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CADMIUM ACCUMULATION IN THE ROOTLESS MACROPHYTE *WOLFFIA GLOBOSA* AND ITS POTENTIAL FOR PHYTOREMEDIATION

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Cadmium (Cd) pollution around the world is a serious issue demanding acceptable solutions, one of which is phytoremediation that is both cost-effective and eco-friendly. Removal of Cd from contaminated water using plants with high growth rates and sufficient Cd accumulation abilities could be an appropriate choice. Here, we investigated a potential Cd accumulator, Wolffia, a rootless duckweed with high growth rate. Cd uptake, accumulation, tolerance, and phytofiltration ability by Wolffia globosa were examined. Furthermore, the effects of arsenic (As) on Cd uptake and phytofiltration by W. globosa were also studied. Cd uptake kinetics showed a linear pattern and a hyperbolic pattern without a plateau in lower (0–2 μM) and higher (0–200 μM) Cd concentration ranges, respectively, suggesting rapid Cd uptake by W. globosa. Cd accumulation ability by W. globosa was higher at Cd concentrations < 10 μM than at > 10 μM . All the five species of Wolffia exposed to 1 μM Cd for 5 days accumulated > 500 mg Cd kg⁻¹ DW. Ten gram fresh W. globosa could diminish almost all the Cd (2 μM) in a 200 mL solution. This enormous accumulation ability was mostly due to passive adsorption of Cd by the apoplast. Arsenic had no significant effect on Cd uptake and phytofiltration. The fresh fronds also showed a great As extracting ability. The results indicated that Wolffia is a strong Cd accumulator and has great Cd phytoremediation potential. Therefore, this plant can be used in fresh aquatic environments co-contaminated by low-levels of Cd and As.

Supplemental materials are available for this article. Go to the publisher's online edition of International Journal of Phytoremediation to view the supplemental file.

KEY WORDS: cadmium, uptake kinetics, accumulation, phytoextraction, phytoremediation, arsenic, *Wolffia globosa*

INTRODUCTION

Cadmium (Cd), listed as number 7 (of 275) in the priority list of hazardous materials (ASTDR 2011), is highly toxic to humans and plants. It can cause a variety of

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human diseases, such as renal tubular dysfunction, pulmonary emphysema and osteoporosis/osteomalacia, without an efficient chelation treatment for reducing Cd body burden (Wagner 1993; Satarug *et al.* 2010). Significant amounts of Cd-containing waste continue to be produced and discharged into water systems due to its increasing industrial use (Jarup 2003). The high toxicity and great solubility in water make Cd a significant pollutant (Lockwood 1976). So far there is no evidence of its essentiality in plant growth. Moreover, Cd can be taken up and accumulated by many plants through pathways for essential elements (Shah 2011), through which it enters into the food chain.

Many solutions have been developed since the recognition of the adverse effects of Cd pollution. However, traditional water treatments, such as precipitation, flocculation, ion exchange, and membrane filtration, can be expensive, ineffective at low metal concentrations, or not eco-friendly, with subsequent huge quantity of toxic sludge to dispose (Ahluwalia and Goyal 2007; Halttunen, Salmine, and Tahvonen 2007). In addition, these techniques cannot be utilized for *in situ* Cd removal. In contrast, phytoremediation is an emerging cost-effective and eco-friendly technology that utilizes plants to extract, transform or stabilize a number of pollutants in water, sediments or soils (Prasad, Greger, and Aravind 2006). These processes can be conducted directly at the sites of pollution, with some simple protocols of plant harvesting in the case of phytoextraction. Therefore, Cd phytoextraction by some hyperaccumulators could be a “greener” and more sustainable way of Cd removal from water. However, in most cases these Cd hyperaccumulators grow slowly with low biomass, thereby limiting its wide use in remediation. In addition, within the less than 10 species of well-studied Cd hyperaccumulators (Dahmani-Muller *et al.* 2000; Reeves and Baker 2000; Yang *et al.* 2004; Vogel-Mikuš, Drobne, and Regvar 2005; Wei *et al.* 2005), none of them is an aquatic plant suitable for Cd extraction from aquatic environments. Therefore, aquatic plants that possess high biomass or a rapid growth with sufficient Cd accumulation will have the potential for Cd phytoremediation of contaminated aquatic environments.

