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Application of anodic stripping voltammetry to predict the bioavailable/toxic concentration of Cu in natural water[☆]

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Abstract

In this paper, the concentration of labile Cu measured by differential pulse anodic stripping voltammetry with a double acidification method (DAM-DPASV) was used to predict the growth inhibition of Cu on the alga *Scenedesmus obliquus* (*S. obliquus*). The influences of alkalinity, Cl^- , and different concentrations of ethylene diamine tetraacetic acid (EDTA) and fulvic acid (FA) on the labile concentration and toxicity of Cu were investigated in reconstituted water and natural water. The results showed that Cl^- and alkalinity did not affect labile Cu concentration, but decreased the toxicity of Cu to *S. obliquus* in the Cu concentration range observed in fresh water. The influences of alkalinity and Cl^- could be modeled if Cu-carbonate complexes and Cu-chloride complexes were considered as non-toxic species. Organic complexes of Cu with EDTA and FA did not contribute either to the labile concentration of Cu or to the toxicity of Cu on *S. obliquus*. The growth inhibition of Cu on *S. obliquus* in natural water could be predicted by using the bioavailable concentration of Cu, or $[\text{Cu}^*]$, which could be calculated from the labile Cu concentration using the DAM-DPASV method and the concentrations of Cu-carbonate and Cu-chloride complexes obtained from the MINTEQA2 model.

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1. Introduction

Bioavailability and toxicity of trace metals to aquatic organisms depend on the physical and chemical speciation of metals (Allen and Hansen, 1996). Therefore the species of a metal, rather than its total concentration, is the key to understanding its effect on the biota (Allen, 1993). Previous studies on several trace metals and species of aquatic organisms have demonstrated that the organism's response to metals could be modeled by using its free ion activity (FIAM) (Morel, 1983; Morel and Hering, 1993; Campbell, 1995). The FIAM has generally been interpreted to imply that a constant degree of biological effects will occur at a constant che-

mical activity of the free ion of a metal (e.g. divalent transition-metal cations). These studies have led to the conviction that the concentration of free ionic metal is the key factor on metal toxicity. While it might be tempting to conclude that the free-ion alone is responsible for toxicities observed, free-ion activity does not appear to be a good predictor of toxicity across some water quality conditions (e.g. water hardness and dissolved organic matter), and does not explain competition between the metal ions and other cations (Playle et al., 1992; Erickson et al., 1996). Based on these considerations, biotic-ligand model (BLM), a recently proposed surface-interaction model of metal binding to fish gill, simultaneously incorporates trace metal speciation and competition between the concerned metal ion and other cations in the toxicity assessment (Di Toro et al., 2001; Santore et al., 2001). Thus, adopting BLM could help predict the acute toxicity of Cu to fish on a mechanistic basis and in various conditions of water hardness. But there are a number of factors that should

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be investigated to improve the model's predictability (US Environmental Protection Agency, 1999). For example, in testing the model's ability to predict Cu toxicity, it is assumed that DOC is 10% humic acid and the conditional stability constant of Cu with humic acid is not a site-specific parameter (US Environmental Protection Agency, 2000).

Anodic stripping voltammetry (ASV) is a powerful technique for trace element speciation study, and can be used to provide information regarding the labile/inert metal fraction (Florence, 1986). This procedure therefore gives a good approximation of electro-active metal speciation, which is based on the deposition of labile metals on the surface of an electrode according to the kinetics of their uptake processes by cells of organisms (Morrison et al., 1990; Florence et al., 1992). The correlation between ASV-labile concentrations of Cu and toxicity derived from bioassays may be useful in predicting the Cu toxicity. Some aquatic components, such as FA, can affect the stripping current not only through changes in aqueous Cu speciation, but also through adsorption of organic components on the surface of the electrode (e.g. mercury drop). To avoid the interferences of organic components on stripping current, a double acidification method (DAM) has been recommended (Florence, 1992). Generally, strong organic complexes, such as Cu-EDTA and Cu-FA, are neither labile nor bioavailable to organisms (Allen and Hansen, 1996). Weak organic complexes are not competitive to inorganic complexes because of much lower

ligand concentrations in natural waters. It could be reasonably assumed that the labile concentration of Cu should consist of mainly inorganic species. However, only part of the inorganic species are bioavailable or are in toxic forms. Therefore, it is necessary to exclude the concentrations of Cu-carbonates and Cu-chlorides from the measured labile concentration and use a defined term, i.e. $[Cu^*]$, to predict the toxicity of Cu in a complex water system (Wang et al., 2003). In the study, this hypothesis was tested by Cu-spiking lake water and bioassay using the alga *S. obliquus*.

