

Comparison of heavy metal accumulation by a bloom-forming cyanobacterium, *Microcystis aeruginosa*

ZENG Jin^{1*}, ZHAO DaYong², JI YongBan¹ & WU QingLong¹

¹ State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China;

² State Key Laboratory of Hydrology-Water Resources and Hydraulic Engineering, College of Hydrology and Water Resources, Hohai University, Nanjing 210098, China

Received December 28, 2011; accepted March 30, 2012; published online August 16, 2012

Microcystis aeruginosa is the dominant species during cyanobacterial blooms in freshwater lakes. In the present study, we compared the bioaccumulation characteristics of cadmium (Cd) and zinc (Zn) in *Microcystis* cells. In short-term uptake tests, a rapid sorption of Cd and Zn occurred in the first few minutes, with a subsequent slower internalization process. No obvious difference was observed between Zn and Cd in terms of their short-term uptake kinetics. In efflux experiments, elimination of Zn from the cells was faster than that of Cd. In the 72-h exposure tests, the intracellular Cd concentrations increased with exposure time whereas the intracellular Zn concentrations always reached a plateau. The cellular Cd showed greater variation than the cellular Zn at various free Cd²⁺ or Zn²⁺ concentrations. The differences in Cd and Zn accumulation and elimination indicated that *Microcystis* cells had a higher bioaccumulation capacity for Cd than for Zn. In field studies, the bioconcentration factor (BCF) of Cd in lake-harvested *Microcystis* was more than 10 times higher than those of other metals. The results of the present study strongly suggested that the bloom-forming *Microcystis* may affect the Cd transportation and biogeochemical cycling in eutrophic freshwater ecosystems.

Microcystis aeruginosa, cadmium, zinc, bioaccumulation, bioconcentration factor

Citation: Zeng J, Zhao D Y, Ji Y B, et al. Comparison of heavy metal accumulation by a bloom-forming cyanobacterium, *Microcystis aeruginosa*. Chin Sci Bull, 2012, 57: 3790–3797, doi: 10.1007/s11434-012-5337-2

The bioaccumulation behaviors of heavy metals have attracted substantial attention. When toxic metals are discharged into natural waters, appropriate methods are required to remove these metal pollutants. Conventional methods including physiochemical and biological technologies have been employed to remove toxic metals from aqueous solutions [1,2]. Phytoplankton is suggested to be potential and attractive biosorbents to concentrate heavy metals due to their ubiquitous presence in aquatic ecosystems and high binding affinity to metals [1]. Generally, dried phytoplankton biomass is often used in the removal of metals with concentrations greater than several grams per liter [3,4]. In the case of low soluble metal concentrations in natural waters, phytoplankton cells play important roles in

metal bioaccumulation and transportation, which are of interest and need further investigation. Cadmium (Cd) and zinc (Zn) are major metal pollutants commonly found in industrial effluents. Cd is a non-essential element for most organisms and can be accumulated in organisms and subsequently result in severe toxicity. Zn is an essential micro-nutrient at low concentrations, which is also potentially hazardous to organisms when present at higher concentrations [5,6]. The Cd and Zn have similar chemical properties in natural aquatic environments; thus, a comparative study of these two substances might have relevant ecological implications.

Bioaccumulation of metals by different groups of phytoplankton has been investigated in previous studies [7–9]. Among these algal biosorbents, cyanobacteria are attractive because they are ubiquitous in nature and exhibit good

*Corresponding author (email: jzeng@niglas.ac.cn)

metal sorption properties [8,10]. *Microcystis* spp. are dominant species during cyanobacterial blooms in eutrophic freshwater lakes [11]. Some evidence has indicated that *Microcystis* hold great potential to be an effective bio-sorbent for heavy metal removal from contaminated waters [3,8]. Metal bioaccumulation by bloom-forming *Microcystis* cells may thus exert a major influence on the cycling of trace metals in eutrophic waters [12]. Therefore, for better understanding the fate of metals in aquatic environment, it is necessary to investigate the bioaccumulation of metals by the bloom-forming *Microcystis*.

The aim of the present study was to compare the differences between Cd and Zn regarding their bioaccumulation characteristics in *Microcystis* cells. The properties and capacities of living *Microcystis* cells to accumulate and eliminate Cd were specifically evaluated. Short-term (120-min) uptake, 10-h efflux, and 72-h accumulation tests were conducted. Metal concentrations in lake water and lake-harvested bloom-forming *Microcystis* assemblages were also measured in field investigations and the bioconcentration factor (BCF) was calculated. Radiotracer techniques were employed to quantify the accumulation and elimination of metals in the cells.

1 Materials and methods

1.1 Cell culture

The unicellular freshwater cyanobacterium, *Microcystis aeruginosa*, was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The stock cultures were routinely maintained in BG₁₁ medium [13] and cultured at temperature of 25°C, light irradiance of 35 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, and light:dark regime of 12:12 h. Cell density was counted daily with a hemocytometer under a microscope.

Concentrations of the stock Cd and Zn solutions were checked using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (Perkin-Elmer, Elan 9000, USA). Radiotracer techniques were employed to facilitate the metal bioaccumulation measurements in short-term uptake and efflux tests. All experimental glassware and polycarbonate bottles were soaked in 10% HCl solution overnight and then rinsed thoroughly with ultrapure water (Milli-Q, 18.2 M Ω cm, Millipore, USA).

