USE OF ANODIC STRIPPING VOLTAMMETRY IN PREDICTING TOXICITY OF COPPER IN RIVER WATER

ZIJIAN WANG,* SHENGBIAO HUANG, and QING LIU
State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Shuangqing Road 18, Haidian District, Beijing, 100085, China

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Abstract—The labile concentration and toxicity of Cu as influenced by alkalinity and different concentrations of ethylenediaminetetraacetic acid (EDTA) and naturally derived fulvic acid (FA) were determined by bioassays carried out in the culture media for Daphnia magna (D. magna). The labile concentration of Cu was obtained by differential pulse anodic stripping voltammetry with a double-acidiﬁcation method (DAM-DPASV). Changes in water alkalinity did not affect the labile concentration of Cu, but increase in alkalinity did reduce the mortality of D. magna. In the presence of EDTA and FA, both labile concentration of Cu and mortality were reduced. By excluding Cu-carbonate complexes from the labile concentration, a bioavailable concentration of Cu ([Cu*]) was obtained and was used to predict the acute toxicity of Cu on D. magna. For natural waters, the labile concentration of Cu was measured by DAM-DPASV, and [Cu*] was calculated using MINTEQ A2 software (developed by the U.S. Environmental Protection Agency) based on the anion composition of waters. This procedure was tested for waters and sediment elutriates sampled from the Le An River (Jiangxi Province, China) that were severely polluted by the discharges from a copper mine. The results showed that [Cu*] was a good indicator for Cu toxicity and could be used under ﬁeld conditions.

Keywords—Copper speciation  Acute toxicity  Daphnia magna  Natural waters

INTRODUCTION

Bioavailability and toxicity of trace metals to aquatic organisms depend on the physical and chemical speciation of the metals [1]. Therefore, the speciation of a metal, rather than its total concentration, is the key for understanding its effect on the biota [2]. Previous studies on several metals and varieties of aquatic organisms have demonstrated that the response of organisms to metals could be modeled by using their free-ion activity [3–5]. To rationalize these experimental results and to explain what was perceived as “the universal importance of free-ion activities in determining the biological effect of all cationic trace metal,” Morel [5] formulated the free-ion activity model (FIAM) for metal-organism interaction. The FIAM has generally been interpreted to imply that a constant degree of biological effect will occur at a constant chemical activity of the free ion of a metal for divergent transition metal cations. These studies suggest that the concentration of free-ionic metal is the key factor in determining the toxicity. It might be tempting to conclude that the free ion alone is responsible for the observed toxicities, but this does not appear to be supported by the previously published work [6].

Free-ion activity does not appear to be a good predictor of toxicity under certain water-quality conditions (e.g., water hardness), and it does not explain competition of the metal ion in question with other cations [7]. The formation of a biotic-ligand model (BLM), a recent surface-interaction model of metal binding to fish gill, simultaneously incorporates metal speciation with its toxicity and competition between the metal ion under study and other cations [8,9]. Thus, adopting the BLM model could help to predict acute toxicity of Cu to fish on a mechanistic basis and under various conditions of water hardness. However, several factors should be investigated over time that may improve the model’s predictability and our understanding of its strengths or limitations [10]. For example, in testing the model’s ability to predict Cu toxicity, it was assumed that the dissolved organic carbon (DOC) was 10% (w/w) humic acid and that the conditional stability constant of Cu with humic acid was not considered to be a site-speciﬁc parameter [10].