Wolffia, in the family of Lemnaceae (duckweed), is a rootless free-floating aquatic plant consisting of small fronds without xylem or phloem cells. Its simplicity in structure makes it an interesting model plant for studies of elemental behavior in plants (Zhang *et al.* 2009). In addition, the high growth rate and potential to take up large amounts of pollutants (Hillman and Culley 1978) make it an excellent candidate for heavy metal phytoremediation. Previous studies have shown that *W. globosa*, the smallest plant in this genus, has a potential to accumulate Cd (Garg and Chandra 1994; Boonyapookana *et al.* 2002), and this plant has also been identified as having potential in As phytoremediation (Zhang *et al.* 2009). However, to our knowledge, so far there is little systematic study on Cd uptake, accumulation, tolerance, and phytoremediation ability by *W. globosa*. In the present study, we therefore characterized these four aspects in *W. globosa*. Furthermore, the phytoremediation potency by *W. globosa* under conditions of complex contamination by Cd/As, which is common in real environments (Williams *et al.* 2009; Wang *et al.* 2010), was also discussed.

MATERIALS AND METHODS

Plant Culture

W. globosa L. was collected from ponds in Wuhan, Hubei province, China, while *W. australiana*, *W. cylindracea*, *W. columbiana*, and *W. arrhiza* species were kindly supplied by Prof. Dr. Elias Landolt (ETH, Zurich). These plants were grown hydroponically in

a greenhouse at ambient temperatures (20–32°C) and relative humidity (60–85%) under sunlight in Xiamen, China. The basal nutrient solution was modified 1/5 Hoagland solution with a composition as follows: 2.0 mM KNO₃, 0.2 mM KH₂PO₄, 0.4 mM MgSO₄, 0.5 mM Ca(NO₃)₂, 4.0 μM FeSO₄-EDTA, 2.0 μM MnCl₂, 0.07 μM CuSO₄, 0.14 μM ZnSO₄, 9.7 μM H₃BO₃, and 0.1 μM Na₂MoO₄ (pH was adjusted to 6.0 with KOH or HNO₃). Nutrient solution was renewed once every week. All the experiments except specifically noted were carried out in a ray radiation incubator (GZP-450S, Jinghong, Shanghai, China) with the following conditions: 14 h light period with a light intensity of 50 μmol photons m⁻² s⁻¹, 30:25°C day: night temperature, and 60% relative humidity.

Cd Accumulation and Tolerance in *W. globosa*

Four replicates of 5 g (fresh weight, FW) *W. globosa* were weighed into containers with 500 mL nutrient solution containing 0, 1, 5, 10, 50, or 100 μM Cd(NO₃)₂ (pH was adjusted to 6.0 with KOH). The surface area of the solution in the container was around 210 cm². The containers were placed randomly in the ray radiation incubator as mentioned above. Nutrient solution was renewed every day. After 7 days, the plants were harvested. The harvesting process included: collection, rinsing with an ice-cold desorption solution (2 mM MES, 5 mM CaCl₂) for 15 min and blotting dry. Fresh weight of each replicate was recorded. The fresh fronds were then immediately weighed into microwave-digestion tubes for further digestion and Cd concentration determination.

Cd Uptake Kinetics in *W. globosa* with or without As

Three replicates of 2.0 g (FW) *W. globosa* were incubated in 250 mL conical flasks with 100 mL uptake solution (pH was adjusted to 6.0 with KOH) containing 2.0 mM MES, 0.5 mM Ca(NO₃)₂ and 0, 0.05, 0.1, 0.5, 1, or 2 μM Cd(NO₃)₂, with or without 10 μM Na₃AsO₄ (As(V)) or NaAsO₂ (As(III)). The flasks were shaken at 100 rpm at 30°C in a shaking incubator (TS-211CGZ, Tunsuc, Shanghai, China) for 30 min. The plants were then harvested and weighed into microwave-digestion tubes for further digestion and Cd concentration determination. Cd uptake kinetics with higher concentrations (0, 1, 5, 10, 50, 100, and 200 μM) of Cd without As were also measured in the same manner.