1.2 Short-term uptake of Cd and Zn by *Microcystis*

The 120-min uptake tests were conducted to examine the short-term uptake kinetics of Cd and Zn by *Microcystis*. The designated concentrations of stable Cd and Zn were added into the polycarbonate bottles along with radioisotopes ¹⁰⁹Cd (in 0.1 mol L⁻¹ HCl, specific activity of 3.96 mCi mg⁻¹) and ⁶⁵Zn (in 0.1 mol L⁻¹ HCl, specific activity of 20 mCi mg⁻¹), respectively. Four metal concentrations were included and every concentration had three replicates.

Nitrilotriacetic acid (NTA) was used to control the metal speciation. The free metal ion concentration was calculated on the basis of the dissolved metal concentrations and NTA concentrations by using the MINEQL+ software [14]. The cells in exponential growth phase were harvested and re-suspended in the test medium with an initial cell density of 5×10^5 cells mL⁻¹. After incubation for 3, 10, 20, 30, 60, and 120 min, a 10-mL aliquot of the sample solution was taken from each bottle and gently filtered (<50 mmHg, 1 mmHg=133.32 Pa) through a 1- μm polycarbonate membrane to harvest the cells. The cells on membrane were washed three times with 10 mL of 0.1 mmol L⁻¹ EDTA solution to remove the loosely adsorbed metals. The non-EDTA-extractable fraction corresponded to intracellular metals. Radioactivity retained in the cells was measured with a gamma counter (Wallac 1480 NaI, Turku, Finland).

1.3 Efflux of Cd and Zn by *Microcystis*

The Cd and Zn efflux experiments were conducted to examine the metal retaining in *Microcystis*. The cells were pre-exposed at the two different Cd or Zn concentrations with respective radiotracers (¹⁰⁹Cd and ⁶⁵Zn) for 1 d. Then the cells were collected by centrifugation, washed with EDTA solution, and resuspended in BG₁₁ medium (without Cd or Zn) containing 1×10^{-5} mol L⁻¹ EDTA. The EDTA was used to chelate with the eliminated Cd or Zn and prevent their re-entering into the cells. At different time intervals (0, 1, 2, 4, 6, 8, and 10 h), the cells were filtered, washed, and assayed for the radioactivity as described above. The percentages of metals retained in the cells were calculated based on the radioactivity.

1.4 Long-term bioaccumulation of Cd and Zn by *Microcystis*

The 72-h tests were performed to contrast the differences between Cd and Zn in their bioaccumulation characteristics in *Microcystis* cells during a long-term exposure. There were six metal concentrations in the experiments. The collected cells were added to the equilibrated medium at an initial cell density of 4.0×10^5 cells mL⁻¹. During the 72-h incubation period, the cell density was counted at 0, 10, 24, 48, and 72 h for growth rate measurement. At the same time, a 100-mL cell culture from each replicate bottle was collected and rinsed with EDTA solution. The collected cell pellets were digested following the methods described by Ho et al. [15]. Briefly, the cells were predigested at room temperature for 2 h with 3 mL HNO₃ (69%, superpure), then digested within a heat block at 50°C for 5 h, 85°C for 48 h, and 100°C for 1 h. The reagent blanks were digested using the same protocol. The Cd and Zn concentrations were analyzed with ICP-MS, which represented the intracellular metal concentrations. The dissolved metal concentrations measured at the end of the experiments were about

70%–95% of the initial metal concentrations. The free M^{2+} concentration was controlled by the NTA ligand, which was suggested to keep almost constant in the medium during the experiments.

1.5 Metal concentrations in lake-harvested *Microcystis*

Bloom-forming *Microcystis* were collected from Lake Taihu, one of the largest, shallow, freshwater lakes in China. The *Microcystis* colonies were collected at the northern shore of Lake Taihu, where dense blooms were assembled at the surface water. The bloom assemblages were concentrated, rinsed with ultra-pure water, stored at -70°C , freeze-dried. A 0.1 g sample of dried *Microcystis* powder was digested following the methods described above. Water samples were also collected for measuring the dissolved metal concentrations (filtered through $0.45\ \mu\text{m}$ Millipore filters) at the same place where the bloom assemblages were collected. The metal concentrations were then analyzed with ICP-MS.

1.6 Data analysis

For the short-term uptake kinetics, the Michaelis-Menten (M-M) equation was used to fit the relationship between the metal uptake rate and the free M^{2+} concentration:

$$V = \frac{V_{\max}c}{K_m + c}, \quad (1)$$

where V is metal uptake rate, V_{\max} is maximum uptake rate when the metal-binding ligands are saturated, c is free M^{2+} concentration ($[M^{2+}]$), and K_m is half-saturation constant, defined as the metal concentration when V equals $0.5V_{\max}$.

To assess the differences between treatments, t -test was performed with SPSS software (SPSS Inc., Chicago). Statistical significance was acceptable at the $P \leq 0.05$ confidence level.

2 Results and discussion

2.1 Short-term uptake kinetics of Cd and Zn by *Microcystis*

The short-term (120-min) uptake of Cd and Zn as a function of time is shown in Figure 1. After the *Microcystis* cells have been exposed to the experimental medium containing various Cd or Zn concentrations, a rapid bioaccumulation process occurred in the first 10 min, with a subsequent slower uptake period over a contact time from 20 to 120 min. Furthermore, despite the fact that the bioaccumulation of Cd and Zn by *Microcystis* was more rapid at higher free M^{2+} concentrations within the initial 10-min rapid uptake period (Figure 1), the trends and patterns of the uptake dynamics were similar among different treatments.

