cu toxicity tests using *C. dubia*, *C. dubia* ephippia, and MetPLATE™ test kits with BLM predictions. The LC_{50} and EC_{50} values are in µg/L Cu. Graphical EC_{50} values were estimated by linear interpolation, and TSK LC_{50} values were determined by the Trimmed Spearman-Karber method.

Simulated Test Water	BLM Predicted LC_{50}	Traditional TSK LC_{50}	Ephippia TSK LC_{50}	MetPLATE™ Graphical EC_{50}
Pluton water	6.3	6.8	4.8	14
Pluton water-hard	11	8.7	7.5	31
Pluton water-hard plus iron	10	3.2	--	31
Pluton water-elevated DOC	54	43	39	40
Pluton water-elevated DOC plus iron	42	10	12	39
Sedimentary water (A)	26	29	20	26
Sedimentary water-hard (A)	24	19	19	66
Sedimentary water-hard plus iron (B)	35	3.5	3.3	43
Sedimentary water-elevated DOC	89	86	94	111
Sedimentary water-elevated DOC plus iron	44	35	30	78

The LC_{50} results are consistently comparable between traditional *C. dubia* toxicity tests and *C. dubia* ephippia tests. This finding indicates that the *C. dubia* ephippia can be used in lieu of traditional toxicity tests for these types of test solutions. The MetPLATE™ results are more variable. For some test solutions (e.g., PW, PW+DOC, SW(A), and SW+DOC), results between MetPLATE™ and *C. dubia* toxicity tests are similar, and both MetPLATE™ and *C. dubia* test results illustrate the protective effect of DOC on Cu toxicity. However, in solutions with added Ca and Fe, there are significant discrepancies between the MetPLATE™ and *C. dubia* toxicity test results. For example, MetPLATE™ results show less Cu toxicity when Ca (and hardness) is added to the solution, whereas this protective effect of Ca on Cu toxicity is not readily apparent from the *C. dubia* results. Also, the response to the addition of Fe is variable in the MetPLATE™ results. It appears that the *E. coli* test organism used in the MetPLATE™ test kit has somewhat different responses than *C. dubia* to environmental variables that can affect Cu toxicity.