Cd Phytofiltration by *W. globosa* with or without As

Fresh fronds of *W. globosa* were collected from stock culturing and freeze-dried. Half of the dry fronds were ground with a motor mill. Meanwhile, fresh *W. globosa* was transferred to a 5 L plastic container filled with 2 mM MES and 0.5 mM Ca(NO₃)₂ (pH was adjusted to 6.0 with KOH) and incubated for 24 h. Then three replicates of 10 g fresh fronds and 0.46 g dry fronds (the same biomass as 10 g fresh fronds, ground or unground), respectively, were then transferred into 1 L conical flasks filled with 200 mL treatment solutions containing 2 mM MES, 0.5 mM Ca(NO₃)₂ and 2 μM Cd(NO₃)₂ (pH was adjusted to 6.0 with KOH), with or without 10 μM As(V). Control treatments without *W. globosa* were carried out at the same time. The flasks were covered with membranes with small holes to minimize evaporation and were placed randomly in the ray radiation incubator as mentioned above. At 0, 1, 3, 6, 12, 24, 48, and 72 h, 1 mL solution was taken from each flask and replaced with fresh Cd solution. Cd and As concentrations in the solution samples were determined by inductively coupled plasma mass spectrometer

(ICP-MS) (Agilent 7500cx, Agilent Technologies, USA). At 72 h, the fresh *W. globosa* was harvested and weighed into microwave-digestion tubes for further digestion and Cd concentration determination.

Cd Accumulation by 5 Species of *Wolffia*

Five species of *Wolffia*, namely *W. globosa*, *W. australiana*, *W. cylindracea*, *W. columbiana*, and *W. arrhiza*, were chosen to study the ability to accumulate Cd. Four replicates of 5 g (FW) fronds of each species were weighed into containers with 500 mL nutrient solution containing 1 μM $\text{Cd}(\text{NO}_3)_2$ (pH was adjusted to 6.0 with KOH). The surface area of the solution in the container was around 210 cm^2 . The containers were placed randomly in the ray radiation incubator as described above. Fresh weights were recorded and nutrient solution was renewed every day. Five days later, fronds were harvested and weighed into microwave-digestion tubes for further digestion and Cd concentration determination. Cd concentrations on FW basis were transferred into concentrations based on dry weight (DW) according to the water contents of the 5 species.

Plant Tissue Analysis and Cd Determination

Around 0.2 g (FW) of *W. globosa* were weighted into 50 mL Teflon microwave-digestion tubes, steeped in 2 mL nitric acid (GR, Merck, Germany) and allowed to stand overnight at room temperature. One milliliter H_2O_2 (GR, Sinopharm, Shanghai, China) was added into the mixture 10 min before the digestion. The tubes were then heated in a microwave accelerated reaction system (CEM Microwave Technology Ltd, Matthews, NC, USA). The digestion program was as follows: 55°C for 10 min, 75°C for 10 min, 95°C for 30 min, with 5 min ramp time between each stage. The digests were diluted to 50 mL with Milli-Q water (18.2 $\text{M}\Omega$) and filtered with 0.45- μm nylon filters before Cd determination. Cd concentrations were determined by ICP-MS. For quality control, three replicates of reagent blanks and certified reference materials (Chinese rice flour, GBW 10010, National Research Center for Standard Materials, China) were included in each digestion run. The recoveries of Cd from Chinese rice flour were between 93–105%. Cd concentrations in the solutions were also determined by ICP-MS after filtration with 0.45- μm nylon filters.