Anodic stripping voltammetry (ASV) is a powerful techni qu for the study of trace element speciation, which can be used to provide speciation information regarding the labile/inert discrimination [11]. Fulvic acid (FA) binds with Cu, thereby making it no longer labile in ASV measurements. When Cu is bound to FA in natural aquatic environments, it is no longer available for biota. This procedure thus provides a good approximation of labile metal speciation. This leads to the deposition process of labile metal on the surface of an electrode approximating as closely as possible the kinetics of its uptake processes by the cells of organisms [12,13]. The correlation between ASV-labile concentrations of Cu and toxicity derived from bioassay may, therefore, be useful in predicting its toxicity. However, some aquatic components, such as FA, can affect the stripping current not only through affecting aqueous Cu species but also through adsorbing organic components on the surface of an electrode (e.g., mercury drop). To avoid interference by organic components on stripping current, a double-acidiﬁcation process is recommended [14]. By assuming that strong organic complexes of Cu are neither labile nor bioavailable, leading to acute toxicity [1], and that only part of inorganic complexes are bioavailable or toxic, the acute toxicity of Cu in natural water can be predicted. This prediction will be based on measurements obtained by differential pulse ASV with the double-acidiﬁcation method (DAM-DPASV) and calculated by a MINTEQ A2 software (developed by the U.S. Environmental Protection Agency) and will exclude Cu-carbonate complexes from labile speciation of Cu.
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In the present study, we have developed the approach and a model to predict the acute toxicity of Cu on *Daphnia magna*.

**MATERIALS AND METHODS**

**Sampling and test solution preparations**

Nine samples were taken from the Le An River. The sampling sites were chosen along the downstream from Haikou to Panlong Temple. Site L04 was 20 km downstream from site L01, where the largest open-cast Cu mine in China (Dexing Copper mine) is located and whose discharges flow into the Le An River at the convergence with the Dawu River. Site L07 was 50 km downstream and at the convergence with the Jihui River (39 km long), which is another metal-polluted river with several small sulfide mines and smelters. Site L09 was 230 km downstream and in the delta area of Poyang Lake, the largest freshwater lake in China. The metal pollution and the ecological consequences in the Le An River have been reported previously [15–17].

A 20-L water sample was filtered through a Millipore 0.45-

μm filter (Bedford, MA, USA) and collected in a precleaned polyethylene bottle. The samples were stored at 4°C and used within one week. Sediment samples were collected from the same locations on the Le An River and at the same time as the water samples. Sediment samples were stored at 4°C in the dark and used within four weeks. Sediment elutriate was prepared by extracting wet sediment with four volumes of distilled water and shaking at 200 rpm for 12 h, followed by isolation of the aqueous fraction by centrifugation at 2,500 g for 30 min at 4°C. After sampling, concentration of DOC was measured by high-temperature combustion method on a total organic carbon analyzer (TOC; Phoenix 8000; Tekman Dokmann, Cincinnati, OH, USA). Major anions were measured by ion chromatography (Series 4500i, Dionex, San Francisco, CA, USA). The FA was extracted and separated from Le An River sediment and purified according to the method developed by Wang [18].

Stock solution of Cu was prepared in double-distilled, deionized water (ddH2O) from analytical Cu sulfate and acidified with HNO3 (10−2 mol/L). The test solutions were prepared by diluting the stock solution with reconstituted water con-
Chemical measurements

The DAM-DPASV measurements were performed with a Model 303A static mercury drop electrode assembly plus a Model 303 stirrer coupled with a polarographic analyzer (Model 263; EG&G, Princeton, NJ, USA). The mercury electrode used as the working electrode was washed with 10% nitric acid and dddH2O twice. To avoid metal contamination, all plastic and glassware were washed with 1:1 (w/w) nitric acid followed by dddH2O. In all experiments, sodium nitrate (0.1 mol/L) was used to maintain a constant ionic strength of the solutions. The specific setting used for the differential pulse mode was as follows: scan rate, 10 mV/s; drop size, medium; pulse height, 50 mV; step time, 0.3 s; scan width, 2 mV; initial potential, −1.0 V; and final potential, +0.15 V versus Ag-AgCl. A rotation rate of 4,000 rpm was used. During each step of titration, the electrode surface was wiped clean with plain filter paper and rinsed with dddH2O. Measurements were carried out in 10 ml of working solution in a cell at 23 ± 1°C. Then, the samples were degassed with oxygen-free N2 for 15 min. Samples were plated at −1.0 V for 300 s, followed by a 15-s quiescent period before the film was stripped by scanning the potential in the positive direction, using the differential pulse mode, to a final potential of +0.15 V. The double-acidification procedure involved a deposition at pH 2 for 20 s; then, before completion of the 300-s deposition and with the cell circuit uninterrupted, 50 μL of HNO3 (5 mol/L) were added through the standard addition port. After recording the stripping voltammogram, a new run was initiated for the same solution; this time, the pH during both deposition and stripping runs was adjusted to 2.0. The percentage of labile Cu in the total Cu could be obtained as