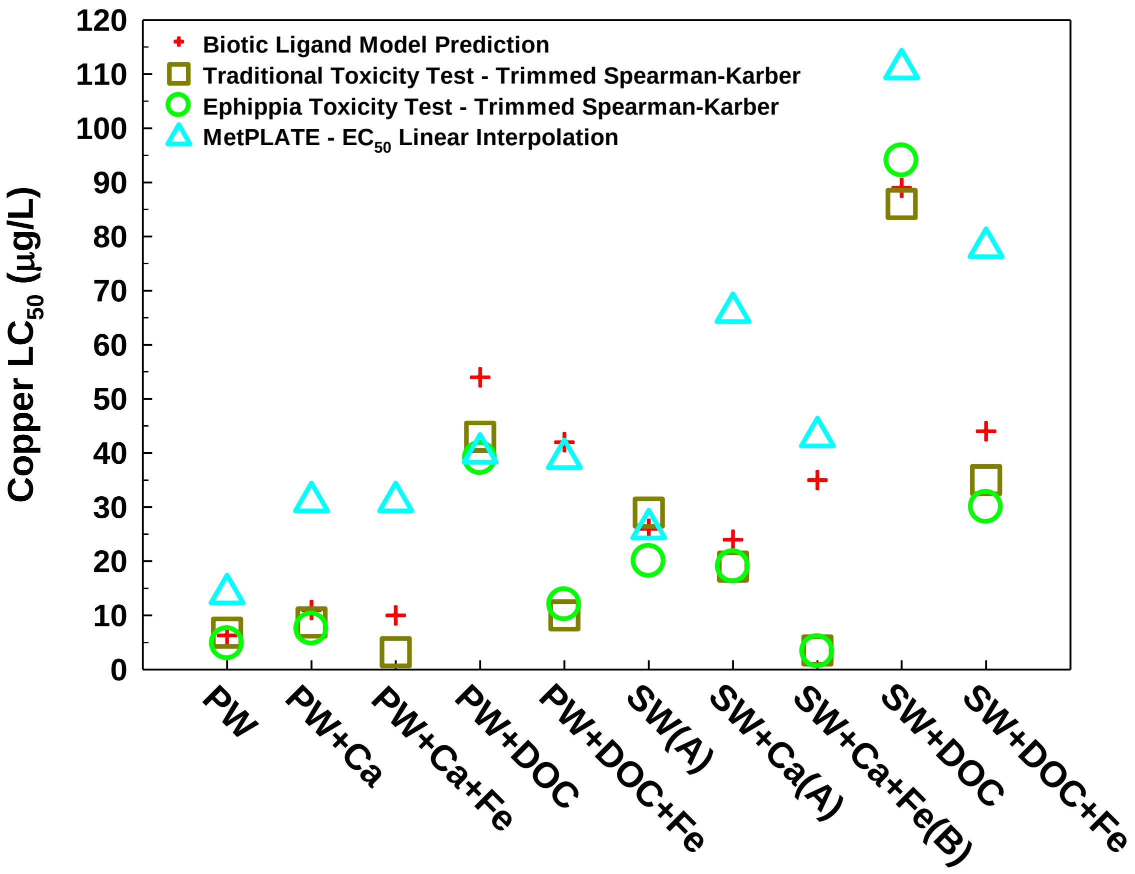

Figure 9. Comparison of results from Cu toxicity tests using *C. dubia*, *C. dubia* ephippia, and MetPLATE™ test kits with BLM predictions. MetPLATE™ values are EC_{50} values, and were estimated by linear interpolation.

Detailed toxicity data for the *C. dubia* ephippia test and the MetPLATE™ kit are shown in Fig. 10-13. The *C. dubia* ephippia tests appear to have more variability between the data points than do the traditional *C. dubia* toxicity tests (compare Fig. 10 and 11 with Fig. 5 and 6). However, overall trends are similar for the traditional and ephippia *C. dubia* tests. MetPLATE™ results are variable. For the simulated sedimentary test water (Fig. 13), there are overlapping data for the various solutions. For the simulated pluton test water (Fig. 12), there are distinct trends that show the protective effects of Ca (hardness) and DOC on Cu toxicity, but do not show any effect of added Fe to the DOC test solutions. More work is needed to elucidate these differences between MetPLATE™ and *C. dubia* toxicity test results. Comparison of MetPLATE™ results with BLM predictions is problematic because the BLM predictions are specifically for *C. dubia*.

