

Using enriched stable isotope technique to study Cu bioaccumulation and bioavailability in *Corbicula fluminea* from Taihu Lake, China

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Abstract In this study, we measured trace metals (Cd, Cr, Cu, Ni, Pb, and Zn) in water and sediment from representative sites of Taihu Lake, with focus on the analysis of trace metal accumulation in *Corbicula fluminea* (bivalve). The results showed that the quality of water in Taihu Lake was generally good and the correlation was not found between Cu bioaccumulation in *C. fluminea* and the concentration in water and sediment. Thus, using the stable isotope tracer method, we studied Cu uptake from the water phase, the assimilation of Cu from the food phase, and the efflux of Cu in vivo by *C. fluminea*. The result revealed that this species exhibited a relatively lower efflux rate constant of Cu compared with other zoobenthos species. Using a simple bioenergetics-based kinetic model, Cu concentrations in the *C. fluminea* were calculated with the measured efflux rate. We put forward a novel method, which was taking the influence of biological kinetic on metal bioaccumulation into account to explain the field survey data.

Keywords Stable isotope · Trace metal · *Corbicula fluminea* · Bioavailability · Taihu Lake

Introduction

Taihu Lake, situated in the Changjiang (Yangtze) delta, is the third largest freshwater lake in China, with its lake area

measuring 2,338 km² (Zhu et al. 2013). It supplies drinking water for several important cities, such as Shanghai, Huzhou, and Suzhou (Qin et al. 2007). Due to the important role that Taihu Lake plays, it has been extensively studied in recent years (Mao et al. 2008; Su et al. 2010; Zhang et al. 2013). Since the 1970s, increasing levels of wastewater have been discharged into Taihu Lake without treatment, thereby introducing a large amount of trace metals (Shen et al. 2007). Many surveys on trace metals in water and sediment have been conducted (Shen et al. 2007; Bing et al. 2011; Yu et al. 2012a). Trace metal accumulation in vivo, such as in phytoplankton, zooplankton, and zoobenthos (Yu et al. 2012a), as well as fish (Chi et al. 2007) and *Corbicula fluminea* (Peltier et al. 2008), has been previously reported.

The regularity of metal bioaccumulation in aquatic organism is generally influenced by the living environment. Yu et al. (2012a) found that the general spatial distribution of heavy metal concentrations in different aquatic organisms (planktons and zoobenthos) in the Taihu Lake was generally consistent with the metal distribution in the water column and sediment. In addition, Shoultz-Wilson et al. (2010) pointed out that greater water alkalinity and hardness tended to negatively correlate with metal accumulation, while organism size had a positive correlation with tissue concentrations of *C. fluminea* and *Elliptio hopetonensis* in the Altamaha River system. The results also showed that significant relationships existed between bivalve tissue Cd, Cu, and Pb contents and concentrations of those metals in fine sediments. However, these researches focused on the correlation between metal bioaccumulation and environmental factors, such as water chemistry and metal concentration in water and sediment. On the contrary, the influence of biological characteristic of organism on metal bioaccumulation had been largely unexplored when illustrating the field survey result.

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In recent years, the biokinetic model developed rapidly in illustrating how and why trace metal bioaccumulation differed among metals, species, and environments (Luoma and Rainbow 2005). Wang et al. (1997) employed a bioenergetic-based kinetic model to determine the relative contributions of different Cr species (Cr(III) and Cr(VI)) to the overall accumulation of Cr in mussels. Much research on heavy metal bioavailability has been performed using the radioisotope tracer method; however, for some trace metals, such as Cu, the gamma-emitting radioisotope is not readily available because of its very short half-life. Recently, with the stable isotope technique, Croteau and Luoma characterized dissolved Cu and Cd uptake (2007) and predicted dietborne Cu, Cd, and Ni toxicity in the freshwater snail (*Lymnaea stagnalis*) (2009). Croteau et al. (2004) revealed copper accumulation and loss dynamics in *Corbicula* with the stable isotope technique, while the assimilation efficiency (AE) from ingested food in *Corbicula* has not been determined in his study.

In this study, we applied the stable isotope technique to investigate the mechanism of Cu accumulation in *C. fluminea* collected from Taihu Lake. We also measured the heavy metal content in water, sediment, and *C. fluminea* in the said lake. As we conducted both laboratory experiments and field surveys in Taihu Lake, we substantiated the Cu bioaccumulation in *C. fluminea* from the perspective of biological characteristic rather than environmental factors. A novel method that taking the effect of biological kinetic on metal bioaccumulation into account to explain the field survey data was put forward.

Materials and methods

Sample collection and measurement

Water, sediment, and *C. fluminea* samples were collected from six locations in Taihu Lake (Fig. 1) from August 3, 2010 to August 10, 2010. The water samples were collected using acid-washed high-density polyethylene (HDPE) bottles manually and transported to the laboratory in coolers. The water samples were filtered with a 0.45- μm filtration membrane to remove the suspended particles and then preserved at 0–4 °C. In total, 0.5 mL of 68 % HNO_3 was mixed with 4.5 mL of water sample to make the metal exist in ionic form.