Statistical Analysis

The means and standard deviations (SD, $n = 3$) or standard errors (SE, $n = 4$) were calculated by Microsoft Office Excel 2010. Data variances were analyzed using one-way ANOVA with SPSS 16.0. When a significant difference was observed among treatments ($P < 0.05$ or $P < 0.01$), data were submitted to multiple comparisons using the LSD test.

RESULTS

***W. globosa* Accumulated Significant Amount of Cd while Having Moderate Cd Tolerance**

The growth of *W. globosa* was significantly ($P < 0.01$) inhibited at Cd concentrations $> 5 \mu\text{M}$ (Figure 1). The dose-response data could be fitted into a log-logistic equation with $R^2 = 0.995$. Based on the equation, the effective ambient concentration of Cd that caused

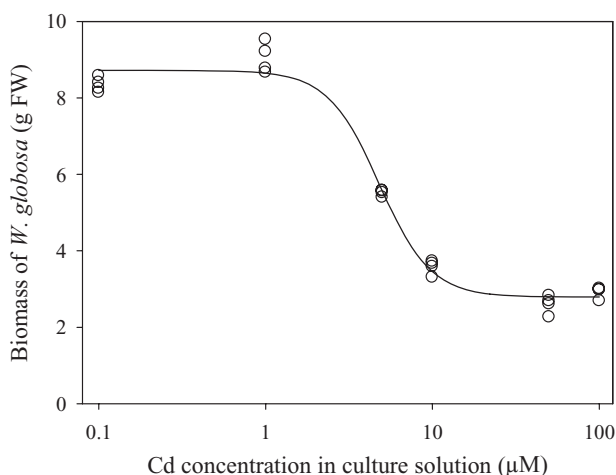


Figure 1 Biomass of *Wolffia globosa* exposed to different concentrations of cadmium (Cd) for 1 wk. Data are individual replicates. The line is the fitted log-logistic curves. To allow log transformation, a small value (0.1) was added to the zero Cd concentration in the control treatment.

a 50% inhibition in growth (EC_{50}) was estimated to be $4.80 \mu\text{M}$. When the biomass of the fronds was plotted against Cd concentrations in the plant, a log-logistic equation ($R^2 = 0.996$) was also obtained (data not shown). The EC_{50} based on the internal Cd concentration was estimated to be $137.62 \text{ mg Cd kg}^{-1} \text{ FW}$.

Cd concentrations in the fronds of *W. globosa* increased significantly ($P < 0.01$) with increasing Cd concentrations in the solution (Figure 2). The accumulation could be divided into two phases, both were linear to the corresponding Cd concentrations in the solutions ($R^2 = 0.999$). The Cd accumulation ability of *W. globosa* exposed to $\text{Cd} < 10 \mu\text{M}$ was higher than those exposed to $\text{Cd} > 10 \mu\text{M}$ (Figure 2). When exposed to $1 \mu\text{M}$ Cd for 1 week, Cd concentration in the fronds was $22.42 \text{ mg kg}^{-1} \text{ FW}$ with a bioconcentration

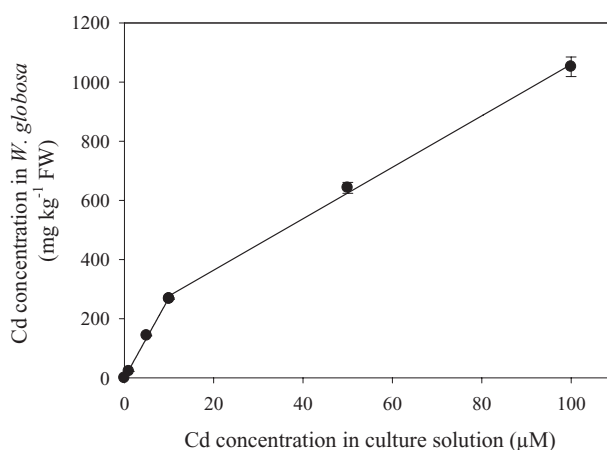


Figure 2 Cadmium (Cd) concentration in *Wolffia globosa*. Fresh fronds of *W. globosa* were incubated in elevated concentrations of Cd (pH = 6.0) for 1 wk, and the solutions were renewed every day. Each point is presented as mean \pm SE (n = 4)