\[
\text{Labile-Cu} (%) = 100 \left[1 - \left( \frac{c_1 - c_2}{c_2} \right) \right]
\]

where \(c_1\) is the concentration of Cu measured with deposition and stripping at pH 2, \(c_2\) is the deposition at pH 6.8 and stripping at pH 2, and \(c_T\) is the total Cu concentration. The calibration was carried out at Cu concentrations ranging from 0.5 to 20 μg/L in 10 increments in reconstituted water (NaNO3, 0.1 mol/L; HEPES, 0.06 mol/L). For each sample, triplicates
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Table 2. Concentrations of main cations of water samples in Le An River (Jiangxi Province, China)

| Sample sites | Ca (mg/L) | K (mg/L) | Mg (mg/L) | Fe (mg/L) | Na (mg/L) | S (mg/L) | As (mg/L) | Sr (mg/L) | Cd (mg/L) | Cu (mg/L) | Zn (mg/L) | Mn (mg/L) | Al (mg/L) |
|--------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| L01          | 9.1      | 2.5      | 4.2      | ND       | ND       | ND       | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L02          | 24.6     | 18.5     | 12.5     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L03          | 2.2      | 4.2      | 10.4     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L04          | 3.0      | 4.7      | 12.7     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L05          | 2.1      | 4.7      | 12.7     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L06          | 2.8      | 4.7      | 12.7     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L07          | 8.6      | 4.7      | 12.7     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L08          | 28.5     | 4.7      | 12.7     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |
| L09          | 10.7     | 4.7      | 12.7     | 15.7     | 13.0     | 31.6     | ND       | ND       | ND       | ND       | ND       | ND       | ND       |

The concentrations of Zn in water samples were measured in acidified filtrates by a graphite furnace atomic absorption spectrometer (Model 3100; Perkin-Elmer, Norwalk, CT, USA). Concentrations of Cu, Pb, and Zn in sediments were measured by graphite furnace atomic absorption spectrometer after microwave digestion [20].

Acute toxicity bioassays

Bioassays for the acute toxicity of Cu were performed on D. magna. The static bioassay procedure described by the U.S. Environmental Protection Agency guideline for test chemicals was adapted with triplicate samples [21]. The test species D. magna was obtained from the Institute of Hydrobiology (Wuhan, China) and adapted in the laboratory for a period of one month. Healthy D. magna, not more than 24 h old at the beginning of the test, did not have to be fed during the test. Ten animals were used for each concentration in the 50 ml of test solution in a 100-ml beaker. The test temperature was 23 ± 1°C. For the validity of the test, no more than 10% immobility (or mortality) of the animals was accepted as the control. The dissolved oxygen concentration at the end of the test was maintained at 60% or greater air saturation.

Data on 96-h median lethal concentration (LC50) determined by the immobilization (or mortality) tests were used as acute toxicity data. The LC50s and associated 90% confidence intervals for each replicate toxicity bioassay were calculated by trimmed Spearman-Karber analysis [22]. Toxic probit (TP) was transformed from mortality according to the method of probit unit [23]. Regression analysis was performed using Sigmaplot® 4.0 (SPSS, Chicago, IL, USA).

Cu speciation

The concentrations of different Cu species present in test solutions, other than that containing FA, could be calculated from the spiked concentrations of Cu, the composition of the reconstituted water, and the known stability constants of Cu complexes using MINTEQ A2 (Ver 4.1). For water samples and test solution containing FA, inorganic Cu speciation could be calculated from the labile concentration of Cu, assuming that Cu associated with strong organic ligands was nonlabile in DPASV measurements and that Cu associated with weak organic ligands was negligible in comparison to inorganic complexes of similar stability constants. This hypothesis is valid because DPASV has long been used to obtain the apparent stability constants of Cu with FA, both by metal titration and by proton titration [24].