Sediment samples were also collected manually to get the representative sample in Taihu Lake and preserved in HDPE containers. Prior to analysis, the sediment samples were dried at 50 °C for 48 h, ground, and sieved through a 63- μm mesh. The total metal concentrations in the sediment were measured after digesting by the mixture of HNO_3 – HClO_4 (Fan et al. 2002). To ensure analytical quality, the “National Certified Reference Material-lacustrine sediment” (GSD-10, with the content of metal elements known) was processed as an

independent sample using the same method. The recoveries of the reference material for all metals were 90–118 %. Besides, the fractions of trace metals Cr, Cu, Pb, and Zn in sediment were analyzed using sequential extraction procedure (Tessier et al. 1979; Li et al. 1995), which partitioned the particulate trace metals into five fractions: exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to organic matter, and residual.

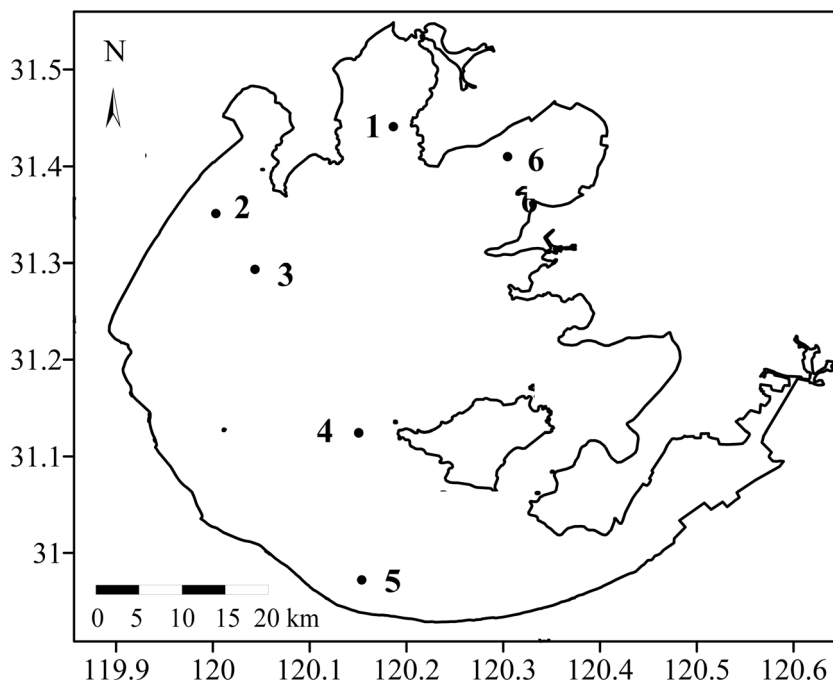
C. fluminea of 5- to 10-mm shell length were found and collected in the same sites as the water and sediment samples. The collection, transportation, and preservation methods of *C. fluminea* were similar with the procedure described in previous report (Croteau et al. 2004). In order to measure the tissue concentrations more accurately, *C. fluminea* were transferred to ultrapure water (Milli-Q system water, >18 M ohm cm^{-1}) for 48 h to discharge the gut contents. The soft tissue of *C. fluminea* was dried until constant weight at 80 °C and then digested by 68 % HNO_3 at 110 °C for 12 h. Two subsamples of specified reference oyster powder (1566a, National Institute of Standards and Technology, Gaithersburg, MD) were digested simultaneously to verify the recovery rate in each process. All labware was cleaned by soaking it in 15 % nitric acid for 24 h and then rinsed with ultrapure water for several times to reduce accidental metal contamination (Croteau et al. 2004). Three replicates were performed in each sampling point for water, sediment, and biological samples. The metal content in all samples were measured by inductively coupled plasma mass spectroscopy (ICP-MS; TJA PQ ExCell, Thermo Fisher Scientific, USA).

Dissolved uptake of Cu in *C. fluminea*

The *C. fluminea* samples used for the exposure experiment were collected from sampling site 2 in Taihu Lake and cultivated in filtered Taihu Lake water. In our laboratory, we fed the *C. fluminea* with *Scenedesmus obliquus* and Taihu Lake algae. Before the beginning of the experiments, 10 individuals of *C. fluminea* were digested by 68 % HNO_3 at 110 °C for 12 h to get the background concentrations of ^{65}Cu in organism. In our study, the pre-existing concentration of ^{65}Cu in organism was corrected, and all experimental data reported in this study represented the net accumulation of ^{65}Cu . The calculation and correction for the background ^{65}Cu is described in detail in our previous study (Fan et al. 2011).