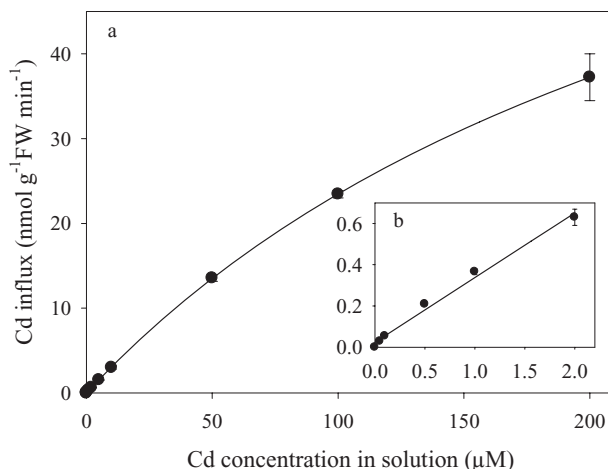


Figure 3 Concentration-dependent uptake kinetics of cadmium (Cd) by *Wolffia globosa*. The Cd concentration range was 0–200 μM for (a) and 0–2 μM for (b). The uptake time was 30 min and pH = 6.0. Each point is presented as mean \pm SD ($n = 3$)

factor (BCF, the ratio of frond Cd concentration to solution Cd concentration) of 199 based on the fresh weight and showing no morphological symptoms of Cd-toxicity. When the Cd concentration increased to 5 μM , Cd accumulation in the fronds reached 143.12 mg kg^{-1} FW with a BCF of 255, with only slight chlorosis. The BCF decreased thereafter (data not shown), although Cd concentrations in the fronds increased with the increasing Cd concentrations in the solution. Fronds exposed to 10 μM Cd had the same extent of chlorosis as those exposed to 5 μM Cd. However, when external Cd concentrations reached 50 μM or 100 μM , *W. globosa* turned dark green with some abnormal necrotic fronds.

Cd Uptake by *W. globosa* Was Rapid and Unaffected by As

In the higher concentration range (0–200 μM), short-term Cd uptake by *W. globosa* showed a hyperbolic pattern (Figure 3). The uptake data could be fitted satisfactorily into a Michaelis-Menten equation ($R^2 = 1.000$). The values for V_{max} and K_m were 89.91 $\text{nmol}\cdot\text{g}^{-1}$ FW $\cdot\text{min}^{-1}$ and 283.01 μM , respectively. In the lower concentration range (0–2 μM), Cd uptake by *W. globosa* was linear in relation to the external Cd concentrations ($R^2 = 0.996$).

As(V) or As(III) did not significantly change the uptake kinetics of Cd by *W. globosa*. The uptake data could be fitted satisfactorily by a linear regression model, with R^2 ranging from 0.996 to 0.999 (Table S1). There were no significant differences for the values of slopes and intercepts between Cd treatments and Cd/As treatments (Table S1).

W. globosa Possessed a Great Ability of Cd Phytofiltration Which Was Not Affected by As

When incubated with fresh *W. globosa*, Cd concentration in the solutions (with or without As(V)) decreased dramatically with increasing uptake time (Figure 4, Figure S1). At 72 h, almost all the Cd (99.5% and 99.4% for the two treatments, respectively) in the solutions was extracted by fresh *W. globosa*. Cd extraction by fresh *W. globosa* was