The Cd and Zn uptake rates (Figure 2) were calculated based on the slopes of the short-term uptake curves. Two linear stages were calculated respectively, the initial stage

from 0 to 10 min and the second stage from 20 to 120 min. Based on the log values, both stages showed a linear relationship between the metal uptake rate and free $[M^{2+}]$, and no plateau was observed at higher M^{2+} concentrations. Based on the Michaelis-Menten (M-M) uptake kinetics, the calculated maximum uptake rates (V_{\max}) were 1.80 , 4.22×10^{-2} , 1.73 , and $4.96 \times 10^{-2}\ \text{mmol kg}^{-1}\ \text{min}^{-1}$ for Cd in 0–10 min, Cd in 20–120 min, Zn in 0–10 min, and Zn in 20–120 min, and the calculated half-saturation constants (K_m) were 2.85×10^{-6} , 7.71×10^{-7} , 1.24×10^{-6} , and $4.25 \times 10^{-7}\ \text{mol L}^{-1}$ for Cd in 0–10 min, Cd in 20–120 min, Zn in 0–10 min, and Zn in 20–120 min. It was obviously that the V_{\max} values of Cd or Zn in the initial stage (0–10 min) were extremely higher ($P < 0.05$) than those of the second stage (20–120 min). However, the V_{\max} showed no significant difference ($P > 0.05$) between Cd and Zn treatments in the same stage (in 0–10 or 20–120 min). Similarly, the K_m values of Cd or Zn in the initial stage (0–10 min) were significantly higher ($P < 0.05$) than those of the second stage (20–120 min), and no significant differences were observed between Cd and Zn treatments in the same stage (in 0–10 or 20–120 min).

Based on the 120-min uptake tests and the results from previous studies [16,17], it was suggested that metal short-term uptake by *Microcystis* involved two stages (Figure 1). The initial stage (0–10 min) corresponds mainly to a rapid passive sorption or surface complexation process (physicochemical sequestration), and the second stage (20–120 min) corresponds mainly to a slower active internalization process (metabolically-dependent uptake) [18,19]. Once the external metals were adsorbed onto the cell wall, internal uptake began immediately [17]. The observed slower uptake in the second stage might be attributed to the transportation of surface-bound metals into the cytoplasm. Swift and Forciniti [20] investigated the biosorption of lead by an active culture of cyanobacterium *Anabaena cylindrica* and they proposed that metals can be quickly adsorbed in the cell envelope initially and slowly internalized into the cells in a subsequent step. Smiejan et al. [21] examined the short-term (14 min) internalization of Cd by a freshwater bacterium *Rhodospirillum rubrum*; they found that the Cd accumulated by the cells increased linearly with time at lower ambient Cd concentrations while the accumulated Cd increased more rapidly during the initial 9 min at higher ambient Cd concentrations. These results are consistent with our present data. No obvious difference was observed between Zn and Cd in terms of their short-term uptake kinetics.

2.2 Efflux of Cd and Zn by *Microcystis*

The percentages of metals retained in *Microcystis* cells in efflux experiments are shown in Figure 3. Generally, the retained metals decreased with depuration time in the Cd-free or Zn-free medium. The efflux curves showed a rapid decrease in the first 2 h and a slower decrease during 2