One hundred and eighty individuals of *C. fluminea* (10 mm long) were fed for 1 h with algae that have not been labeled. Next, the *C. fluminea* samples were transferred to Taihu Lake water labeled with 5, 10, 20, 40, and 60 $\mu\text{g L}^{-1}$ ^{65}Cu . The stable Cu isotope ^{65}Cu (99.2 %) used in this study was purchased from Isotopex Company (San Francisco, USA). At 2, 4, 6, and 8 h, nine *C. fluminea* samples were collected from the exposure solution and rinsed for about 2 min in ultrapure water to remove the metal absorbed on the animals. The nine

Fig. 1 Location of sampling points in Taihu Lake. The six numbers and black spots represent the places where we collected water, sediment, and *C. fluminea*



samples were divided into three groups at random, and each treatment has three individuals of *C. fluminea*. We selected a relatively short exposure time to reduce efflux of the label. The soft tissue was digested by 68 % HNO₃ at 110 °C for 12 h, and the content of ⁶⁵Cu was measured by ICP-MS. Two subsamples of specified reference oyster powder (1566a, National Institute of Standards and Technology, Gaithersburg, MD) were digested simultaneously to verify the recovery rate in each process. The recovery rates of the certified reference material were between 93 and 110 %. The dry weight concentration factor (DCF) was calculated as the ratio of the ⁶⁵Cu concentration in *C. fluminea* (μg g⁻¹ dry weight) to the dissolved ⁶⁵Cu concentration in the test solution (μg L⁻¹) (Lam and Wang 2006).

Cu assimilation in *C. fluminea*

To study the effects of different foods to the assimilation of *C. fluminea*, we fed the samples with *S. obliquus* and Taihu Lake algae at the concentration of 10⁵ cells mL⁻¹. *S. obliquus* and Taihu Lake algae were cultured in Wilkins-Chalgren (WC) medium (containing CaCl₂ 0.25 mM, MgSO₄ 0.15 mM, NaHCO₃ 0.15 mM, K₂HPO₄ 0.05 mM, NaNO₃ 1 mM, H₃BO₃ 0.1 mM, and trace metals and vitamins) (Guillard 1975) for approximately 10 days, centrifuged for approximately 25 min (4,500 rpm), and then suspended in amended WC culture medium (without Cu, Zn, and EDTA); the initial concentration was 2 × 10⁵ cells mL⁻¹. ⁶⁵Cu was added into the medium, giving a final concentration of 200 μg L⁻¹. *S. obliquus* and Taihu Lake algae were centrifuged after labeling for 3 days and then resuspended with

unlabeled natural water thrice. The natural water used in the current study was obtained from Huo Qi Ying Bridge, China (latitude 39°58'5.58", longitude 116°16'53.3"). Natural water was filtered through a 1.2-μm membrane prior to use. Using ICP-MS, Cu was detected in the water at the concentration of 3.5 μg L⁻¹.

The *C. fluminea* samples were transferred to a beaker full of Taihu Lake water after the gut was cleared. They were fed with labeled *S. obliquus* and Taihu Lake algae for 20 min in the dark and then taken out and cleaned with ultrapure water for 1 min. The samples were transferred to ultrapure water and purified continuously for 72 h. At 0, 6, 12, 24, 36, 48, 60, and 72 h, nine samples were collected and divided into three groups randomly. The soft tissue of samples was dried at 80 °C for approximately 12 h. The ⁶⁵Cu concentration of soft tissue was measured by ICP-MS as explained above. At each time point, we replaced the water to prevent the discharged ⁶⁵Cu from entering the body through water and food phases.

Efflux of waterborne and dietborne Cu from *C. fluminea*

In this study, the efflux of Cu in *C. fluminea* was measured following both dissolved and dietary uptake. For the dietary phase exposure, we labeled *S. obliquus* using the same method as in the above-described assimilation experiment. The labeled *S. obliquus* was fed to *C. fluminea* twice daily at the concentration of 1 × 10⁵ cells mL⁻¹ during the uptake period. The water was replaced to reduce the potential uptake of Cu from the aqueous phase.

For the dissolved phase exposure, the *C. fluminea* samples were exposed to Taihu Lake water spiked with ⁶⁵Cu at the

concentration of $20 \mu\text{g L}^{-1}$. They were exposed for 20 h every day. Food was not added during this period to avoid the absorption of Cu onto the food surface. The samples were returned to nonspiked Taihu Lake water under the *S. obliquus* density of $1 \times 10^5 \text{ cells mL}^{-1}$ for another 4 h and then rinsed in nonspiked Taihu Lake water for 5 min to remove the loosely adsorbed *S. obliquus*. Next, the samples were finally transferred back to the spiked water to begin another 24-h cycle.

Both exposures lasted for 3 days. The *C. fluminea* samples were then collected and depurated for 5 days in nonspiked water. During the depuration, nine samples were collected and digested every 12 h to measure the retained ^{65}Cu by ICP-MS. The water and food were replaced at every time point. The efflux rate constant (k_e) of Cu was calculated from the slope of the linear regression between the natural log of the percentage of ^{65}Cu retained and the time of depuration between 2 and 5 days. The biological retention half-life ($t_{1/2}$) of Cu was calculated as $t_{1/2} = 0.693/k_e$ (Fan et al. 2011).