2. Materials and methods

2.1. Sampling and test solution preparations

Seven natural water samples were collected from Guanting Reservoir in the NW part of Beijing, which is the second largest drinking water source in Beijing. The sampling sites are illustrated in Fig. 1. Twenty liters of water sample were filtered through Millipore 0.45 μm filter and collected in a pre-cleaned polyethylene bottle. After sampling, the concentration of dissolved organic C (DOC) was measured by a high-temperature combustion method on a TOC meter (Phoenix 8000, Tekmar-Dohrmann, Ohio, USA). Major anions in water samples were measured by ion chromatography (Model 4500i, Dionex, CA, USA). The total concentrations of dissolved metals in water samples were measured in

Fig. 1. Schematic illustration of the research area and sampling sites.

acidified filtrates by graphite furnace atomic absorption spectrometry (GFAAS, Perkin-Elmer 3100, CN, USA).

A stock solution of Cu was prepared in double-distilled deionized water (dddH₂O) from analytical reagent (CuSO₄·7H₂O, Beijing Chem. Co.). The stock solution was acidified to 10^{−4} mol/l HNO₃. To prepare test solutions from natural water, Cu was sequentially spiked from the stock solution to the filtered water samples to a final concentration of 0.89 mg/l in 7 increments. The spiked water samples were stored in 4 °C and used in a week for the DAM-DPASV measurements and *S. obliquus* bioassay.

The test solutions containing concentrations of different alkalinity, chloride, EDTA, and FA were prepared by adding appropriate amounts of stock solutions of NaHCO₃, NaCl, Na-EDTA, and FA to reconstituted water with composition recommended by OECD (OECD, 1984). In the experiments, FA was extracted and separated from the sediments of Guanting Reservoir and purified following the method developed by Wang (1987). Humic substances are the major component of the natural organic C in water (Burkhard, 2000). Concentrations of FA (in units of mg/L DOC) in the stock solutions were measured after filtering stock solutions of FA through 0.45 µm membrane filters by high-temperature combustion (Phoenix 8000, Tekmar-Dohrmann, Ohio, USA). The test solutions were spiked with increasing doses of Cu from its stock solution. All test solutions (including synthetic solutions and spiked samples) were buffered with *N*-[2-hydroxyethyl]-piperazine-*N'*-[2-ethanesulfonic acid] (HEPES, Sino-American Biotec, Beijing). The pH was adjusted with 0.5 mol/l NaOH to 6.8 prior to use. Concentration of HEPES was 0.006 mol/L in test solutions. It has been demonstrated that HEPES (<0.01 mol/l) forms weak complexes with Cu and does not interfere with Cu speciation in solution (Vasconcelos et al., 1996). The test solutions were allowed to equilibrate for at least 24 h at a constant temperature of 23 ± 1 °C before DAM-DPASV measurements and bioassay.

2.2. Measurements of the total and labile concentrations of Cu

The instrument consisted of an EG & G Princeton Applied Research Model 263 polarographic analyzer, with a Model 303 static Hg drop electrode assembly plus a Model 303 stirrer (EG&G, NJ, USA). In the voltammetry measurements, the Hg electrode as a working electrode was washed with 10% HNO₃ and dddH₂O twice. To avoid metal contamination, all glass and plastic ware were washed with 1:1 HNO₃ followed by dddH₂O. In all experiments, 0.1 mol/l NaNO₃ was used to keep ion strength. Measurements were carried out in 10 ml working solution in a cell at 23 ± 1 °C. The specific setting used for the differential pulse mode was:

scan rate of 10 mV/s; drop size, medium; pulse height of 50 mV; step time of 0.3 s; and scan width of 2 mV; initial potential, −1.0 V; final potential, +0.15 V versus Ag–AgCl. A rotating rate of 4000 rpm was used. During each step of titration, the electrode surface was wiped clean with plain filter paper and rinsed with dddH₂O. Ten millilitre aliquots of the sample were transferred to an acid-cleaned polarographic cell. Then, the samples were degassed with O₂-free N₂ for 15 min. The 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 (DPASV). The double acidification procedure (DAM) involved a deposition at pH 6.8 for 20 s before completion of the 300 s deposition, and with the cell circuit uninterrupted. Fifty microlitres of 5 mol/l HNO₃ were added through the standard addition port to the cell. After recording the stripping voltammogram, a new run was initiated for the same solution; this time the pH of the solution during both deposition and stripping run was below pH 2.0. The electrode labile concentration was calculated according to previous work (Florence, 1992). The Cu measurement of reconstituted water (0.1 mol/l NaNO₃ and 0.006 mol/l HEPES) gave a linear relationship between the peak height and Cu concentrations of 0.5–20 µg/l in 10 increments. Triplicate samples were measured for each solution, and the average was used to determine the labile concentration of Cu. The precision of labile Cu measurement by the DAM-DPASV method was ±9% in the test solution with 2 µg/l Cu (*n* = 6).