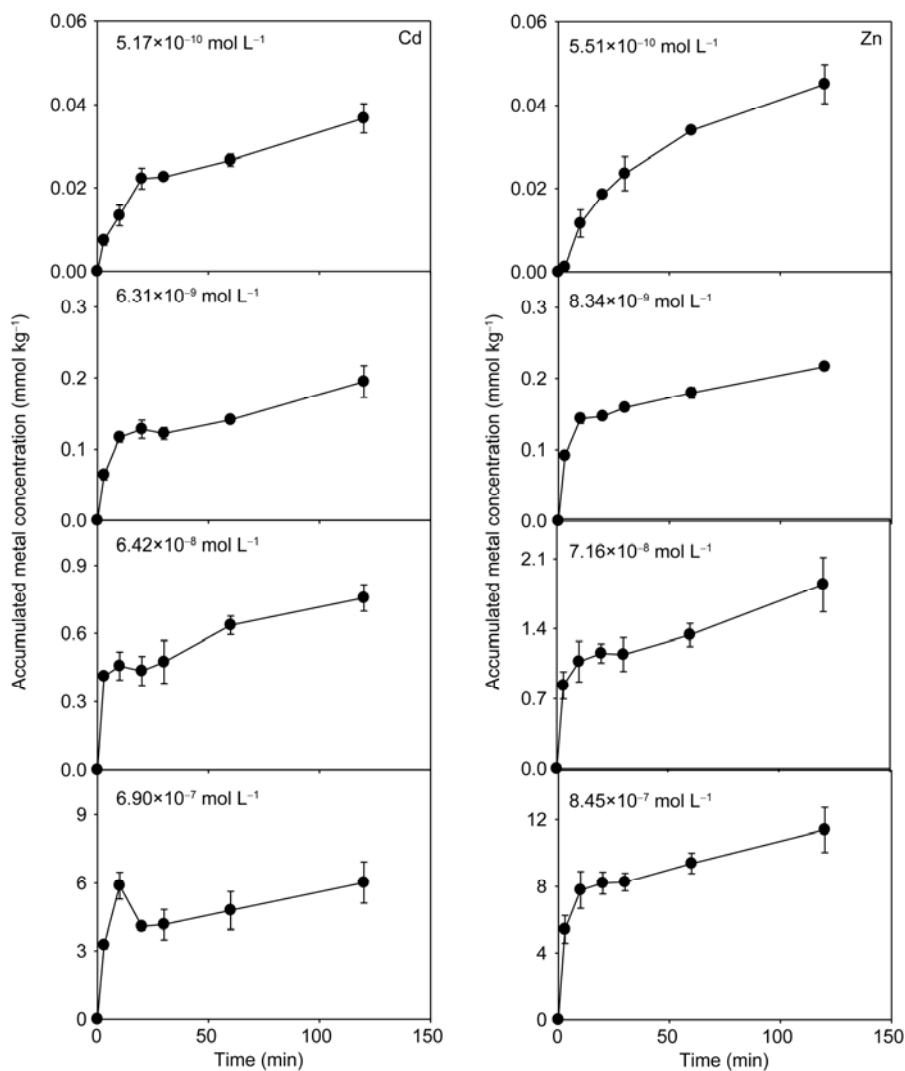


Figure 1 Accumulated Cd or Zn contents in *Microcystis aeruginosa* as a function of exposure time under different free Cd²⁺ or Zn²⁺ concentrations during the 120-min uptake tests. Error bars represent the standard deviation of replicate samples ($n = 3$).

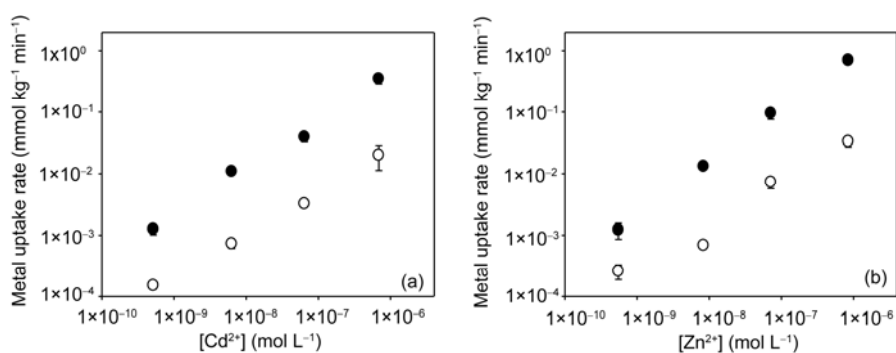


Figure 2 Uptake rate of Cd (a) and Zn (b) in 0–10 min (●) and 20–120 min (○) by *Microcystis aeruginosa* as a function of free metal ion concentrations. Error bars represent the standard deviation of replicate samples ($n = 3$).

to 10 h. After 10 h of depuration, the Cd retained in the cells were more than 75% and the Zn retained were approximately 50% for different concentration treatments, which suggested that Cd tended to be retained in the cells while Zn

was easier to be eliminated from the cells. Furthermore, the cells pre-exposed at higher metal concentrations seemed to show more loss of cellular metals. For example, the retained Zn in *Microcystis* cells were 55% and 39% for 5.51×10^{-10}

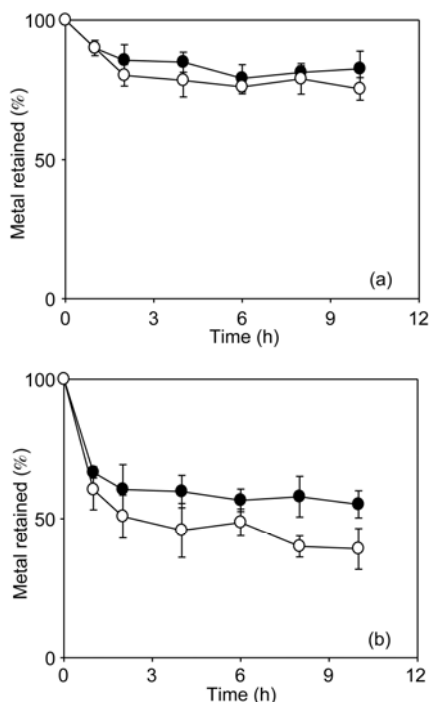


Figure 3 Retention of internalized Cd (a) and Zn (b) by *Microcystis aeruginosa* cells over 10 h of efflux in the presence of different free $[Cd^{2+}]$ or $[Zn^{2+}]$ (●: 2.32×10^{-10} mol L^{-1} $[Cd^{2+}]$ or 5.51×10^{-10} mol L^{-1} $[Zn^{2+}]$; ○: 2.64×10^{-8} mol L^{-1} $[Cd^{2+}]$ or 3.35×10^{-7} mol L^{-1} $[Zn^{2+}]$). Error bars represent the standard deviation of replicate samples ($n = 3$).

and 3.35×10^{-7} mol L^{-1} $[Zn^{2+}]$ treatments, respectively.

Efflux is an important pathway to reflect the retaining capacity of cellular metals. In the 10-h efflux tests of the present study, elimination of Zn from the cells was faster than that of Cd (Figure 3), which suggested that *Microcystis* exhibited higher capability to retain Cd in the cells. Wang and Dei [22] reported that the calculated Cd efflux constants in green alga *Ulva lactuca* were significantly lower than those of Zn, suggesting that Cd was difficult to be eliminated from the cells.

2.3 Long-term bioaccumulation of Cd and Zn by *Microcystis*

The accumulated Cd and Zn concentrations in *Microcystis* cells exposed to various free Cd^{2+} or Zn^{2+} concentrations at 0, 10, 24, 48, and 72 h time points are shown in Figure 4. Generally, the intracellular metal concentrations increased with exposure time, but a marked difference was observed between Cd and Zn treatments. For $[Cd^{2+}]$ ranged from 4.33×10^{-11} to 1.94×10^{-8} mol L^{-1} , the accumulated Cd concentrations increased linearly during the 72-h exposure period. At higher Cd concentrations ($[Cd^{2+}] > 5.78 \times 10^{-8}$ mol L^{-1}), the accumulated Cd concentrations increased during the initial 24 h and then leveled off. For Zn, the accumulated Zn concentrations were not significantly different during the 72-h exposure period when the $[Zn^{2+}]$ ranged from $3.91 \times$

10^{-11} to 6.28×10^{-10} mol L^{-1} . At $[Zn^{2+}]$ higher than 3.93×10^{-8} mol L^{-1} , the accumulated Zn concentrations increased dramatically during the initial 10 h, followed by a plateau when the exposure time ranged from 10 to 72 h.