RESULTS AND DISCUSSION

Deriving the expression for the relationship between Cu speciation and acute toxicity

Influences of alkalinity and different concentrations of EDTA and FA derived from the Le An River on the acute toxicity of Cu to D. magna are shown in Figure 1. Addition of NaHCO₃, EDTA, and FA to test solutions inhibited significantly the Cu toxicity. Similar observations on the inhibition effects of Cu to different organisms have been reported in previous studies [25].

The toxicity of Cu to D. magna increased with spiked concentrations of Cu and decreased with spiked concentrations of
NaHCO₃, EDTA, and FA. This suggests that the complexation of Cu with HCO₃⁻, EDTA, and FA reduced the free-ion concentration of Cu in the aqueous phase according to the FIAM model, thereby reducing the potential of Cu uptake by the organisms. Thus, Cu, when present in the complex form with HCO₃⁻, EDTA, and FA, could be considered as nonbioavailable or nontoxic.

When NaHCO₃ was spiked into the reconstituted water (Fig. 1a), the dominant species of Cu were Cu²⁺, CuOH⁺, Cu(OH)₂aq, CuCO₃aq, CuHCO₃⁺, CuCl⁻, CuCl₂, and CuSO₄aq. Under constant pH, the decrease in toxicity was due to the increase of CuCO₃aq and CuHCO₃⁺. The linear regression between LC50 (µg/L) and concentration of NaHCO₃ (mg/L) could be expressed as

\[ \text{LC50 (µg/L)} = 0.12\text{[NaHCO₃ (mg/L)]} + 12.22 \]

\[ (r² = 0.9796, n = 6) \]  

When EDTA was spiked into the reconstituted water (Fig. 1b), the dominant species of Cu were Cu²⁺, CuOH⁺, Cu(OH)₂aq, CuCO₃aq, CuHCO₃⁺, CuCl⁻, CuCl₂, and CuSO₄aq, and Cu-EDTA complexes. When the mole concentration of EDTA was greater than that of Cu, Cu precipitation other than Cu-EDTA could be negligible. Under constant pH and alkalinity, the decrease in toxicity was likely due to the increase in Cu-EDTA concentration. The linear regression between LC50 (µg/L) and concentration of EDTA (mg/L) could be expressed as

\[ \text{LC50 (µg/L)} = 234.63\text{[EDTA (mg/L)]} + 12.85 \]

\[ (r² = 0.9926, n = 6) \]  

When FA was spiked into the reconstituted water (Fig. 1c), the dominant species of Cu were Cu²⁺, CuOH⁺, Cu(OH)₂aq, CuCO₃aq, CuHCO₃⁺, CuCl⁻, CuCl₂, CuSO₄aq, and Cu-FA complexes. The decrease in toxicity was due to the increase in Cu-FA concentration. The linear regression between LC50 (µg/L) and concentration of FA (mg/L) could be expressed as

\[ \text{LC50 (µg/L)} = 16.52\text{[FA (mg/L)]} + 17.81 \]

\[ (r² = 0.9919, n = 4) \]  

For test solutions in which both NaHCO₃ and FA were present, LC50s under different concentrations of NaHCO₃ and FA were obtained, and the results are shown in Figure 2. By multiregression analysis, the relationship between LC50 and concentrations of NaHCO₃ and FA could be expressed as

\[ \text{LC50 (µg/L)} = 17.55\text{[FA (mg/L)]} + 0.21\text{[NaHCO₃ (mg/L)]} + 11.79 \]

\[ (4) \]

The intercept in Equation 1 represents the bioavailable concentration of Cu that causes the LC50 for \( D. \ magna \), which should be 12.22 µg/L. Equations 2 and 3 indicate that the LC50s for Cu in reconstituted water containing 48 mg/L of NaHCO₃ in absence of EDTA or FA should be 12.85 and 17.81 µg/L, respectively. If the influence of carbonate is excluded, the bioavailable concentration of Cu that causes the LC50 for \( D. \ magna \) should be 7.09 and 12.05 µg/L from Equations 2 and 3, respectively. In Equation 4, the LC50 or bioavailable concentration of Cu in the absence of alkalinity and FA should be 11.79 µg/L, which was quite similar to those in Equations 1 and 3. In addition, from Equation 4, the influence of FA on toxicity is 83-fold greater than that of alkalinity.