Labile concentrations of Cu in spiking test solutions (0.006 mol/l HEPES and 0.1 mol/l NaNO₃) and natural water samples (0.006 mol/l HEPES and 0.1 mol/l NaNO₃) were measured by DAM-DPASV. Total dissolved Cu in test solutions and natural water samples was also measured in acidified filtrates by graphite furnace atomic absorption spectrometry (GFAAS, Perkin Elmer 3100, CN, USA).

2.3. Bioassay using *Alga* (*S. obliquus*)

Stock culture of *S. obliquus* was maintained in media recommended by OECD (OECD, 1984). Twenty millilitres of medium were placed into 50 ml borosilicate Erlenmeyer flasks, pH adjusted (6.8) with HEPES buffering solutions. Copper was spiked to freshly prepared medium on the day of use, to give a gradient range of algal responses from no effect to total inhibition of growth. After allowing Cu to equilibrate in the water sample for 1 h, an aliquot of the stock cell culture was added to give inoculums of 5 × 10³ cells/ml. The flasks were placed on an 80 rpm oscillatory shaker in a chamber with a temperature at 23 ± 1 °C, and with a quantum flux of 4000 Lux at intervals of 12 h light and 12 h dark. After 96 h, OD values were measured using a spectrophotometer at

wavelength 681 nm (UV-120-02, Shimadzu, Japan). Median effective concentrations (EC_{50} s) were calculated using the Trimmed Spearman-Kärber method (Hamilton et al., 1977). Toxic probit was transformed to percentage of growth inhibition following the method of probit unit (Zhou and Zhang, 1989). Regression analysis was performed using Sigmaplot® 4.0 (SPSS, Chicago, IL, USA).

2.4. Cu speciation

The concentrations of Cu speciation in test solutions, other than that containing FA, can be calculated on the composition of ions in the reconstituted water and the spiked concentrations of Cu using MINTEQA2 (v4.1, U.S. EPA). The influences of other cations in the test solutions were ignored. Labile concentrations of Cu in the test solutions were measured prior to the addition of alga.

For spiked natural water samples and test solution containing FA, inorganic Cu species can be calculated from the labile concentrations of Cu measured, assuming that strong organic complexes were non-labile in the DAM-DPASV measurement and concentrations of

weak organic complexes are negligible. 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 proton titration (Florence, 1986; Wang and Stumm, 1987). For weak organic complexes, the concentrations of organic ligands in natural water are much lower than those of inorganic components, although their stability constants are close to each other.

Using the DAM-DPASV measurements, complexation capacity of Cu in Guanting Reservoir waters could be determined following the method described in earlier literature (Carter et al., 1992).

3. Results and discussion

3.1. Correlation between Cu speciation and toxicity to *S. obliquus*

The influences of different anions on the growth inhibition of Cu to *S. obliquus* have been screened in the experiments. The influences of alkalinity (expressed as concentration of HCO_3^-), Cl^- , EDTA and FA (expressed as DOC) on alga growth are shown in Fig. 2. The

Fig. 2. Variation in EC_{50} s expressed by different Cu speciation as a function of ligand concentration. Cu species were: ◆ $[Cu_T]$, ● $[Cu^{2+}]$, ▲ $[Cu^*]$. Different ligands were used: (a) $NaHCO_3$ expressed as HCO_3^- , (b) $NaCl$ expressed as Cl^- , (c) EDTA, (d) FA expressed as DOC.

inhibition on *S. obliquus* decreased with increasing spiked concentrations of carbonate, Cl^- , EDTA and FA. The addition of EDTA, and FA from the water samples of Guanting Reservoir, to test solution inhibited significantly the toxicity of Cu to *S. obliquus*. The influences of inorganic and organic components on the Cu toxicity to alga have been reported by a previous study (Oeaver and Rogers, 1996). In the current experiment, influences of phosphate on Cu toxicity were not tested, because a small modification of phosphate concentration might cause a significant increase in alga growth.