The accumulated Cd and Zn concentrations in *Microcystis* cells as a function of free $[M^{2+}]$ after 72-h exposure are shown in Figure 5. The intracellular Cd concentrations increased largely from 0.015 to 27.90 mmol kg^{-1} (more than 1000 folds) at $[Cd^{2+}]$ ranged from 4.33×10^{-11} to 2.05×10^{-7} mol L^{-1} , and the intracellular Zn concentrations increased slightly from 0.42 to 2.67 mmol kg^{-1} (less than 10 folds) at $[Zn^{2+}]$ ranged from 3.91×10^{-11} to 5.21×10^{-7} mol L^{-1} . Based on the log values, the accumulated Cd concentrations were approximately linearly correlated with free $[Cd^{2+}]$ (4.33×10^{-11} mol $L^{-1} < [Cd^{2+}] < 5.78 \times 10^{-8}$ mol L^{-1}) after 72-h exposure, and a plateau was observed when $[Cd^{2+}] > 5.78 \times 10^{-8}$ mol L^{-1} . At the lower $[Cd^{2+}]$ treatments (4.33×10^{-11} mol $L^{-1} < [Cd^{2+}] < 1.94 \times 10^{-8}$ mol L^{-1}), the intracellular Cd concentrations increased linearly with exposure time and did not reach equilibrium during the 72-h exposure period. Therefore, the relationship between Cd bioaccumulation and free $[Cd^{2+}]$ might change after a long time exposure. In contrast, the intracellular Zn concentrations maintained quite stable even at lower $[Zn^{2+}]$, and which were not directly proportional to aqueous free $[Zn^{2+}]$ ranged from 3.91×10^{-11} to 6.28×10^{-10} mol L^{-1} and a first-order uptake flux was not observed.

The growth curves of the *Microcystis* cells exposed to various Cd or Zn concentrations are shown in Figure 6. Generally, the growth of the cells decreased progressively with elevated metal concentrations. At lower metal concentrations ($[Cd^{2+}]$ ranged from 4.33×10^{-11} to 7.59×10^{-10} mol L^{-1} or $[Zn^{2+}]$ ranged from 3.91×10^{-11} to 5.70×10^{-9} mol L^{-1}), the cell density increased linearly with incubation time, and its specific growth rate was about 0.37–0.41 d^{-1} . At the higher metal concentrations ($[Cd^{2+}] > 5.78 \times 10^{-8}$ mol L^{-1} or $[Zn^{2+}] > 1.90 \times 10^{-7}$ mol L^{-1}), the growth of the cells was completely inhibited after 72 h of exposure. Metal toxicity to the cells was time-dependent at higher metal concentrations, which might be attributed to the enhanced intracellular metal concentrations. Previous studies demonstrated that the intracellular metal concentration can be used to predict the metal toxicity to the algal cells [23].

The differences between Cd and Zn in their accumulation characteristics by *Microcystis* during the 72-h exposure period suggested that different regulation mechanisms may exist in *Microcystis* cells. Zinc is a biologically necessary element; thus, Zn homeostasis is always thought to be responsible for the observed relatively constant quotas of cellular Zn [24–26]. Although new Zn is entering the cells during the exposure period, an equivalent amount of Zn may be excreted to match the rate of Zn uptake. Therefore, the internal Zn content approaches equilibrium. As mentioned above, elimination of Zn from the cells was faster than that of Cd (Figure 3). Therefore, the accumulated Zn