Results and discussion

Concentrations of trace metals in water and sediment in Taihu Lake

The concentrations of trace metals in water and sediment from different points of Taihu Lake are shown in Fig. 2. As shown in Fig. 2a, the concentrations of trace metals in water were in the order $\text{Zn} > \text{Cu} > \text{Ni} > \text{Cr} > \text{Pb} > \text{Cd}$. Zn and Cu had the highest concentrations compared with the other elements in lake water. The concentration of Pb in water was lower compared with the Surface Water Environmental Quality Standard (GB 3838-2002, Table 1), which suggested that Pb was bound to sediment more readily than other trace metals (Yu et al. 2012b). The level of trace metal Cd, usually regarded as one of the most toxic elements in water, was only approximately $0.05 \mu\text{g L}^{-1}$. Overall, the concentrations of trace metals in water were very low. In contrast to the Surface Water Environmental Quality Standard (Table 1), we found that the contents of Cd, Cr, Cu, Pb, and Zn satisfied water quality standard I (which does not require Ni), which indicated that the quality of water in Taihu Lake was good overall.

The concentrations of trace metals in sediment were in the order $\text{Zn} > \text{Cu} > \text{Cr} \approx \text{Ni} > \text{Pb}$ (Fig. 2b), almost the same as that in water. Deng et al. (2011) calculated the sediment quality criteria (SQC, Table 1) of Cd, Cu, Pb, and Zn using the US EPA freshwater quality criteria, which are based on the aquatic toxicity of the final chronic levels of heavy metals. They calculated the SQC of Cd, Cu, Pb, and Zn of Taihu Lake basin as 6.42, 55.3, 20.6, and $201.5 \mu\text{g g}^{-1}$, respectively. By contrast, our survey results showed that the concentrations of Zn and Cu in Taihu Lake sediment were less than 50 % of the

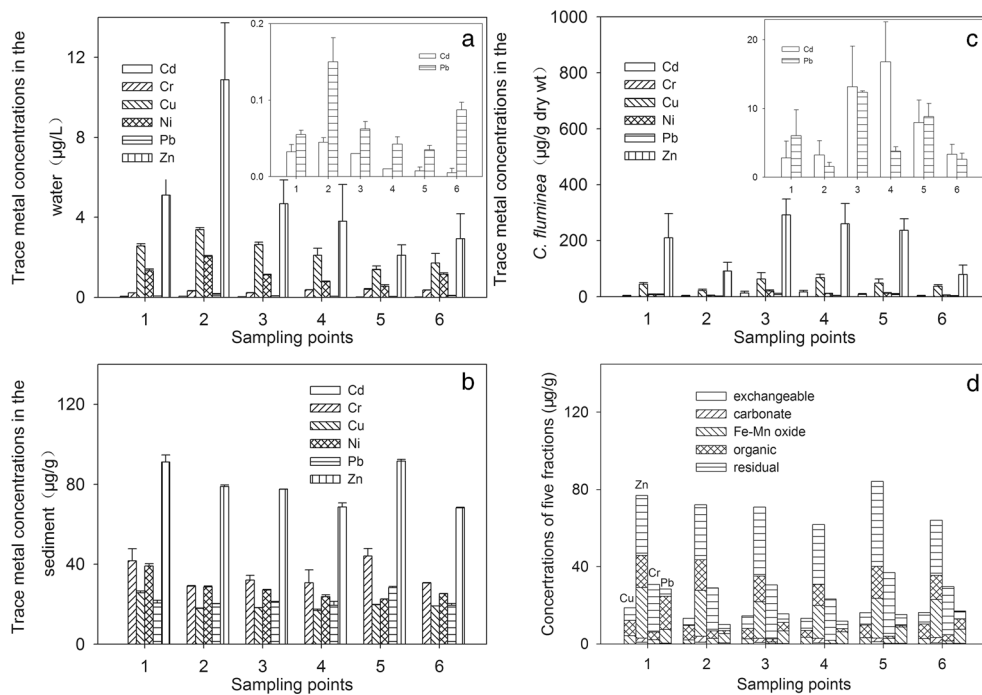
SQC. The concentration of Pb in sediment was almost equal to or even exceed (site 5) the SQC, indicating that Pb posed potential toxicity risk. This may be due to the discharge of Pb-containing industrial effluent and the use of leaded gasoline in the steamship. Overall, the concentrations of trace metals were relatively low and varied with little fluctuation in sediment.

