

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

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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 MINTEQA2 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 divalent 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 technique 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 MINTEQA2 software (developed by the U.S. Environmental Protection Agency) and will exclude Cu-carbonate complexes from labile speciation of Cu.

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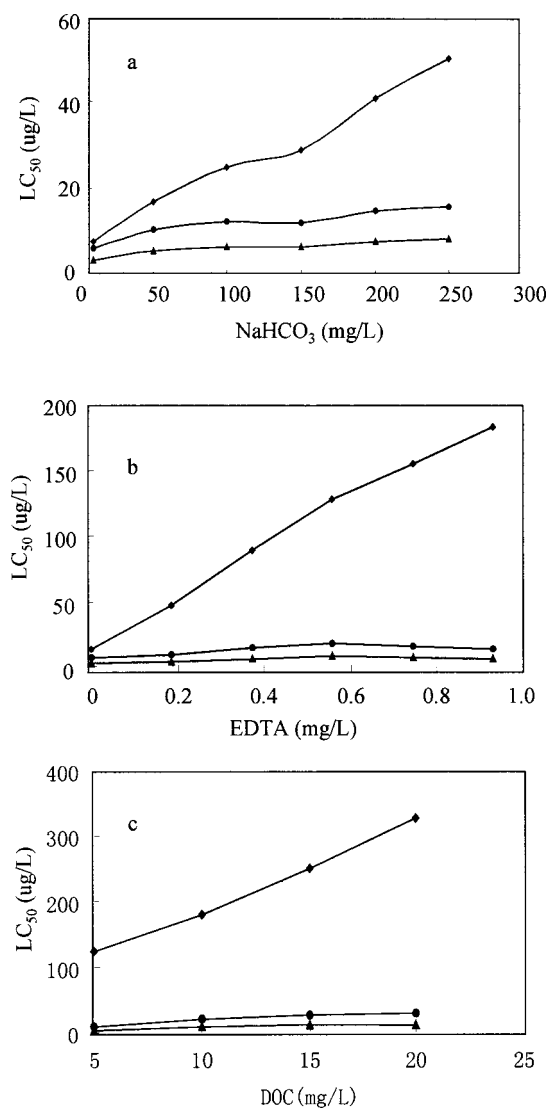


Fig. 1. Median lethal concentrations (LC50s) expressed as total Cu concentrations (\blacklozenge), free Cu concentrations (\blacktriangle), and bioavailable Cu (\bullet) as a function of ligand concentrations. Three different ligands were used: (a) NaHCO_3 , (b) ethylenediaminetetraacetic acid (EDTA), and (c) fulvic acid (FA) expressed as dissolved organic carbon (DOC).

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-

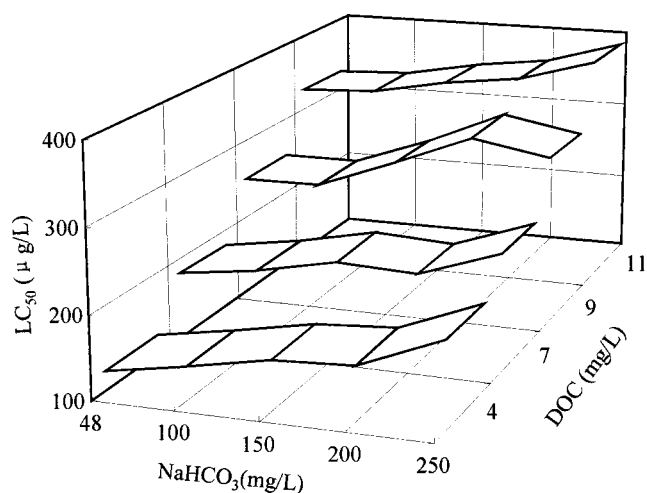


Fig. 2. Toxicity of Cu to *Daphnia magna* as a function of alkalinity (expressed as NaHCO_3), fulvic acid, and dissolved organic carbon (DOC). The median lethal concentrations (LC50s) are expressed in terms of total Cu concentrations.

μm filter (Bedford, MA, USA) and collected in a pre-cleaned 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 $2,500\text{ g}$ 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 Dorkmann, 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, de-ionized water (ddH_2O) from analytical Cu sulfate and acidified with HNO_3 (10^{-4} mol/L). The test solutions were prepared by diluting the stock solution with reconstituted water con-

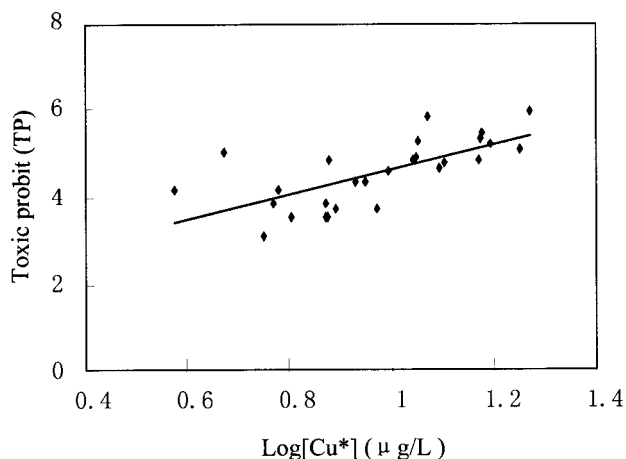


Fig. 3. Relationship between toxic probit (TP) and logarithmic concentration of bioavailable Cu ($[\text{Cu}^*]$). Toxic data ($n = 28$) were collected from Figures 1 and 2.