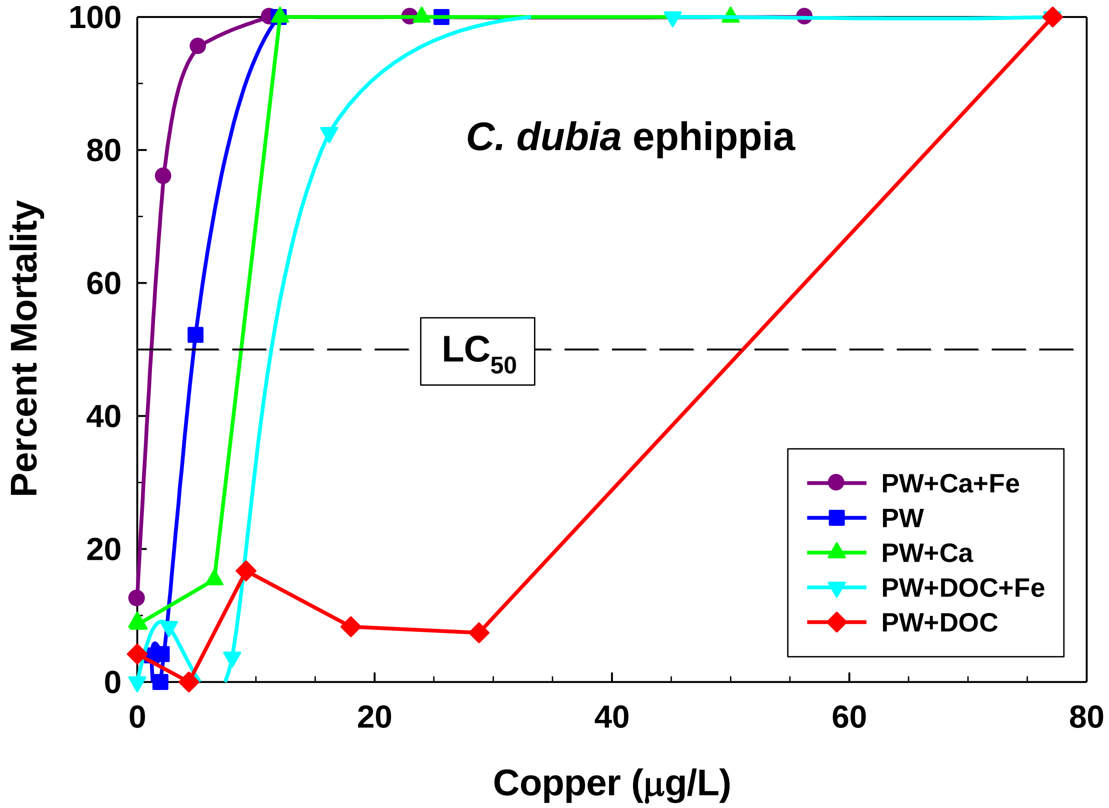

Figure 10. Results from *C. dubia* ephippia toxicity tests in different test solutions based on stream-water samples from drainage basins underlain by plutonic rock. The LC$_{50}$, shown by the dashed line, shifts as a result of differences in the chemical composition of the test solutions.

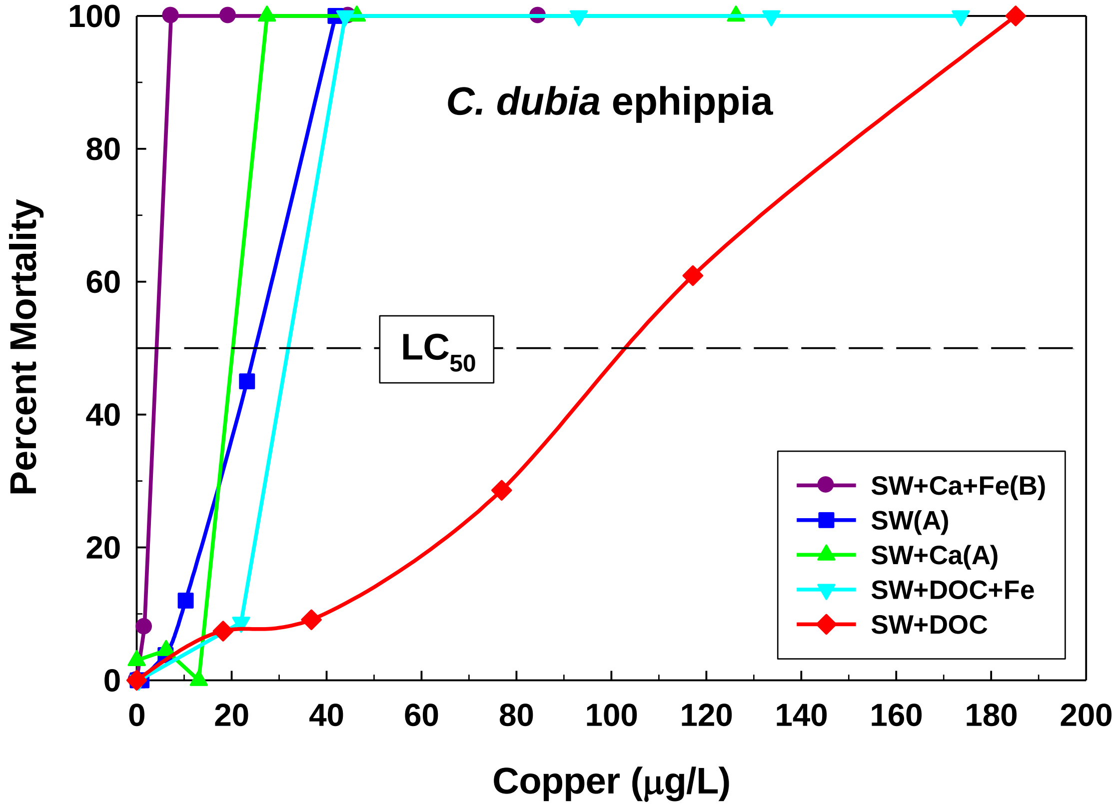

Figure 11. Results from *C. dubia* ephippia toxicity tests in different test solutions based on stream-water samples from drainage basins underlain by sedimentary rock. The LC_{50}, shown by the dashed line, shifts as a result of differences in the chemical composition of the test solutions.