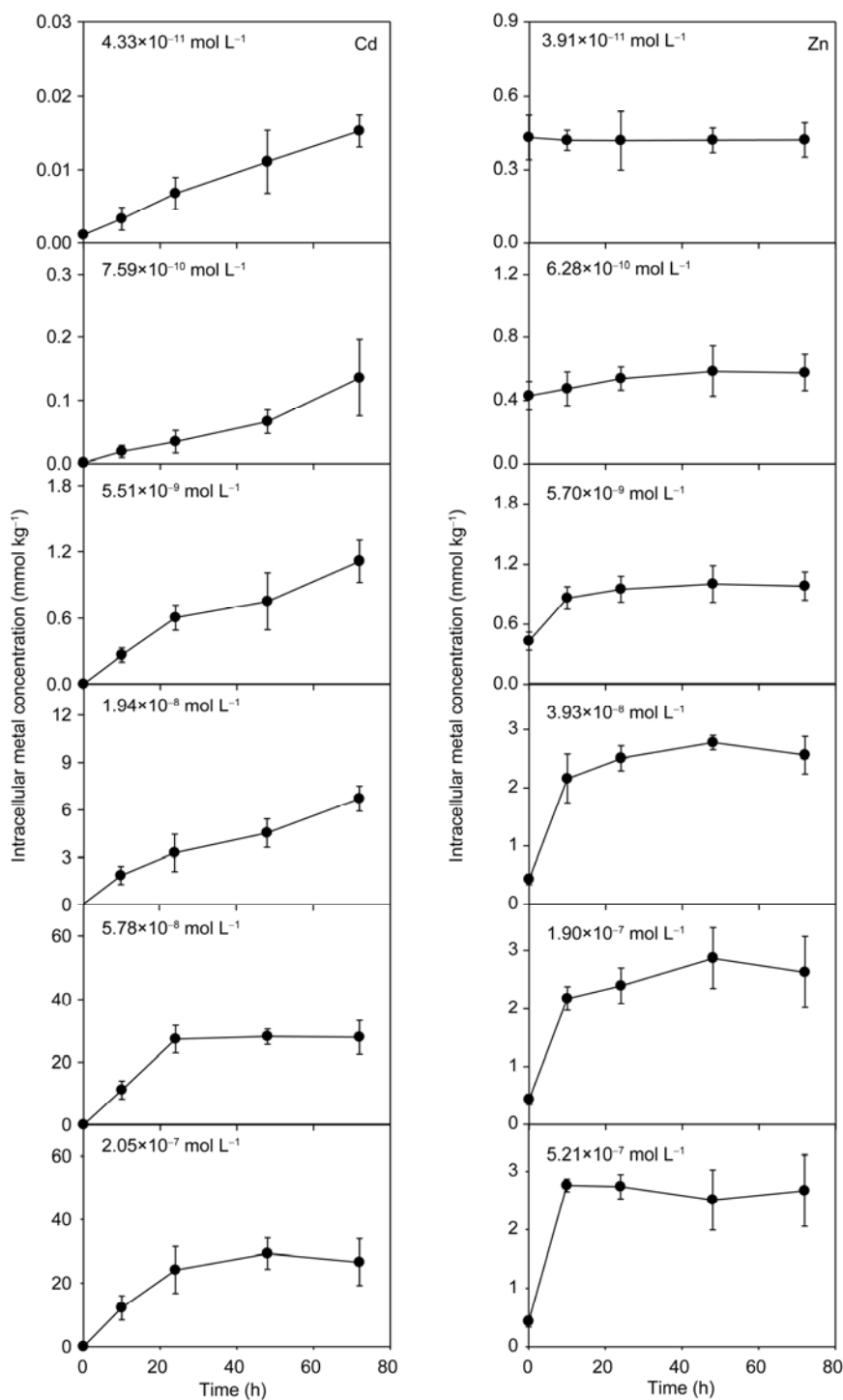


Figure 4 Changes of the accumulated Cd or Zn concentrations in *Microcystis aeruginosa* as a function of exposure time under different free Cd^{2+} or Zn^{2+} concentrations during the 72-h exposure. Error bars represent the standard deviation of replicate samples ($n = 3$).

concentrations remained approximately constant after 10 h exposure due to the Zn homeostasis in *Microcystis* cells (Figure 4). Hassler and Wilkinson [27] found that the intracellular Zn concentrations were nearly independent of the free $[\text{Zn}^{2+}]$ in the media. Knauer et al. [16] also demonstrated that phytoplankton was able to maintain relatively constant cellular Zn contents over several orders of magni-

tude variations of $[\text{Zn}^{2+}]$ in external medium. In contrast, Cd is a toxic metal for most organisms. The import of Cd across the cytoplasmic membrane into the cytoplasm is suggested to be facilitated by Zn transport systems [24]. The internalized Cd can be subsequently bound to metallothionein and is not readily excreted from the cytoplasm [6]. Therefore, the intracellular Cd concentration increased

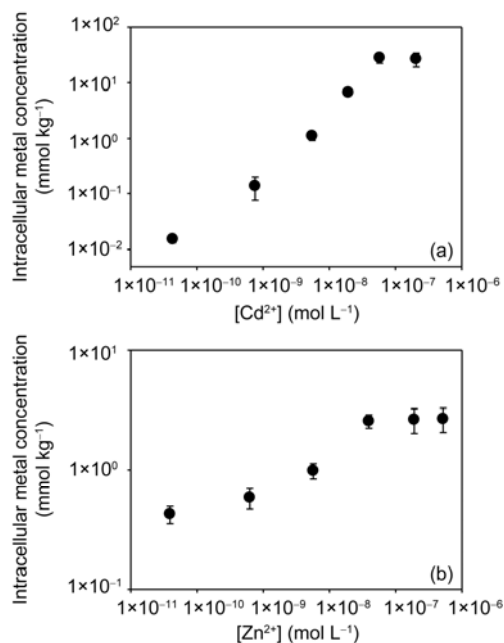


Figure 5 The accumulated Cd (a) and Zn (b) concentrations in *Microcystis aeruginosa* as a function of free Cd^{2+} and Zn^{2+} concentrations after 72-h exposure. Error bars represent the standard deviation of replicate samples ($n = 3$).

progressively with exposure time and no saturation was observed at lower Cd concentrations (Figure 4), which is likely due to the lack of sophisticated strategies in *Microcystis* cells to export excess intracellular Cd.

2.4 Metal concentrations in lake-harvested *Microcystis*

Lake Taihu is characterized by its eutrophic status and frequent cyanobacterial blooms in recent decades, and *Microcystis* spp. are the dominant species during blooming in this lake [11]. Metal concentrations in lake water and bloom materials were summarized in Table 1. The bioconcentration factor (BCF) was calculated based on the metal concentrations in lake-harvested *Microcystis* and the dissolved metal concentrations of lake water as listed in Table 1. The BCF value of Cd (101680) was remarkably higher (more than 10 times higher) than those of other metals. This observation is noteworthy and strongly suggested that *Microcystis* biomass was a promising biosorbent candidate for removing Cd from aqueous solution even at lower Cd concentrations.