In our model, [Cu*] in test solution consists of inorganic species other than CuCO₃aq and CuHCO₃⁺. By definition, LC50s could be expressed as the total spiked concentration of Cu ([Cu₅₄]), concentration of free-ion Cu ([Cu²⁺]), and [Cu*]. The expressions are illustrated in Figure 1. The LC50 expressed as [Cu₅₄] is a function of alkalinity and organic components. However, LC50s expressed as either [Cu²⁺] or [Cu*] could give a constant value, independent of variation in alkalinity and organic components. In fact, [Cu²⁺] was proportional to [Cu*] under constant alkalinity. The LC50 could be predicted by FIAM or BLM models regarding [Cu²⁺]. However, the difficulty in using FIAM or BLM models to predict Cu toxicity in natural water is due to determination of the site-specific apparent complexation constant for Cu-DOC or Cu-FA complexes. Therefore, we could use [Cu*] to predict the acute toxicity of Cu on \( D. \ magna \). By excluding Cu-carbonate complexes from the labile concentration, [Cu*] was obtained and used to predict the acute toxicity of Cu on \( D. \ magna \). By performing a DAM-DPASV measurement on natural water sample, the labile concentration of Cu was obtained, which consists mainly of inorganic Cu species. The value of [Cu*] could be obtained by exclusion of Cu-carbonate complexes using MINTEQ A2 calculation based on measured composition of anions in the waters.

The available toxicity data (except in cases where mortality was <10% or >90%) obtained from laboratory experiments with different concentrations of NaHCO₃, EDTA, and FA were transformed into TP, and the relationship between TP and [Cu*] was obtained by linear regression analysis (Fig. 3). The regression equation could be used in predicting Cu toxicity in natural water, where mortality can hardly be exactly 50%:

\[ TP = 2.86 \log([Cu*]) \text{ (µg/L)} + 1.75 \]

\[ (r² = 0.4695, n = 28) \]  

Measuring water-quality parameters and acute toxicity

The pH, concentrations of cations and anions, concentrations of heavy metal pollutants, and concentration of DOC (mg-C/L) in Le An River waters are shown in Tables 1 and 2. The copper concentration was highest at site L04 (73.2 µg/L) but lower at site L06 (16.6 µg/L). Previous investigation indicated that, among heavy metals, aqueous concentrations of Cu, Pb, and Zn during the dry season [15] and of Cu during the rainy season [26] exceeded the environmental quality standards for surface water (GB 3838-88, China).

The regional backgrounds of metals in Le An River sediments were 34, 117, and 45 mg/kg for Pb, Zn, and Cu, respectively. Accordingly, the Le An River sediments were heavily polluted by heavy metals (Table 3). Metals that had sediment concentrations significantly greater than their background levels were Zn, Pb, and especially, Cu. At site L04, the concentration of Cu reached 2,878 mg/kg (the highest level), and at site L07, the concentration of Cu was 1,012 mg/kg.

In most of the natural freshwaters, Cu was mainly bound to organic substances [12]. Because of the presence of naturally derived organic matter, the DPASV labile concentrations of Cu could not be directly measured in water samples from several other locations (data not shown). The DAM was applied to eliminate the effects of complexing agents on the ASV process and of surfactants on both deposition and stripping processes in natural water [14]. The labile concentration of Cu was obtained from the measured stripping current in DAM-DPASV. From the labile concentration, the bioavailable concentrations of Cu could be obtained.
Results of the acute toxicity bioassays for the Le An River waters are shown in Table 4. The river water was slightly toxic at site L04 (mortality, 6.7%) and extremely toxic at site L06 (mortality, 100%). Mortality predicted by the bioavailable concentration of Cu using Equation 5 was quite consistent with that measured using bioassays for Le An River waters (Table 4). At sites L01 to L05 and L07 to L09, toxicities could be neither measured nor predicted, because the mortality of less than 10% was considered to be insignificant.