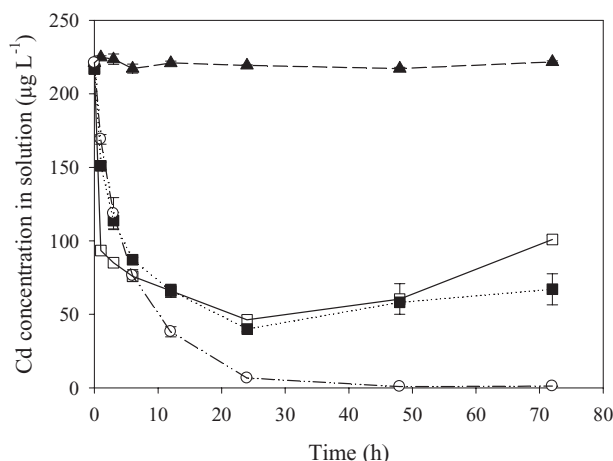


Figure 4 Changes in cadmium (Cd) concentrations in the solutions. Fresh and dry *W. globosa* were independently incubated in $2 \mu\text{M}$ Cd for 3 days and the solutions were sampled at different time points. ▲: Cd without *W. globosa*; □: Cd with ground dry *W. globosa*; ■: Cd with unground dry *W. globosa*; ○: Cd with fresh *W. globosa*. Data are means \pm SD ($n = 3$).

initially rapid, followed by a slower phase of absorption. Most of the Cd absorption (65.4% and 64.4% for the two treatments, respectively) occurred during the first 6 h (Figure 4, Figure S1). Cd concentration in the control treatments without *W. globosa* stayed the same during the whole experiment. In the treatments with fresh *W. globosa*, more than 99% of Cd in the original solution was accumulated by the fronds (data not shown), making the Cd concentration 4.41 mg kg^{-1} FW in Cd treatment and 4.34 mg kg^{-1} FW in Cd/As(V) treatment, with no significant difference between the two concentrations. No toxicity symptom was observed for the fresh fronds in the two treatments during the 3-day culture.

Cd adsorption by dry *W. globosa* (ground and unground) was also investigated in the same method as for fresh *W. globosa*. Cd concentration in the uptake solutions (without As(V)) incubated with unground dry duckweed decreased in the same manner as the fresh fronds treatments in the first 6 h ($P > 0.05$) (Figure 4). Then the decrease of the Cd concentration slowed down more than that of the treatment with fresh *W. globosa*. Cd concentration reached its lowest point at $40 \mu\text{g L}^{-1}$ after 24 h. After this, the Cd concentration in the solution started to increase, with a final Cd concentration of $67 \mu\text{g L}^{-1}$. For the treatments with ground dry duckweed, Cd concentrations decreased dramatically in the first 1 h, reached its lowest point at $46 \mu\text{g L}^{-1}$ after 24 h, and then increased to a final concentration of $101 \mu\text{g L}^{-1}$. There was no significant difference ($P > 0.05$) in terms of Cd concentration in the solutions between Cd treatment and Cd/As(V) treatment with dry duckweed at each time point (Figure 4, Figure S1). Cd adsorbed by dry duckweed accounted for around 80% of Cd accumulated by fresh duckweed.

Arsenic concentration in the solution decreased with the incubation time when treated with fresh *W. globosa*. After 3 days, 79% of As was removed from the solution by the fresh fronds. However, in the treatments with dry duckweed (ground or unground), arsenic concentrations in the solutions remained the same (data not shown).

Cd Accumulation by 5 Species of *Wolffia*

Five species of *Wolffia* (*W. globosa*, *W. australiana*, *W. cylindracea*, *W. columbiana*, and *W. arrhiza*) were incubated in 1 μM Cd for 5 days to investigate their abilities of Cd accumulation. Cd concentrations on the dry weight basis are shown in Figure S2a. Cd concentrations in all the 5 species exceeded 500 mg kg⁻¹ DW. BCFs of the 5 species were from 198 for *W. arrhiza* to 235 for *W. australiana* (on fresh weight basis). No toxicity symptoms were observed in any *Wolffia* species. There were significant differences ($P < 0.01$) between Cd concentrations in the 5 species, with *W. columbiana* accumulating the highest concentration of Cd (Figure S2a). Meanwhile, daily fresh weight records showed a significantly lower ($P < 0.01$) growth of *W. columbiana* compared to the growth of the other 4 species (Figure S2b).