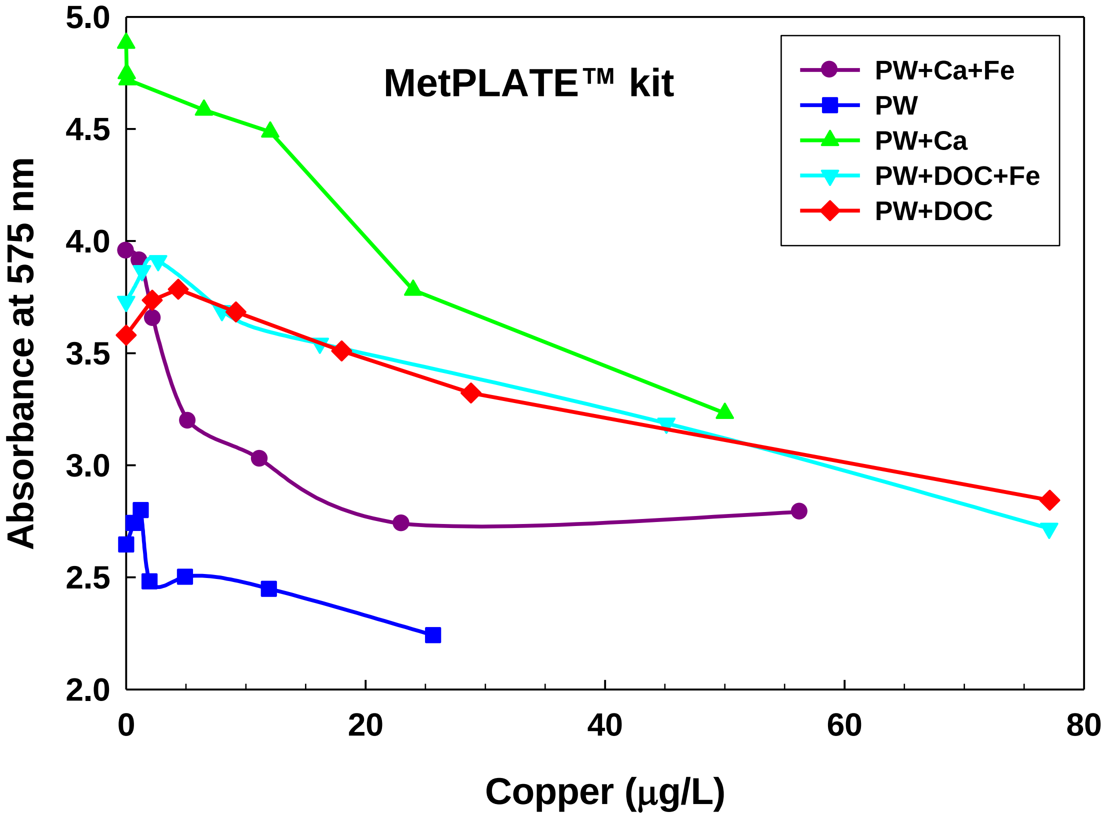

Figure 12. Results from MetPLATE™ test kit toxicity tests in different test solutions based on stream-water samples from drainage basins underlain by plutonic rock. Note that the intensity of the color (absorbance) in the tests indicates the amount of enzyme activity (related to test-organism activity), and is inversely proportional to sample toxicity.