The results could be explained by the FIAM that the consequence of complexation of Cu with HCO_3^- , Cl^- , EDTA and FA was the reduction of free ion activity in the aqueous phase and thus reduced the potential of metal uptake by organisms. Similarly, the results could also indicate that the complexes of Cu with HCO_3^- , Cl^- , EDTA and FA were non-bioavailable or non-toxic. When NaHCO_3 was spiked into the reconstituted water (Fig. 2a), the dominant species of Cu were Cu^{2+} , CuOH^+ , $\text{Cu}(\text{OH})_2\text{aq}$, CuCO_3aq , and CuHCO_3^+ . Under constant pH, the decrease of toxicity should be caused by the increase of CuCO_3aq and CuHCO_3^+ . The linear regression between EC_{50} (mg/l) and the concentration of NaHCO_3 (mg/l) could be expressed as:

$$\text{EC}_{50}(\text{mg/l}) = 0.011 [\text{HCO}_3^-, \text{mg/l}] + 0.3849 (R^2 = 0.9032, n = 10) \quad (1)$$

The toxicity of Cu was significantly reduced when the concentration of Cl^- in aqueous phase was higher than 100 mg/l (Fig. 2b) and the decrease of toxicity correlated well with the increase of concentrations of $\text{CuCl}_n^{-(n-2)}$. The linear regression between EC_{50} (mg/l) and concentration of chloride (mg/l) could be expressed as:

$$\text{EC}_{50}(\text{mg/l}) = 0.0092 [\text{Cl}^-, \text{mg/l}] + 0.284 (R^2 = 0.8904, n = 8) \quad (2)$$

By classification analysis, $\text{CuCl}_n^{-(n-2)}$ complexes could be classified as non-toxic forms. Sarin et al. (2000) showed in their recent publication that the toxicity of Cu was a function of Cl^- concentration. The toxicity of Cu decreased at high Cl^- concentrations in accordance with decreased free Cu^{2+} concentration or increased $\text{CuCl}_n^{-(n-2)}$ complex concentration. Obviously, the results either support FIAM or could be explained as the consequence of formation of non-toxic chemical forms of $\text{CuCl}_n^{-(n-2)}$.

When EDTA was spiked into the reconstituted water (Fig. 2c), the dominant species was Cu-EDTA complexes. When the molar concentration of EDTA was

more than that of Cu, species other than Cu-EDTA could be negligible. Under constant pH and alkalinity, the decrease in toxicity should be due to the increase in Cu-EDTA concentration. The linear regression between EC_{50} (mg/l) and concentration of EDTA (mg/l) could be expressed as:

$$\text{EC}_{50}(\text{mg/L}) = 0.1507 [\text{EDTA, mg/L}] + 0.3477 (R^2 = 0.9454, n = 8) \quad (3)$$

When FA was spiked into the reconstituted water (Fig. 2d), the dominant species was Cu-FA complexes. Cu species other than Cu-FA decreased significantly. The decrease of toxicity should be caused by the increase in Cu-FA concentration or decrease on the free Cu ion concentration. The linear regression between EC_{50} (mg/l) and concentration of FA (expressed as DOC, mg/l) could be expressed as:

$$\text{EC}_{50}(\text{mg/L}) = 0.01 [\text{DOC, mg/l}] + 0.2634 (R^2 = 0.9341, n = 8) \quad (4)$$

For studying the influence of multi-components on the toxicity of Cu, the experiment was carried out accordance with $L_{15}(5)^3$ orthogonal experiment design, in which EC_{50} s under different concentrations of NaHCO_3 , Cl^- and FA were obtained. By multi-regression analysis, the relationship between EC_{50} and concentrations of HCO_3^- , Cl^- and DOC could be expressed as:

$$\text{EC}_{50}(\text{mg/l}) = 0.269 + 0.015 [\text{Cl}^-, \text{mg/l}] + 0.012 [\text{HCO}_3^-, \text{mg/l}] + 0.179 [\text{DOC, mg/l}] \quad (5)$$

It is interesting to note that the intercepts in the empirical Eqs. (1)–(5) are similar, i.e., from 0.26 to 0.37 mg/l. The coefficients in Eq. (5) represent the relative importance of each complexing ligand in natural waters. For example, it showed that 1 mg/l of FA has an equivalent influence on reduction of Cu toxicity as 14.9 mg/l of carbonate

In the authors' hypothesis, only part of the inorganic Cu species contributes to the toxicity. A bioavailable concentration of Cu (or, $[\text{Cu}^*]$) is defined to denote the sum of inorganic species other than Cu-carbonate and Cu-chloride complexes. The calculation formula for the bioavailable concentration of Cu (or, $[\text{Cu}^*]$) could be expressed as

$$[\text{Cu}^*] = [\text{Cu}_{\text{labile}}] - [\text{CuCl}_n^{-(n-2)}] - [\text{CuCO}_3\text{aq}] - [\text{CuHCO}_3^+]. \quad (6)$$