Because the highest concentration of Cu was observed at site L04, not at site L06, some other factors should affect the toxicity to D. magna. Among these factors, concentration of DOC was the highest at site L04 (3 mg/L), whereas it was undetectable at site L06. Consequently, the highest labile concentration of Cu (14.7 μg/L) was observed at site L06, not at site L04. The influence of DOC appears to be responsible for the reduced toxicity at site L04. A mortality of 100% occurred at site L06, where a mortality of 45% was predicted based on the toxicity of Cu. The observed toxicity at site L06 could also be caused by the joint toxic effect of Cu and Zn. The toxic equivalent of Zn at site L06 was 2.6, as calculated from the measured concentration of Zn divided by its LC50 (540 μg/L in reconstituted water). The toxic equivalent of Cu (LC50 = 13.6 μg/L in reconstituted water) was 1.98, which was similar to that of Zn. The influences of other metals on toxicity could be negligible.

Table 3. Concentrations (mg/kg dry wt) of Cu, Pb, and Zn in sediments from different sampling sites in the Le An River (Jiangxi Province, China)

| Element | L01 | L02 | L04 | L05 | L06 | L07 | L08 | L09 |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| Cu      | 36  | 215 | 2,878 | 2,173 | 1,788 | 1,012 | 733 | 523 |
| Pb      | 44  | 81  | 42  | 44  | 29  | 208  | 94  | 68  |
| Zn      | 117 | 226 | 221 | 201 | 202 | 878  | 664 | 451 |

| [Cu]_\text{Meas} (μg/L) | [Cu]_\text{Lab} (μg/L) | [Cu]* (μg/L) | Measured mortality (%) | Predicted mortality (%) |
|-------------------------|-------------------------|---------------|------------------------|-------------------------|
| L01                     | 5.3                     | ND            | ND                     | 0                       |
| L02                     | 5.3                     | 2.6 ± 0.7     | ND                     | 0                       |
| L04                     | 73.2                    | ND            | ND                     | 0                       |
| L05                     | 5.1                     | 1.3 ± 1.3     | 0.6                    | ND                      |
| L06                     | 16.6                    | 14.7 ± 2.5    | 11.5                   | 100 ± 18                | 45                      |
| L07                     | 6.8                     | 5.1 ± 3.2     | 3.8                    | ND                      |
| L08                     | 6.8                     | 5.8 ± 1.9     | 4.5                    | ND                      |
| L09                     | 5.1                     | 3.8 ± 0.6     | 3.2                    | ND                      |

a The concentration of total Cu ([Cu*]) was measured by graphite furnace atomic absorption spectrometry.

b The labile concentration of Cu ([Cu]_\text{Lab}) was measured by a differential pulse anodic stripping voltammetry with a double-acidification method.

c The bioavailable concentration of Cu ([Cu]*) was calculated from [Cu]_\text{Lab} and concentration of anions in water using MINTEQ A2 (developed by the U. S. Environmental Protection Agency).

d The mortality was measured on D. magna (n = 3).

e The mortality was predicted using Equation 5 (see text).

f ND = not detected.
Table 5. Concentrations of total Cu ([Cu]) and bioavailable Cu ([Cu*]) and predicted/measured mortality on *Daphnia magna* in sediment elutriates down the Le An River (Jiangxi Province, China)

| Sampling sites | [Cu] (µg/L) | [Cu*] (µg/L) | Predicted mortality (%) | Measured mortality (%) |
|---------------|-------------|--------------|------------------------|------------------------|
| L01           | ND          | ND           | 0                      | ND                     |
| L04           | 3.8         | 2.6 ± 1.3    | 1                      | 7 ± 3                  |
| L06           | 7.7         | 6.4 ± 1.3    | 19                     | 87 ± 12                |
| L07           | 249.8       | 152.9 ± 19.2 | 100                    | 100 ± 21               |
| L08           | 7.1         | 4.5 ± 1.3    | 8                      | 27 ± 7                 |
| L09           | 9.6         | 5.8 ± 3.2    | 13                     | 7 ± 5                  |