DISCUSSION

Characteristics of Cd Uptake, Accumulation, and Toxicity in *W. globosa*

In the present study, we systematically characterized Cd uptake, accumulation and toxicity in *W. globosa*. Short-term uptake kinetics of Cd by *W. globosa* was rapid at low Cd concentrations (0–2 μM) (Figure 3). The satisfactory linear fitting suggested rapid Cd binding to the cell wall as proposed by Hart *et al.* (2002). Results from the phytofiltration experiment showed that most of the Cd accumulated by *W. globosa* was rapidly adsorbed by the apoplastic component (which will be discussed in detail in the next section), further confirming that the rapid Cd uptake was mainly due to cell wall binding. The adsorbed Cd in apoplast is difficult to be desorbed by a CaCl₂ solution after 15 min treatment as suggested by Zhao *et al.* (2006). The large amount of Cd passively adsorbed by the apoplast could probably overshadow the relatively small amount of Cd actively taken up by *W. globosa*, resulting in high value of K_m (283 μM) derived from a Michaelis-Menten curve without a plateau at high Cd concentrations (0–200 μM). Due to the rapid passive adsorption, active uptake kinetics by *W. globosa* could not be characterized in the present study.

W. globosa showed great Cd accumulation when exposed to Cd for 1 week (Figure 2). The two phases of the accumulation model suggested two different accumulation abilities and phytotoxic responses under different extents of Cd stress. The Cd accumulation ability of *W. globosa* exposed to Cd <10 μM was greater than those exposed to Cd >10 μM , conforming to the toxicity responses, where no toxic symptom or only a slight chlorosis were observed for fronds cultured with Cd <10 μM whereas severe toxic responses occurred in fronds exposed to Cd >10 μM . This result indicated that a critical threshold of Cd accumulation capacity may exist in *W. globosa*, which may be mainly controlled by the Cd adsorption capability of the cell wall. The EC_{50} (4.80 μM) was lower and Cd concentrations in the fronds was higher than those obtained by Boonyapookana *et al.* (2002). These disparities may be due to different treatment conditions. Their study also indicated that *W. globosa* was a good Cd accumulator and utilization of this plant as a remediator in contaminated aquatic environments was recommended. Our study further showed that the utilization could be optimal in environments contaminated with low Cd concentrations (<10 μM).

Growth inhibition was the most dramatic symptom of Cd toxicity in *W. globosa* at Cd concentrations >5 μM (Figure 1). In the leaves of the submerged plant *Elodea canadensis*, Cd was shown to inhibit cell division and affect cell enlargement (Dalla Vecchia *et al.* 2005). Chlorosis is one of the most general symptoms of Cd toxicity in plants (Das, Samantaray,

and Rout 1997), which was also the case for *W. globosa* incubated in solutions containing 5 μM or 10 μM Cd for 7 days. It is assumed that chlorosis in plants under Cd pressure is probably due to a direct or an indirect interaction with foliar iron (Das, Samantaray, and Rout 1997; Thomine *et al.* 2000). However, when Cd concentrations in the solution were 50 μM or 100 μM , the plant turned dark green and necrosis occurred in the fronds. In one species of the same genus, *W. arrhiza*, high levels of Cd induced severe oxidative damage and a decline of enzymatic and nonenzymatic antioxidants (Piotrowska *et al.* 2010). The severe homeostasis interference may be caused by high amounts of Cd actively taken up by the plant cells, when the cell wall binding capacity was saturated.