Therefore, the intercepts in Eqs. (1)–(5) represent the bioavailable concentration of Cu in solutions of different compositions that causes the 50% inhibition on alga growth or EC_{50} . In Fig. 2, EC_{50} s of Cu in the presence of carbonate, Cl^- , EDTA and FA were expressed as a function of $[Cu_T]$, $[Cu^{2+}]$ and $[Cu^*]$, respectively. The expression of EC_{50} s in terms of either $[Cu^{2+}]$ or $[Cu^*]$ resulted in a constant value, which was independent of aqueous compositions. The results indicated that the EC_{50} data could be explained either by FIAM or by the hypothesis that only the bioavailable or toxic fraction of Cu $[Cu^*]$ in solution could be responsible for the observed toxicity.

The difficulty in using FIAM and BLM to predict Cu toxicity in natural water is the determination of the site-specific apparent complexation constant for the complex of Cu and DOC (or FA). Therefore, $[Cu^*]$ may be used to predict the growth inhibition of Cu on *S. obliquus*. This approach is similar to those using inorganic concentrations of Cu to predict the acute toxicity on fish or invertebrates (Allen and Hansen, 1996), but it takes into

the account that part of inorganic Cu species which may not contribute to the toxicity. In practice, the labile concentration of Cu, or $[Cu^{2+}]$, can be calculated from the stripping currents in the DAM-DPASV measurements. The bioavailable concentration of Cu, or $[Cu^*]$, could be calculated from the $[Cu^{2+}]$ taking away the concentrations of non toxic inorganic species obtained from the MINTEQA2 model.

For predicting the toxicity of Cu in natural water using $[Cu^*]$, the available toxic data (confidence interval 90%) in the simulation experiments with different concentrations of $NaHCO_3$, EDTA and FA were transformed into toxic probit (TP). The results are given in Fig. 3. The regression equation was shown as follows, which would be used in predicting Cu toxicity in natural water for mortality equals 50%.

$$TP = 18.35 [Cu^*, \text{mg/l}] + 3.08 \quad (R_2 = 0.9371, n = 37) \quad (7)$$

3.2. Measuring water quality parameters and Cu toxicity in Guanting reservoir waters

A case study was carried out in Guanting Reservoir in Beijing. After sampling, water quality parameters, such as pH, DOC, anions, and major and trace elements, were measured. The results are shown in Tables 1 and 2.

In most of the natural freshwaters, Cu was mainly bound to organic substances. Because of the presence of natural derived organic matter, the concentration of labile Cu could not be directly measured by DPASV. A double acidification procedure was introduced to eliminate the effects of complexing agents on the ASV stripping process and surfactants on both deposition and stripping processes in natural water (Florence, 1992). The labile concentration of Cu was calculated from the measured stripping current in the DAM-DPASV measurements. The concentration of $[Cu^*]$ was calculated by assuming that the labile metal was inorganic species, which could be calculated from the labile Cu concentration using the DAM-DPASV method and the con-

Fig. 3. Relationship between measured TP by bioassays and measured $[Cu^*]$. Different plotting symbols were used to denote each of the 4 ligands tested (◆ expressed as HCO_3^- , ● [expressed as Cl^- , ▲ expressed as DOC, ◇ expressed as EDTA]. In this figure, toxic data ($n = 39$) were taken from Fig. 2.

Table 1
Physicochemical properties and concentrations of major anions and cations of water samples in Guanting Reservoir waters (mg/l)

Sampling sites	Locations	pH	DOC	HCO_3^-	Cl^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
G01	40°18'02"N, 115°37'37"E	8.55	0.57	0.73	3.1	5.2	76.2	5.5	0.3	4.0	12.5
G02	40°16'11"N, 115°37'37"E	8.49	1.40	0.74	4.7	9.2	88.8	4.3	0.3	3.2	12.5
G03	40°15'07"N, 115°36'09"E	8.56	1.19	0.69	2.3	5.4	78.2	4.7	0.3	4.2	11.8
G04	40°20'41"N, 115°42'52"E	8.55	1.49	0.54	2.5	8.7	87.5	5.0	0.4	3.3	10.4
G05	40°21'44"N, 115°45'39"E	8.21	1.24	0.71	2.8	4.8	74.4	4.8	0.5	4.4	11.2
G06	40°27'16"N, 115°59'34"E	8.46	2.39	0.73	4.1	6.4	69.4	5.3	0.4	5.0	11.0
G07	40°21'20"N, 115°31'46"E	8.14	0.17	0.75	2.9	5.8	76.5	4.5	0.4	5.6	16.6

centrations of Cu-carbonate and Cu-chloride complexes obtained from the MINTEQA2 model.