Chemical fractionation of heavy metals in the sediment can be used to evaluate the pollution level (Shen et al. 2007; Fan et al. 2002). As shown in Fig. 2d, we found that most Cu was associated with organic and residual fractions in the sediment. Cu could easily complex with organic matter probably due to the high stability constant of organic Cu compounds (Li et al. 2001). The Fe–Mn oxide fraction was the next important phase for Cu. The exchangeable and carbonate fractions were found to have minor contributions to Cu. The major portion of Zn was bound to the residual fraction in the sediment. The next most important fraction of Zn in the sediment was the Fe–Mn oxide fraction, followed by the organic fraction. The Zn complex with Fe–Mn oxide has a relatively higher stability constant than that with carbonate. The exchangeable and carbonate fractions were found to account for a small proportion of Zn. This result is in agreement with the findings reported by Li et al. (2001) for sediment from Pearl River, China. The trace metal Pb was mainly associated with the Fe–Mn oxide, followed by the organic fraction and residual fraction. In addition, the exchangeable and carbonate fractions were found to be minor contributors to Pb. The percentages of Cr associated with different fractions were in the order residual > organic > Fe–Mn oxide > carbonate > exchangeable. Of these five fractions, the most important phase was the residual fraction, which accounted for 90 % of all Cr. The high levels of Cr in the residual fraction suggest that Cr in sediment is less mobile than Cu, Pb, and Zn.

Accumulation of trace metals in *C. fluminea* in Taihu Lake

The accumulation of trace metals in *C. fluminea* in different points of Taihu Lake is shown in Fig. 2c (Cr in *C. fluminea* was not detected). We found that the concentrations of trace metals in *C. fluminea* were in the order $\text{Zn} > \text{Cu} > \text{Ni} > \text{Cd} \approx \text{Pb}$ for all sampling points as well as that Cu or Zn had higher content levels than Cd, Pb, or Ni. Under heavy-metal unpolluted conditions, shellfish accumulated more essential elements, such as Cu and Zn, and fewer nonessential elements, such as Pb and Cd (Sun and Wang 2003). This can be primarily attributed to the fact that the background content of essential elements is higher than that of nonessential elements in natural environments (Amiard et al. 1987). Statistical analysis between five metals (Zn, Cu, Ni, Cd, and Pb) showed that only the contents of Zn and Cu in vivo have a significant positive correlation (Pearson test, $P < 0.05$). Peltier et al. (2008) also found strong positive correlations between Cu and Zn concentrations in tissues of *C. fluminea*.

Fig. 2 Cd, Cr, Cu, Ni, Pb, and Zn concentration in water sample (a), sediment sample (b), *C. fluminea* (c) and different geochemical phase of sediment sample collected from Taihu Lake. (Cd in sediment and Cr in *C. fluminea* were not detected). For a, b, and c, data are expressed as mean ± standard deviation (SD) (n=3)



Comparative data for metal accumulation in *C. fluminea* between our survey results and other reports are shown in Table 1. Compared with the *C. fluminea* population in the Yangtze River estuary (Sun and Wang 2003) and Altamaha River system (Shoultz-Wilson et al. 2010), the *C. fluminea* in Taihu Lake has a higher accumulation of Pb. This may be related to the high content of this trace element in sediment. Bilos et al. (1998) assessed the magnitude of trace metal pollution in *C. fluminea* in an estuary and found that accumulations of Cd and Ni were relatively low compared

with that in our study. Peltier et al. (2008) found higher Zn and lower Cd bioaccumulation in *C. fluminea* when they identified the potential sources of contamination using trace element concentrations in an urban river. Overall, the Cu accumulation of *C. fluminea* in Taihu Lake is relatively higher than that of *C. fluminea* in other countries.

As discussed above, the level of metal Cu or Zn bioaccumulation in *C. fluminea* was much higher than level of Cd, Pb, or Ni. Moreover, considering the importance of Cu for aquatic organism, we selected Cu as the representative element to

Table 1 Trace metal concentrations in water, sediment, and *Corbicula fluminea* compared with SWEQS, SQC, and metal content in *C. fluminea* from different environments

		Trace metal content ($\mu\text{g L}^{-1}$; $\mu\text{g g}^{-1}$; $\mu\text{g g}^{-1}$ dry weight)						References
		Cd	Cr	Cu	Ni	Pb	Zn	
Water	Water	0	0.2–0.4	1.4–3.4	0.6–2.0	0–0.2	0.6–10.9	
	Mean	0	0.3	2.3	1.2	0.1	4.3	
	SWEQS	1	10	10	–	10	50	
Sediment	Sediment	nd	28.9–44.0	16.8–25.6	22.4–39.0	19.3–28.2	68.1–91.6	
	Mean	nd	34.6	19.4	27.6	21.4	79.3	
	SQC	6.42	–	55.3	–	20.6	201.5	
<i>Corbicula fluminea</i>		2.8–16.8	nd	22.3–68.4	3.8–20.2	1.5–12.3	79.3–291.3	This study
		2.13–7.79	1.35–2.55	40.02–93.23	–	0.98–3.28	143.04–209.33	Sun and Wang 2003
		0.5–1.9	1.3–11	28–89	1.3–6.4	–	118–316	Bilos et al. 1998
		0.8–4.1	–	32.0–87.7	–	–	189.4–544.0	Peltier et al. 2008
		0.731–4.77	–	34.7–152	1.45–9.23	0.143–1.31	92.6–179	Shoultz-Wilson et al. 2010