taining NaHCO₃ (48 mg/L), MgSO₄ (30 mg/L), CaSO₄·2H₂O (30 mg/L), and KCl (2 mg/L) before use. The test solutions of different alkalinity (expressed as [NaHCO₃]) or containing different amounts of ethylenediaminetetraacetic acid (EDTA), and/or FA were prepared by adding appropriate amounts of stock solutions of NaHCO₃, Na-EDTA, FA, and Cu to the reconstituted water. Concentration of FA (mg-C/L) was measured after filtering through 0.45-μm membrane filters, and TOC was measured. All test solutions were buffered with N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES; Sino-American Biotec, Beijing, China), and the pH was adjusted with NaOH (0.5 mol/L) to 6.8 before use. In the test solutions, the concentration of HEPES was 0.006 mol/L. It has been demonstrated that HEPES, when present in concentrations of less than 10 mmol/L, forms weak complexes with copper and does not interfere with copper speciation in solution [19]. The test solution was allowed to equilibrate for at least 24 h at a constant temperature of 23 mean ± SEM 1°C before DAM-DPASV measurements and bioassay.

The DAM-DPASV measurements were performed to obtain the labile concentrations of Cu. Bioassays using *D. magna* were performed to obtain the acute toxicity in the test solutions and in river water samples and sediment elutriates.

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 6.8 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

$$\text{Labile-Cu (\%)} = 100[1 - (c_1 - c_2)/c_T]$$

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

Table 1. Physicochemical properties and concentrations (mg/L) of main anions of water samples in Le An River (Jiangxi Province, China)

Sampling sites	pH	Inorganic carbon	Total hardness	DOC ^a	HCO ₃ ⁻	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻	F ⁻	PO ₄ ³⁻
L01	7.0	10	102.34	1.1	0.75	3.4	1.2	2.1	ND ^b	0.34
L02	6.6	9	148.39	ND ^b	0.64	3.0	25.5	43.1	ND	0.12
L04	3.2	4	317.25	3.0	ND	4.4	97.9	272.4	0.6	0.06
L05	6.9	9	189.33	2.0	0.75	3.3	6.2	72.4	0.1	0.17
L06	7.1	11	199.56	ND	1.06	4.4	3.6	113.8	0.3	0.14
L07	7.1	8	143.28	ND	0.68	3.3	4.1	146.6	0.1	0.19
L08	7.1	7	179.10	ND	0.58	2.8	6.4	41.4	0.1	0.26
L09	7.1	9	133.04	1.0	0.87	7.3	2.6	22.4	0.1	0.34

^a DOC = dissolved organic carbonate.

^b ND = not detected.

Table 2. Concentrations of main cations of water samples in Le An River (Jiangxi Province, China)

Sam- pling sites	mg/L										μg/L									
	Ca	K	Mg	Fe	Na	As	S	Sr	Cd	Cu	Zn	Mn	Al	Ni	Cr	Pb				
L01	9.1	2.5	2.0	ND ^a	3.1	0.20	0.96	0.04	0.14	5.3	8	ND	28.2	6.9	5.4	53.7				
L02	24.6	3.8	4.2	ND	4.7	0.08	15.17	0.16	ND	5.2	ND	0.14	14.7	7.7	2.1	78.5				
L04	72.2	15.0	12.6	1.3	8.6	0.03	97.48	0.91	ND	73.2	16	0.43	1697.2	12.6	9.4	63.0				
L05	33.0	6.2	4.7	ND	4.8	0.02	27.79	0.31	3.16	5.1	ND	ND	27.1	7.4	2.8	49.8				
L06	29.9	3.7	7.7	ND	18.1	0.08	37.24	0.11	7.83	16.6	2392	1.11	13.4	19.7	0.9	55.7				
L07	28.5	5.3	5.4	ND	7.6	0.11	27.22	0.21	0.01	6.8	349	0.32	27.0	8.2	2.7	76.2				
L08	21.8	4.7	3.6	ND	5.2	0.04	18.21	0.15	ND	6.8	17	0.01	13.4	7.4	5.1	50.5				
L09	10.7	2.8	2.2	ND	6.5	0.07	4.96	0.04	ND	5.1	ND	ND	26.7	ND	ND	58.3				

^a ND = none detected.

measurements were taken, and the labile concentration of Cu was calculated from the calibration curve. Precision for measurements in DAM-DPASV was $\pm 9\%$ relative standard deviation for test solution containing Cu at 2 μg/L ($n = 6$).