Heavy metals can be selectively removed by the *Microcystis* cells. The bioaccumulation characteristics of different metals in bloom-forming *Microcystis* may have important implications for metal cycling in eutrophic aquatic environments. Parker et al. [28] suggested that the naturally occurred *Microcystis* exhibited substantial abilities for the sorption of copper, cadmium, and nickel. Luoma et al. [29] observed that a spring phytoplankton bloom caused remarkable reductions (more than 50%) in the dissolved Cd and

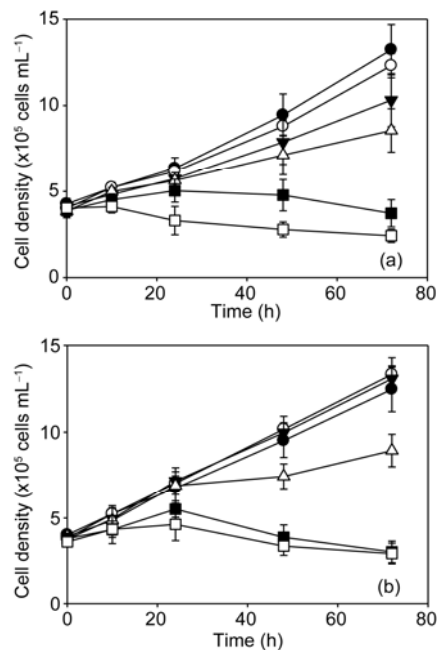


Figure 6 Growth curves of *Microcystis aeruginosa* under varied $[\text{Cd}^{2+}]$ (a) or $[\text{Zn}^{2+}]$ (b) during the 72-h exposure period. Symbols with different shapes indicate different metal concentration treatments (\bullet : 4.33×10^{-11} mol L^{-1} $[\text{Cd}^{2+}]$ or 3.91×10^{-11} mol L^{-1} $[\text{Zn}^{2+}]$; \circ : 7.59×10^{-10} mol L^{-1} $[\text{Cd}^{2+}]$ or 6.28×10^{-10} mol L^{-1} $[\text{Zn}^{2+}]$; \blacktriangledown : 5.51×10^{-9} mol L^{-1} $[\text{Cd}^{2+}]$ or 5.70×10^{-9} mol L^{-1} $[\text{Zn}^{2+}]$; \triangle : 1.94×10^{-8} mol L^{-1} $[\text{Cd}^{2+}]$ or 3.93×10^{-8} mol L^{-1} $[\text{Zn}^{2+}]$; \blacksquare : 5.78×10^{-8} mol L^{-1} $[\text{Cd}^{2+}]$ or 1.90×10^{-7} mol L^{-1} $[\text{Zn}^{2+}]$; \square : 2.05×10^{-7} mol L^{-1} $[\text{Cd}^{2+}]$ or 5.21×10^{-7} mol L^{-1} $[\text{Zn}^{2+}]$). Error bars represent the standard deviation of replicate samples ($n = 3$).

Ni concentrations in South San Francisco Bay, USA, due to the accumulation of metals in the phytoplankton. The results of the present study indicated a high Cd adsorption capacity for the bloom-forming *Microcystis*. Therefore, the naturally abundant *Microcystis* biomass, which is otherwise a nuisance in cyanobacterial blooms, may exhibit favorable potential for application in removal of Cd from natural waters.

3 Conclusions

In conclusion, our study demonstrated that the bloom-forming *Microcystis* showed different performance for Cd and Zn accumulation. The Cd tends to be retained in the cells while Zn is easier to be eliminated from the cells. The intracellular Cd concentrations increased with exposure time, whereas the intracellular Zn concentrations always reached a plateau. The cellular Cd showed greater variation than Zn at various free Cd^{2+} or Zn^{2+} concentrations after a long-term exposure. The BCF value of Cd in the lake-harvested *Microcystis* was significantly higher than those of other metals. These results suggested that the cyanobacterial bloom may offer great potential for accumulation and transportation of Cd in aquatic environments and had implications for understanding the biogeochemical cycling of different metals in eutrophic freshwater ecosystems.

Table 1 Metal concentrations in lake water and lake-harvested *Microcystis* of Lake Taihu and the calculated BCF values^{a)}

Metals	Lake water ($\times 10^{-9}$ mol L ⁻¹)	Lake-harvested <i>Microcystis</i> (mmol kg ⁻¹)	BCF ^{b)}
Cd	0.21 \pm 0.09	0.02 \pm 0.00	101680
Ni	47.07 \pm 8.08	0.36 \pm 0.07	7611
Cu	61.27 \pm 21.60	0.17 \pm 0.03	2755
Zn	81.85 \pm 28.72	0.17 \pm 0.04	2016
Cr	50.26 \pm 15.58	0.06 \pm 0.01	1188
Pb	7.02 \pm 5.37	0.01 \pm 0.00	928

a) Values are mean \pm SD ($n = 3$). b) BCF: Bioconcentration factor, which was calculated based on the accumulated metal concentrations in lake-harvested *Microcystis* and the dissolved metal concentrations in the water.

This work was supported by the National Basic Research Program of China (2008CB418104, 2008CB418102), the National Natural Science Foundation of China (41101052, 41001044), the Natural Science Foundation of Jiangsu Province (BK2011876), the Open Foundation from the State Key Laboratory of Hydrology-Water Resources and Hydraulic Engineering at Hohai University (2010490311), the Open Foundation from the State Key Laboratory of Pollution Control and Resources Reuse at Nanjing University (PCRRF11022), and the Project of Nanjing Institute of Geography & Limnology (NIGLAS2010QD10).