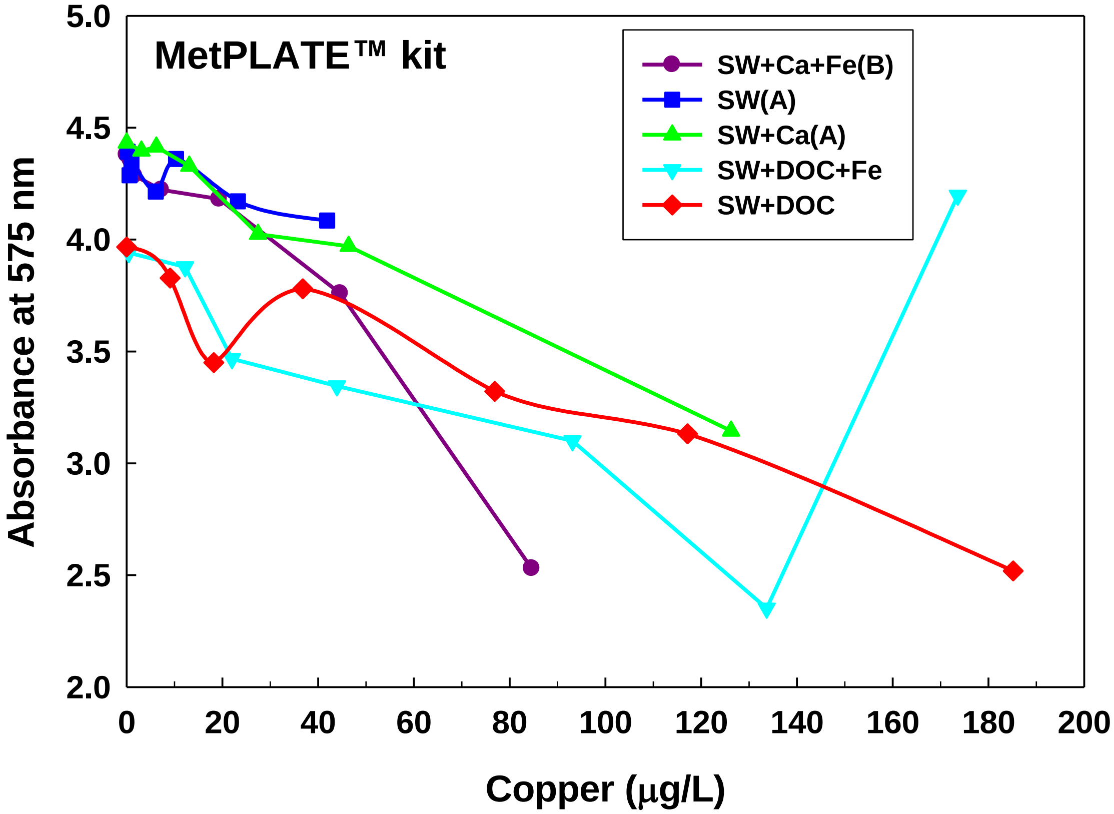

Figure 13. Results from MetPLATE™ test kit toxicity tests in different test solutions based on stream-water samples from drainage basins underlain by sedimentary rock. Note that the intensity of the color (absorbance) in the tests indicates the amount of enzyme activity (and, hence, test-organism activity), and is inversely proportional to sample toxicity.

Conclusions

In this study, BLM Cu toxicity predictions were compared with laboratory Cu toxicity tests for a variety of test solutions. Comparison of BLM predictions with traditional Cu toxicity tests using *C. dubia* show that there is fairly good agreement between predicted LC_{50} values calculated using the BLM and LC_{50} values determined from toxicity tests for a variety of simulated test waters, except for test waters to which Fe was added. The effect on Cu toxicity of increasing the Ca concentration (and hardness) in test solutions appears to be minimal. Iron-rich particulates, formed when Fe was added to the solutions, appear to sequester Cu and render that Cu unavailable to *C. dubia*. For test-water solutions containing elevated DOC, the LC_{50} is 3-5 times greater (less toxic) than the LC_{50} for the lower-DOC test-water solutions.

In the test solutions that were filtered after addition of Fe, there is a decrease in LC_{50} values (increase in toxicity) in results from the *C. dubia* toxicity tests compared with results in the test solutions with no Fe added. The agreement between BLM LC_{50} predictions and results from the toxicity studies is poor for test solutions with added Fe because the BLM does not take Fe into account in its computations. The influence of added Fe appears to have two effects. First, the added Fe causes some of the DOC to precipitate, which is demonstrated by the loss of some of the measured DOC from solution. Second, the added Fe either preferentially binds to metal-binding sites on the DOC or the DOC remaining in solution has less Cu-binding affinity, both of which would result in less Cu binding to the DOC and greater Cu toxicity to *C. dubia*. The BLM can account for changes in Cu toxicity due to loss of DOC from solution, but it is not able to account for competitive Fe binding to DOC or for DOC fractionation. This may be a significant shortcoming of the BLM for predicting site-specific water-quality criteria at mining impacted sites that contain Fe precipitates in the streams.

Comparison of toxicity results between traditional *C. dubia* toxicity tests and *C. dubia* ephippia tests shows that their LC_{50} results are consistently comparable. This finding indicates that the *C. dubia* ephippia tests can be used in lieu of traditional toxicity tests for these types of test solutions. MetPLATE™ test kit results are more variable and appear to be more sensitive to added Ca (increased hardness). The lack of correspondence in results between *C. dubia* toxicological tests and MetPLATE™ test kits requires further investigation, but probably demonstrates different toxicological responses for different test organisms.

Acknowledgments

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