The total concentrations of dissolved Cu, Pb, and 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 $23 \pm 1^\circ\text{C}$. 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-Kärber 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 MINTEQA2 (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

$$\text{LC50 } (\mu\text{g/L}) = 0.12[\text{NaHCO}_3 \text{ (mg/L)}] + 12.22$$

$$(r^2 = 0.9796, n = 6) \quad (1)$$

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, CuSO₄aq, and Cu-EDTA complexes. When the mole concentration of EDTA was greater than that of Cu, Cu speciation 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

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

$$(r^2 = 0.9926, n = 6) \quad (2)$$

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

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

$$(r^2 = 0.9919, n = 4) \quad (3)$$

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

$$\text{LC50 } (\mu\text{g/L}) = 17.55[\text{FA (mg/L)}]$$

$$+ 0.21[\text{NaHCO}_3 \text{ (mg/L)}] + 11.79 \quad (4)$$

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 ([Cu_T]), concentration of free-ion Cu ([Cu²⁺]), and [Cu*]. The expressions are illustrated in Figure 1. The LC50 expressed as [Cu_T] 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 MINTEQA2 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%:

$$\text{TP} = 2.86 \log[\text{Cu}^*] \text{ (}\mu\text{g/L)} + 1.75$$

$$(r^2 = 0.4695, n = 28) \quad (5)$$

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.

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	Sampling site							
	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

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.

Sediments from different sampling sites were used to obtain sediment elutriates, and elutriates were used for acute bioassay and DAM-DPASV measurements. The experiment could simulate the sediment toxicity under aerobic conditions. The total dissolved and labile concentration of Cu in sediment elutriate from the Le An River and the observed toxicity to *D. magna* are shown in Table 5. The toxicity to *D. magna* was predicted from the [Cu*] (Table 5). As shown in Table 5, both the highest

soluble and bioavailable concentrations of Cu occurred at site L07, and both measured and predicted toxicities indicated a mortality of 100% at this site. A mortality of 87% was also observed in sediment elutriate from site L06, where only moderate mortality (19%) was predicted based on the measured [Cu*].

The concentration of Cu in sediment from site L07 was lower than that from site L06 (Table 3), whereas the total dissolved and labile concentration of Cu from site L07 was higher. This indicates that Cu bound to sediment at site L07 was more mobile and bioavailable and that the sediment toxicity could hardly be predicted by its total metal concentration. This suggests that both mobility and bioavailability be taken into account.

To obtain the LC50 of sediment elutriate from site L07, elutriate was sequentially diluted for bioassay. The results of toxic bioassay and DAM-DPASV measurement for 50% sequential dilution of sediment elutriate from site L07 are shown in Table 6. The data show both measured and predicted mortalities of *D. magna* with regard to [Cu*]. As can be seen, the mortality of *D. magna* in the diluted sediment elutriate could be well predicted by assuming that Cu was the toxicant.

From Table 6, it is quite interesting to note that the LC50 for [Cu*] in sediment elutriate was approximately 11.64 µg/L, which is quite close to the laboratory-observed [Cu*] that caused the LC50 in reconstituted water (13.6 µg/L). This indicates that the LC50 expressed as [Cu*] to *D. magna* in freshwater was approximately 12 µg/L, independent of water-quality variations. Similarly, the lowest-observed-effective concentration should be 2.3 µg/L.

CONCLUSIONS

Alkalinity, EDTA, and FA have significant influences on the acute toxicity of Cu to *D. magna*. These influences could be

Table 4. Concentrations of different Cu species and predicted/observed mortality on *Daphnia magna* in the Le An River waters (Jiangxi Province, China)

Sampling sites	[Cu _T] (µg/L) ^a	[Cu _{labile}] (µg/L) ^b	[Cu*] (µg/L) ^c	Measured mortality (%) ^d	Predicted mortality (%) ^e
L01	5.3	ND ^f	ND	ND	0
L02	5.3	2.6 ± 0.7	ND	ND	0
L04	73.2	ND	ND	6.7 ± 3.5	0
L05	5.1	1.3 ± 1.3	0.6	ND	5
L06	16.6	14.7 ± 2.5	11.5	100 ± 18	45
L07	6.8	5.1 ± 3.2	3.8	ND	8
L08	6.8	5.8 ± 1.9	4.5	ND	3
L09	5.1	3.8 ± 0.6	3.2	ND	4

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

^b The labile concentration of Cu ([Cu_{labile}]) 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_{labile}] and concentration of anions in water using MINTEQA2 (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_T]) 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 _T] (μg/L) ^a	[Cu*] (μg/L) ^b	Predicted mortality (%) ^c	Measured mortality (%) ^d
L01	ND ^e	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

^a Measured by graphite furnace atomic absorption spectrometry.

^b Calculated from [Cu_{labile}] and measured alkalinity in elutriate (data not shown).

^c The mortality was measured on *D. magna* ($n = 3$).

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

^e ND = not detected.

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 replace 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) ^a	Predicted mortality (%) ^b	Measured mortality (%) ^c
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

^a Calculated from labile Cu and measured alkalinity in elutriate (data not shown).

^b The mortality was measured on *D. magna* ($n = 3$).

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

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