“–” indicates not available; nd not detected

SWEQS Surface Water Environmental Quality Standard, SQC Sediment Quality Criteria

study the metal bioavailability using enriched stable isotope technique. In addition, the correlation was not found (Pearson test, $P < 0.05$) between Cu bioaccumulation in the *C. fluminea* and the contents in water and sediment. Therefore, the Cu bioaccumulation in vivo was not only controlled by the water quality or metal contamination in sediment. We think that *C. fluminea* has a special active regulation mechanism for trace metal Cu, and research into the bioavailability of Cu to *C. fluminea* is necessary.

Biokinetics of Cu in *C. fluminea* from Taihu Lake

Dissolved uptake of Cu in *C. fluminea*

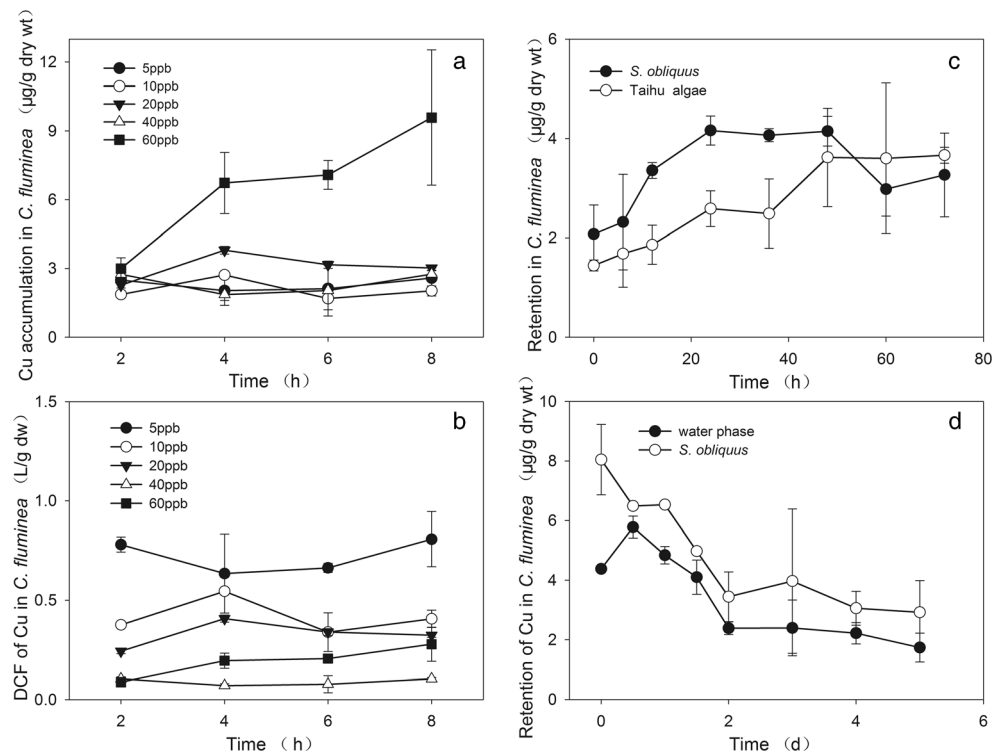
The levels of Cu accumulation in *C. fluminea* caused by exposure to different concentrations for 8 h are shown in Fig. 3a. We found that the accumulation of Cu in vivo was almost unchanged with increasing exposure time within the exposure concentration range of 5–40 $\mu\text{g L}^{-1}$ and increased rapidly at the exposure concentration of 60 $\mu\text{g L}^{-1}$.

The DCFs of dissolved Cu in *C. fluminea* caused by exposure to different concentrations for 8 h are shown in Fig. 3b. The DCF of Cu in vivo was almost unchanged with increasing exposure time when the exposure concentration was in the range of 5–40 $\mu\text{g L}^{-1}$ and increased gradually at the exposure concentration of 60 $\mu\text{g L}^{-1}$. The DCF of dissolved Cu in *C. fluminea* decreased gradually as the exposure concentration increased. Bossuyt and Janssen (2005) obtained similar results in their study of Cu accumulation in *Daphnia*

magna. When the exposure concentration ranged from 12 to 35 $\mu\text{g L}^{-1}$, the DCF of Cu in *D. magna* decreased as the exposure concentration increased. However, when the exposure concentration was in the range of 35–100 $\mu\text{g L}^{-1}$, the DCF remained almost the same. When the exposure concentration reached 150 $\mu\text{g L}^{-1}$, the DCF increased rapidly.