Concentrations of different Cu species, complexation capacity and predicted/measured inhibition to *S. obliquus* in Guanting Reservoir waters are given in Table 3. The highest concentration of dissolved Cu was 8.88 µg/l at site G03, which was lower than the allowable levels of the China National Standards (GHZB-1-1999) (China-EPA, 2000). However, both the highest labile concentration and bioavailable concentration of Cu occurred at Site G07 (5.3 and 0.54 µg/l, respectively). In the comparison between Site G03 and Site G07, the lability and bioavailability of Cu was affected by the concentration of DOC (Table 1), which could bind Cu in water (Table 3).

At sites G03 and G07, measured inhibitions on *S. obliquus* were not observed, while inhibitions of 3 and 4% were predicted using Eq. (7). Inhibition less than 10% was considered as insignificant (confidence interval 90%). At other sites except G06, growth inhibition of Cu on *S. obliquus* could be neither measured nor predicted. The measured growth inhibition (28%) at site

G06 should be caused by toxicants other than Cu (Ma et al., 2001).

To test the hypothesis of other toxicants, the water samples from Guanting Reservoir were spiked with the stock solution of Cu, and EC₅₀s were determined. The spiked solution was then subjected to the DAM-DPASV measurements to obtain labile concentrations of Cu in the spiked water samples. Accordingly, concentrations of bioavailable Cu ([Cu*]) were calculated using Eq. (6).

In Table 4, the observed EC₅₀s were expressed in terms of the total concentration, the labile concentration, as well as the bioavailable concentration of Cu. When expressed as the total concentration, EC₅₀s varied with sampling sites in a range of 0.20–0.55 mg/l. When expressed as the labile concentration, EC₅₀s were in a range of 0.13–0.29 mg/l. EC₅₀ was best expressed in the bioavailable concentration of Cu ([Cu*]) as the indicator,

Table 2
Concentrations of trace metals in Guanting Reservoir waters (µg/l)

Sampling sites	Cr	Mn	Co	Ni	Zn	Cu	Mo	Cd	Pb
G01	6.83	0.67	0.27	2.67	12.1	2.67	6.06	0.11	1.0
G02	6.30	0.93	0.35	3.12	19.2	2.67	7.56	0.14	1.9
G03	4.83	1.20	0.54	3.94	14.5	8.88	7.68	0.14	17.6
G04	8.80	1.05	0.38	4.39	23.0	5.47	6.71	0.69	7.7
G05	3.60	0.91	0.35	2.56	9.09	2.06	2.81	0.10	3.7
G06	3.19	1.48	0.51	3.67	13.2	4.62	2.91	0.13	0.7
G07	4.23	0.46	0.43	2.64	12.2	6.21	5.76	0.11	1.1

Table 3
Concentrations of different Cu speciation, complexation capacity and predicted/measured inhibition of growth to *S. obliquus* in Guanting Reservoir waters

	G01	G02	G03	G04	G05	G06	G07
[Cu _T] (µg/l) ^a	2.7	2.7	8.9	5.4	2.1	4.6	6.2
[Cu _{labile}] (µg/l) ^b	ND ^g	ND	4.4±1.3	2.1±0.7	1.3±1.1	1.1±0.9	5.3±0.9
[Cu*] (µg/l) ^c	ND	ND	0.26	0.11	0.08	0.12	0.54
Measured inhibition (%) ^d	ND	ND	ND	ND	ND	18	ND
Predicted inhibition (%) ^e	ND	ND	3	ND	ND	ND	4
CC (µg/L) ^f	34±21	64±17	31±15	85±24	26±15	128±57	53±34

^a The [Cu_T] was measured by using GFASS in natural waters.

^b The [Cu_{labile}] was measured by using DAM-DPASV.

^c The [Cu*] was calculated from [Cu_{labile}] and concentration of anions in water using MINTEQA2;

^d The inhibition of growth was measured on *S. obliquus* in non-spiked natural water samples.

^e The inhibition of growth was predicted on *S. obliquus* using Eq. (5) through inhibition of growth/toxic probit conversion table.

^f CC (complexation capacity) to Cu in natural water was measured by DAM-DPASV.

^g ND = not detected.