* Measured by graphite furnace atomic absorption spectrometry.
* Calculated from [Cu_{labile}] and measured alkalinity in elutriate (data not shown).
* The mortality was measured on *Daphnia magna* (n = 3).
* The mortality was predicted using Equation 5 (see text).

excluded by defining the bioavailable concentration of Cu, or [Cu*], which could be obtained by a DAM-DPASV measurement and a calibration to exclude Cu-carbonate complexes from labile Cu species. The relationship between [Cu*] and toxicity to *D. magna* could be formulated through laboratory simulation experiments in the presence of NaHCO₃, EDTA, and FA. This relationship could be then be applied to predict the acute toxicity of Cu in the natural water and sediment elutriates from a river that has been severely polluted by a copper mine. The bioavailable concentration of Cu that causes 50% of mortality on *D. magna*, both in the laboratory experiments and under field conditions, was approximately 12 µg/L.

The proposed procedure provides an alternative to the FIAM model. Furthermore, the bioavailable concentration of Cu, or [Cu*], was used to test the free-ion activity in the prediction of toxicity. The information presented in this paper provides a means to measure the bioavailable concentration of Cu in natural water by combining DAM-DPASV measurement and a model calculation to exclude the influences of Cu-carbonate complexes.

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Table 6. Concentrations of bioavailable Cu ([Cu*]) and predicted/measured mortality on *Daphnia magna* in the sequential dilutions of elutriate from site L07 in Le An River (Jiangxi Province, China)

| Dilution proportion | [Cu*] (µg/L) | Predicted mortality (%) | Measured mortality (%) |
|---------------------|-------------|------------------------|------------------------|
| 1/1                 | 152.9 ± 19.2| 100                    | 100 ± 21               |
| 1/2                 | 98.6 ± 14.7 | 100                    | 100 ± 12               |
| 1/4                 | 49.3 ± 8.3  | 95                      | 100 ± 34               |
| 1/8                 | 23.7 ± 5.8  | 75                      | 67 ± 10                |
| 1/16                | 9.6 ± 4.5   | 44                      | 47 ± 8                 |
| 1/32                | 6.4 ± 2.6   | 18                      | 20 ± 6                 |

* Calculated from labile Cu and measured alkalinity in elutriate (data not shown).
* The mortality was measured on *Daphnia magna* (n = 3).
* The mortality was predicted using Equation 5 (see text).
19. Vasconcelos MT, Azenha MA, Lage O, 1996. Electrochemical evidence of surfactant activity of the Hepes pH buffer which may have implication on trace metal availability to culture in vitro. Anal Biochem 241:248–253.
20. Wen XH, Wu LZ, Zhang Y, 1997. Optimized microwave preparation procedure for the elemental analysis of aquatic sediment. Fresenius J Anal Chem 357:1111–1115.
21. U.S. Environmental Protection Agency. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-660/3-75/009. Washington, DC.
22. Hamilton MA, Russo RC, Thurston RV, 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719, Correction 12:417 (1978).
23. Zhou YX, Zhang ZS. 1989. Methods For Aquatic Bioassays. Agricultural Publishing House, Beijing, China.
24. Wang Z, Stumm W, 1987. Heavy metal complexation by surfaces and humic acids: A brief discourse on assessment by acidimetric titration. Neth J Agric Sci 35:231–240.
25. Deaver E, Rodgers JH, 1996. Measuring bioavailable copper using anodic stripping voltammetry. Environ Toxicol Chem 15:1925–1930.
26. Wang Z, Ma M, Du Q, 1994. Toxicity assessment by photobacterium phosphoreum. China J Environ Sci 5:159–164.