Dissolved organic matter (DOM), which had strong complexible ability with metal ions in aquatic environments, played a critical role in the speciation, mobility, bioavailability, and hence biotoxicity of trace metals to aquatic organism (Bai et al. 2008). Yu et al. (2012a) reported that the average value of dissolved organic carbon (DOC) was 26.96 mg L^{-1} in Taihu Lake, China. Therefore, the presences of DOC could complex with ^{65}Cu and reduce the free ion concentration, especially under lower exposure level in dissolved uptake portion of the experiment. As a result, the dissolved uptake of lower exposure concentration was strongly influenced by DOC. On the other hand, the accumulation of trace metal in vivo is the result of the joint action of absorption and discharge processes. The phenomenon of saturation or decline in accumulation indicates that there may be existing regulatory mechanisms of absorption and discharge in organism. Adams et al. (2000) hypothesized that three mechanisms of the accumulation of essential elements in aquatic organisms exist, namely, active regulation, storage, and the mixture of these two. In the mechanism of active regulation, aquatic organisms could absorb trace metals actively to meet the needs of the metabolism when the environmental concentration is low and increase the discharge or reduce the absorption when the concentration is high.

Fig. 3 The dissolved uptake, assimilation, and efflux of Cu in *C. fluminea*. The accumulation (a) and DCF (b) of Cu in *C. fluminea* over time at different exposure concentrations and the retained Cu in *C. fluminea* during the assimilation process in different kinds of food (c) and the efflux process of water and dietary exposure (d). Data are expressed as mean \pm SD ($n=3$)



In the mechanism of storage, the concentration in vivo continually increases, but the increasing extent is lower compared with the change in concentration, indicating that DCF does not significantly change. Thus, we think that the mechanism of Cu regulation in *C. fluminea* falls under active regulation. This may be ascribed to the fact that under high Cu concentrations, cells in vivo could increase the discharge or reduce the absorption of Cu to regulate the accumulation. As a result of aquatic organisms' regulatory ability, the corresponding bioconcentration factors (BCFs) would be inversely related to exposure concentration instead of a constant (Adams et al. 2000).

Cu assimilation in *C. fluminea*

Data on Cu relative retention in vivo over time observed after 20 min of pulse feeding with different kinds of algae are shown in Fig. 3c. When *S. obliquus* were given, the relative retention of Cu increased gradually in the first 24 h, reached a maximum value and stabilized within 24–48 h, and decreased gradually within 48–72 h. When the *C. fluminea* samples were fed with Taihu Lake algae, the relative retention of Cu increased in the first 48 h, reached a maximum value, and then remained stable for 72 h.

The phenomenon shown in Fig. 3c is interesting. In theory, after 20 min of pulse feeding, *C. fluminea* samples were transferred into unlabeled water and underwent the discharge process only. Thus, the relative retention of Cu should decrease gradually depending on time. Among the pulse feedings, in addition to the algae and ions absorbed in vivo by the *C. fluminea*, we think that there was a small amount of solution in the gap between the shell and soft tissue containing low levels of labeled algae and Cu ions. *C. fluminea* could absorb the Cu ions in the solution to increase the accumulation in the discharge process. We indeed observed some green spots in some parts of the soft tissue of *C. fluminea*, which indicated that *C. fluminea* accumulated a certain amount of labeled algae from the pulse feeding. The algae should be discharged in vitro over time, but the efflux was lower than the absorption from the gap, indicating that the relative retention of Cu in *C. fluminea* has a rising process. As shown in Fig. 3c, Cu concentrations in the clams exposed to *S. obliquus* were relatively constant and then declined over time.

Efflux of waterborne and dietborne Cu from *C. fluminea*

The efflux process of Cu absorbed by water and food phases in vivo is shown in Fig. 3d. The retention of Cu decreased rapidly in the first 2 days, and the efflux rate became slow in 2–5 days. The retention percentage of Cu accumulated by the food phase was higher than that accumulated by the water phase. In 5 days, the retention percentages were 36.4 and 30.1 %, respectively. Croteau et al. (2004) also found that the ⁶⁵Cu rapidly escaped ($P < 0.05$) the tissue of *C. fluminea* in

the first 2 days during the loss experiment in their study and that the ^{65/63}Cu in vivo changed inconspicuously ($P > 0.1$) in 2–14 days. The metabolic process has two stages, namely, a fast stage and a slow stage. The efflux rate constant k_e stands for the slow stage rate, and we thus calculated k_e according to the results we obtained within 2–5 days. We determined that the k_e and biological retention half-life ($t_{1/2}$, days) of Cu were $0.052 \pm 0.02 \text{ day}^{-1}$ and 13.33 ± 1.6 days, respectively, after absorption by the water phase. The corresponding constants were $0.075 \pm 0.04 \text{ day}^{-1}$ and 9.24 ± 0.2 day after absorption by the food phase. Obviously, the accumulated Cu from the food phase had a higher efflux rate constant and a smaller biological retention half-life.