Table 4

EC₅₀s expressed by different Cu speciation to *S. obliquus* in Cu spiked Guanting reservoir waters

Sampling sites	EC ₅₀ (mg/l)		
	[Cu _T] ^a	[Cu _{labile}] ^b	[Cu*] ^c
G01	0.31±0.07	0.16±0.03	0.10
G02	0.36±0.11	0.29±0.06	0.11
G03	0.30±0.10	0.18±0.05	0.10
G04	0.46±0.08	0.13±0.03	0.09
G05	0.20±0.09	0.18±0.04	0.11
G06	0.55±0.13	0.15±0.05	0.10
G07	0.34±0.08	0.13±0.06	0.10

^a EC₅₀s of Cu were expressed as function of [Cu_T].

^b EC₅₀s of Cu were expressed as function of [Cu_{labile}].

^c EC₅₀s of Cu were expressed as function of [Cu*].

Fig. 4. Measured EC₅₀s versus complexation capacity in Guanting Reservoir waters ($y = 0.0028x + 0.203$, $R^2 = 0.9753$, $n = 7$). Data were obtained from Tables 3 and 4.

where it ranged from 0.09 to 0.11 mg/l, almost independent of water composition.

This suggested that median inhibition of Cu to *S. obliquus* in Guanting Reservoir water was 0.10 mg/l in terms of [Cu*]. It should be noted that [Cu*] in spiked natural water was obtained in a similar way as it was obtained in reconstituted water. Therefore its value should be comparable to those obtained in laboratory simulations. However while EC₅₀ in terms of [Cu*] in spiked reservoir water was found to be 0.10 mg/l, the range was from 0.26 to 0.37 mg/l in reconstituted water from empirical Eqs. (1)–(5). The difference in EC₅₀s between spiked water and reconstituted water could be caused by the effects of competing cations (i.e. [H⁺], [Ca²⁺] and [Mg²⁺]) were not included in the model. The presence of competing cations in natural waters and in culture medium may decrease the amount of Cu bound to biotic receptor sites (Meyer et al., 1999). Further work is needed to compare [Cu*] expressed EC₅₀s obtained from different types of natural waters.

Using the total Cu concentration, the observed EC₅₀s could be linearly correlated to the measured Cu complexation capacities of water (Fig. 4, $R^2 = 0.802$, $n = 7$). The results showed the significant influence of DOC from Guanting Reservoir on the toxicity of Cu. For example at site G06, complexation capacity was highest (0.128 mg/l), the corresponding EC₅₀ was highest (0.55 mg/l). Generally, the influences of DOC on the toxicity of Cu could be calculated or excluded either by measuring the complexation capacity or measured water-effect ratio (WER) (USEPA, 1994). The current

study provides an alternative to exclude inert forms of Cu from the total concentration.

4. Conclusions

The bioavailable concentration of Cu, or [Cu*], was used to predict growth inhibition of Cu on *S. obliquus* in this paper. The relationship between EC₅₀ on *S. obliquus* and the concentration of Cu could be formulated through laboratory simulation experiments in the presence of NaHCO₃, chloride, EDTA and FA. EC₅₀ in terms of [Cu*] could be obtained by excluding the inert forms of Cu species from the total concentration. A procedure of applying both DAM-DPASV measurements and model calculation to obtain [Cu*] in FA-containing reconstituted waters and spiked natural waters was studied. It was found that the EC₅₀ in terms of the bioavailable concentrations of Cu on *S. obliquus* were in the range of 0.18–0.38 mg/l in reconstituted water, and 0.10 mg/l in spiked natural water. The proposed procedure provides an alternative to the FIAM model. Moreover, it may provide a method to determine the bioavailable concentration of Cu in natural water and to predict Cu toxicity in complex media.