- Ahluwalia S S, Goyal D. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour Technol*, 2007, 98: 2243–2257
- Espinoza-Quiñones F R, Módenes A N, Costa I L, et al. Kinetics of lead bioaccumulation from a hydroponic medium by aquatic macrophytes *Pistia stratiotes*. *Water Air Soil Pollut*, 2009, 203: 29–37
- Chen J Z, Tao X C, Xu J, et al. Biosorption of lead, cadmium and mercury by immobilized *Microcystis aeruginosa* in a column. *Process Biochem*, 2005, 40: 3675–3679
- Han X, Wong Y S, Wong M H, et al. Biosorption and bioreduction of Cr(VI) by a microalgal isolate, *Chlorella miniata*. *J Hazard Mater*, 2007, 146: 65–72
- Nan Z, Li J, Zhang J, et al. Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. *Sci Total Environ*, 2002, 285: 187–195
- Rainbow P S. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environ Pollut*, 2002, 120: 497–507
- Slaveykova V I, Wilkinson K J. Physicochemical aspects of lead bioaccumulation by *Chlorella vulgaris*. *Environ Sci Technol*, 2002, 36: 969–975
- Li P F, Mao Z Y, Rao X J, et al. Biosorption of uranium by lake-harvested biomass from a cyanobacterium bloom. *Bioresour Technol*, 2004, 94: 193–195
- Wang W X, Dei R C H, Hong H S. Seasonal study on the Cd, Se, and Zn uptake by natural coastal phytoplankton assemblages. *Environ Toxicol Chem*, 2005, 24: 161–169
- Baptista M S, Vasconcelos M T. Cyanobacteria metal interactions: Requirements, toxicity, and ecological implications. *Crit Rev Microbiol*, 2006, 32: 127–137
- Wu X, Kong F, Chen Y, et al. Horizontal distribution and transport processes of bloom-forming *Microcystis* in a large shallow lake (Taihu, China). *Limnologica*, 2010, 40: 8–15
- Morel F M M, Price N M. The biogeochemical cycles of trace metals in the oceans. *Science*, 2003, 300: 944–947
- Stanier R Y, Kunisawa R, Mandel M, et al. Purification and properties of unicellular blue-green algae (*order Chroococcales*). *Microbiol Mol Biol Rev*, 1971, 35: 171–205
- Zeng J, Yang L, Wang W X. Cadmium and zinc uptake and toxicity in two strains of *Microcystis aeruginosa* predicted by metal free ion activity and intracellular concentration. *Aquat Toxicol*, 2009, 91: 212–220
- Ho T Y, Wen L S, You C F, et al. The trace-metal composition of size-fractionated plankton in the South China Sea: Biotic versus abiotic sources. *Limnol Oceanogr*, 2007, 52: 1776–1788
- Knauer K, Behra R, Sigg L, et al. Effects of free Cu²⁺ and Zn²⁺ ions on growth and metal accumulation in freshwater algae. *Environ Toxicol Chem*, 1997, 16: 220–229
- Rangsayatorn N, Upatham E S, Kruatrachue M, et al. Phytoremediation potential of *Spirulina (Arthrospira) platensis*: Biosorption and toxicity studies of cadmium. *Environ Pollut*, 2002, 119: 45–53
- Wolterbeek H T, Viragh A, Sloof J E, et al. On the uptake and release of zinc (⁶⁵Zn) in the growing alga *Selenastrum capricornutum* Printz. *Environ Pollut*, 1995, 88: 85–90
- Campbell P G C, Errécalde O, Fortin C, et al. Metal bioavailability to phytoplankton-applicability of the biotic ligand model. *Comp Biochem Phys C-Toxicol Pharmacol*, 2002, 133: 189–206
- Swift D T, Forciniti D. Accumulation of lead by *Anabaena cylindrica*: Mathematical modeling and an energy dispersive X-ray study. *Biotechnol Bioeng*, 1997, 55: 408–418
- Smiejan A, Wilkinson K J, Rossier C. Cd bioaccumulation by a freshwater bacterium, *Rhodospirillum rubrum*. *Environ Sci Technol*, 2003, 37: 701–706
- Wang W X, Dei R C H. Kinetic measurements of metal accumulation in two marine macroalgae. *Mar Biol*, 1999, 135: 11–23
- de Schampelaere K A C, Stauber J L, Wilde K L, et al. Toward a biotic ligand model for freshwater green algae: Surface-bound and internal copper are better predictors of toxicity than free Cu²⁺-ion activity when pH is varied. *Environ Sci Technol*, 2005, 39: 2067–2072
- Blencowe D K, Morby A P. Zn (II) metabolism in prokaryotes. *Fems Microbiol Rev*, 2003, 27: 291–311
- Cavet J S, Borrelly G P M, Robinson N J. Zn, Cu and Co in cyanobacteria: Selective control of metal availability. *Fems Microbiol Rev*, 2003, 27: 165–181
- Hassler C S, Behra R, Wilkinson K J. Impact of zinc acclimation on bioaccumulation and homeostasis in *Chlorella kesslerii*. *Aquat Toxicol*, 2005, 74: 139–149
- Hassler C S, Wilkinson K J. Failure of the biotic ligand and free-ion activity models to explain zinc bioaccumulation by *Chlorella kesslerii*. *Environ Toxicol Chem*, 2003, 22: 620–626
- Parker D L, Mihalick J E, Plude J L, et al. Sorption of metals by extracellular polymers from the cyanobacterium *Microcystis aeruginosa* fo. *flos-aquae* strain C3-40. *J Appl Phycol*, 2000, 12: 219–224
- Luoma S N, van Geen A, Lee B G, et al. Metal uptake by phytoplankton during a bloom in South San Francisco Bay: Implications for metal cycling in estuaries. *Limnol Oceanogr*, 1998, 43: 1007–1016

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.