Comparisons of the efflux rate constant between different aquatic organisms, such as *D. magna* ($0.19\text{--}0.20 \text{ day}^{-1}$) from the food phase (Zhao et al. 2009), scallop *Chlamys nobilis* (0.148 day^{-1}), clam *Ruditapes philippinarum* (0.147 day^{-1}), and green mussel *Perna viridis* (0.131 day^{-1}) from dissolved phase (Pan and Wang 2009), revealed that *C. fluminea* has a relatively lower efflux rate constant. This low efflux rate constant may explain the high Cu accumulation ($22.3\text{--}68.4 \mu\text{g g}^{-1}$) in vivo under low concentrations thereof in Taihu Lake. Pan and Wang (2009) reported that the efflux rate constant plays a decisive role in the concentration of Cu in bivalves compared with other biological kinetic parameters.

To further verify the influence of efflux rate on metal accumulation in *C. fluminea*, Cu concentrations in *C. fluminea* were calculated with the measured efflux rate using the following simple kinetic model (Wang et al. 1999)

$$C_{ss} = \frac{k_u \times C_w}{k_{e,w} + g} + \frac{AE \times IR \times C_f}{k_{e,f} + g} \quad (1)$$

where C_{ss} is the estimated steady-state Cu concentration in *C. fluminea* ($\mu\text{g g}^{-1}$ dry wt), k_u is the uptake rate constant from the dissolved phase ($\text{L g}^{-1} \text{ day}^{-1}$), C_w is the Cu concentration in the dissolved phase ($\mu\text{g g}^{-1}$), $k_{e,w}$ is the Cu efflux rate constant (day^{-1}) from water phase, AE is Cu assimilation efficiency (%) from ingested food, IR is the *C. fluminea* ingestion rate ($\text{g algae g}^{-1} \text{ day}^{-1}$), C_f is the Cu concentration in ingested food ($\mu\text{g g}^{-1}$ dry wt), $k_{e,f}$ is the Cu efflux rate constant (day^{-1}) from the food phase, and g is the growth rate constant (day^{-1}). Metal efflux rate constant k_e is generally one order of magnitude higher than the growth rate constant g , which could be ignored in modeling calculations (Wang et al. 1997). Therefore, C_{ss} could be calculated as

$$C_{ss} = \frac{k_u \times C_w}{k_{e,w}} + \frac{AE \times IR \times C_f}{k_{e,f}} \quad (2)$$

According to Eq. 2, Cu concentrations in *C. fluminea* were calculated with the measured efflux rate and Cu concentration in water and assumed uptake rate constant from the dissolved phase, assimilation efficiency, ingestion rate, and Cu

concentration in food. Among them, the uptake rate constant from the dissolved phase k_u ($0.224 \pm 0.038 \text{ L g}^{-1} \text{ day}^{-1}$), AE from ingested food ($38 \pm 10 \%$), and IR of *C. fluminea* ($0.028 \pm 0.005 \text{ g g}^{-1} \text{ day}^{-1}$) were taken from the estimated data by Croteau and Luoma (2005). Cu concentration in ingested food, ranging from 11.32 to 151.45 $\mu\text{g g}^{-1}$, comes from Cu content in phytoplankton in Taihu Lake measured by Yu et al. (2012a). The mean numeric value of Cu concentration (63.5 ug g^{-1}) for six sampling site in Taihu Lake was used for calculated Cu concentration in *C. fluminea*. The measured mean value of Cu concentration in water was $2.3 \text{ }\mu\text{g L}^{-1}$. As calculated above, the efflux rate constant $k_{e,w}$ and $k_{e,f}$ are $0.052 \pm 0.02 \text{ day}^{-1}$ and $0.075 \pm 0.04 \text{ day}^{-1}$, respectively. According to kinetic model, mean numeric value for calculated Cu concentration in *C. fluminea* was $18.9 \text{ }\mu\text{g g}^{-1}$, which was nearly two fifths of the mean value of measured Cu concentration ($47.6 \text{ }\mu\text{g g}^{-1}$). Therefore, Cu concentrations in the *C. fluminea* calculated by the kinetic model were comparable for a range of values with our measured results in the field. This result also demonstrates that the high Cu accumulation in *C. fluminea* in Taihu Lake is related to the relatively lower efflux rate constant under the same Cu concentration in food.

Conclusions

The qualities of water and sediment in Taihu Lake were generally good, and the concentrations of trace metals in *C. fluminea* were in the order $\text{Zn} > \text{Cu} > \text{Ni} > \text{Cd} \approx \text{Pb}$ for all sampling points in Taihu Lake. However, the correlation was not found between Cu bioaccumulation in *C. fluminea* and the contents in water and sediment. Using the stable isotope tracer method, we found that compared with other aquatic organisms, *C. fluminea* has a relatively lower efflux rate constant. The modeling calculation demonstrates that the Cu accumulation in *C. fluminea* in Taihu Lake was affected by efflux rate constant. Therefore, the influence of biological kinetic on metal bioaccumulation should not be ignored in aquatic organism.

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