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References

- Allen, H.E., Hansen, D.J., 1996. The importance of trace metal speciation to water quality criteria. *Water Environ. Res.* 68, 42–54.
- Allen, H.E., 1993. The significance of trace speciation for water, sediment and soil quality standards. *Sci. Total Environ.* 134, 23–34.
- Burkhard, R.L., 2000. Estimating dissolved organic carbon partition coefficients in nonionic organic chemicals. *Environ. Sci. Technol.* 34, 4663–4668.
- Campbell, P.G.C., 1995. Interactions between trace metals and aquatic organisms. In: Tessier, A., Turner, D.R. (Eds.), *Metal Speciation and Bioavailability in Aquatic Systems*. John Wiley & Sons, New York, NY, USA, pp. 45–102.
- Carter, R.J., Hoxey, A., Verheyen, T.V., 1992. Complexation capacity of sediment humic acids as a function of extraction technique. *Sci. Total Environ.* 125, 25–31.
- China National Environmental Protection Agency, 2000. *Environmental Quality Standard for Surface Water*. GHZB-1-1999. China-NEPA, Beijing, China.
- Di Toro, D.M., Allen, H.E., Bergman, H.L., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ. Toxicol. Chem.* 20, 2383–2396.
- Erickson, R.J., Benoit, D.A., Mattson, V.R., 1996. The effects of water chemistry on the toxicity of copper to Fathead Minnows. *Environ. Toxicol. Chem.* 15, 181–193.
- Florence, T.M., 1986. Electrochemical approaches to trace element speciation in waters. *Analyst* 111, 489–505.
- Florence, T.M., 1992. Trace element speciation by anodic stripping voltammetry. *Analyst* 117, 551–553.
- Florence, T.M., Morrison, G.M., Stauber, J.L., 1992. Determination of trace element speciation and the role of speciation in aquatic toxicity. *Sci. Total Environ.* 125, 1–13.
- Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman–Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* 11, 714–719.
- Ma, M., Zhao, H.F., Wang, Z.J., 2001. Mutagenic activity of organic pollutants extracted from waters in Guanting Reservoir and Yongding River. *J. Hygiene Res.* 30, 355–357.
- Meyer, J.S., Santore, R.C., Bobbitt, J.P., DeBrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., DiToro, D.M., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free ion activity does not. *Environ. Sci. Technol.* 33, 913–916.
- Morel, F.M.M., 1983. *Principles of aquatic chemistry*. John Wiley & Sons, New York, NY, USA.
- Morel, F.M.M., Hering, J., 1993. *Principles and Applications of Aquatic Chemistry*. John Wiley & Sons, New York, USA.
- Morrison, G.M., Florence, T.M., Stauber, J.L., 1990. The effects of complexing agents and surfactants on the deposition and stripping processes in DPASV of metals. *Electroanalysis* 2, 9–14.
- Oeaver, E., Rodgers, J.H., 1996. Measuring bioavailable copper using anodic stripping voltammetry. *Environ. Toxicol. Chem.* 15, 1925–1930.
- Organization for Economic Cooperation and Development (OECD), 1984. Algal growth inhibition test. In: *OECD Guideline for Testing of Chemicals*. 201.
- Playle, R.C., Gensemer, R.W., Dixon, D.G., 1992. Copper accumulation on gills of Fathead Minnows: Influence of water hardness, complexation and pH on the gill micro-environment. *Environ. Toxicol. Chem.* 11, 381–391.
- Santore, R.C., Di Toro, D.M., Paquin, P.R., 2001. Ligand model of the acute toxicity of metals 2: application to acute copper toxicity to freshwater fish and daphnia. *Environ. Toxicol. Chem.* 20, 2397–2402.
- Sarin, C., Hall, J.M., Cotter-Howells, J., Killham, K., Cresser, M.S., 2000. Influence of complexation with chloride on the responses of a LUX-marked bacteria bioassay to cadmium, copper, lead and mercury. *Environ. Toxicol. Chem.* 19, 259–264.
- US Environmental Protection Agency, 1994. *Water Quality Standards Handbook*. US Environmental Protection Agency, Office of Water, Washington, DC, USA.
- US Environmental Protection Agency, 1999. *Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments*. USEPA-822-E-99-001. Technical Report. Office of Water of the EPA Science Advisory Board, Washington, DC, USA.
- US Environmental Protection Agency, 2000. *A Sab Report: Review of the Biotic Ligand Model of the Acute Toxicity of Metals*. EPA-SAB-EPE-00-006. Prepared by the Ecological Processes and Effects Committee of The Science Advisory Board Washington, DC, USA.
- Vasconcelos, M.T., Azenha, M.A., Lage, O.M., 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.
- Wang, Z.J., 1987. Interaction between heavy and metals and humic acid-quasi-particle surface complexation model. *Acta Scientiae Circumstantiae (China)* 7, 313–318.
- Wang, Z.J., 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.
- Wang, Z.J., Huang, S.B., Liu, Q., Ma, M., 2002. Measuring the toxic concentration of copper in natural water and sediment elutriate by using anodic stripping voltammetry and daphnia magna bioassay. *Environ. Toxicol. Chem.* 21(9), 1788–1795.
- Zhou, Y.X., Zhang, Z.S., 1989. *Methods For Aquatic Bioassays*. Agricultural Publishing House, Beijing, China. 114–121.