Cd Phytofiltration by *W. globosa* and Possible Mechanism

W. globosa has a substantial ability to extract Cd from the solutions (Figure 4). After 3 days incubation with fresh *W. globosa*, Cd in the solutions was decreased to a final concentration of around 1 $\mu\text{g L}^{-1}$, below the guideline value set by WHO (2004) (3 $\mu\text{g L}^{-1}$). Cd reduction could be entirely ascribed to accumulation by *W. globosa*. Furthermore, extraction behavior of unground dry fronds was the same as the fresh fronds in the first 6 h (Figure 4), suggesting that Cd accumulated by fresh *W. globosa* was rapidly adsorbed passively by the cell wall. Cd adsorbed by dry fronds accounted for 80% of the Cd accumulated by fresh fronds, implying that only 20% of Cd in the fronds was actively taken up by *W. globosa*. As proposed by Wagner (1993), the binding capacity of cell walls under low-level Cd exposure may be highly significant. In the roots of rice and *Zea mays*, and leaves of *E. canadensis*, Cd was primarily bound to the negatively-charged cell wall (Dabin *et al.* 1978; Klan *et al.* 1984; Dalla Vecchia *et al.* 2005). Pectic substances and hemicellulosic wall polymers could be the candidates for Cd binding (Wagner 1993). Protein and carbohydrate contents of *W. globosa* (named as *W. arrhiza* by Bhanthum and McGarry, 1971) were 19.8% and 43.6% of the dry weight, respectively, which could donate negatively-charged binding groups for Cd adsorption. When ground, these groups were directly exposed to Cd binding and subsequently causing a faster Cd decrease in solution, compared to unground fronds (Figure 4).

Effect of As on Cd Uptake and Phytoextraction by *W. globosa*

The As concentration used in the present study (10 μM) was relevant to the concentration ranges usually found in polluted ground water (Nordstrom 2002). Arsenic (As(V) or As(III)) at this concentration had no significant effect on the short-term uptake of Cd (0-2 μM) by *W. globosa* (Table S1). In *W. globosa*, As(V) is mainly taken up by phosphate transporters, while some aquaporin channels might participate in As(III) uptake (Zhang *et al.* 2009). The transporters or channels mediating uptakes of As were probably not evolved in the process of rapid adsorption of Cd onto the cell wall.

W. globosa can take up As(V) and then excrete As(III) after rapid As(V) reduction (Zhang *et al.* 2009), causing co-existence of As(V) and As(III) in the solution. Therefore, only As(V) was chosen to study the effect of As on Cd extraction by *W. globosa*. At a concentration of 10 μM , As(V) did not have a significant effect on Cd accumulation by fresh or dry fronds (Figure 4, Figure S1). However, in some studies on the combined effects of As and Cd on plant growth and element uptake, arsenic decreased Cd accumulation in plants such as wheat seedlings (Liu *et al.* 2007), *Spirodela polyrrhiza* (Seth, Chaturvedi, and Misra 2007) and rice seedlings (Sun *et al.* 2008), while it enhanced Cd uptake in

Solanum nigrum grown at low level of Cd (Sun, Zhou, and Diao 2008). The different effects may be due to variations between plant species. The result in the present study also showed that 79% of As in the solution can be removed by fresh *W. globosa*, showing a considerable ability of As phytofiltration, which is consistent with previous work (Zhang *et al.* 2009). However, dry fronds (ground or unground) showed no adsorption ability of As. This phenomenon is not surprising, because in the pH range of 6.0–8.0 (pH range of the incubation matrix), As(III) exists as an uncharged species, while As(V) occurs as oxyanionic species, both of which could not be adsorbed by the negatively-charged cell wall. The As removal by fresh fronds but not by dry fronds suggested that essentially all the As accumulated by *W. globosa* was due to active uptake by the plant cytoplasm. The substantial extraction abilities of both Cd and As by fresh *W. globosa* suggested potential utilization of this plant in aquatic environments co-contaminated by these two elements.

Advantages of *Wolffia* as Cd Phyto-remediator

All the 5 species of *Wolffia* studied displayed considerable Cd accumulation capacities, among which *W. columbiana* accumulated the highest concentration of Cd on the DW basis, explaining its lowest growth rate (Figure S2). *Wolffia* species proliferate primarily through vegetative budding of new fronds from parent fronds and the cultures can achieve near exponential growth rates (Stomp 2005). Under the conditions of the present study, the growth rates of these plants were relatively low with biomass doubling time of approximately one week. However, under natural conditions with higher light intensities and temperatures, greater growth rates could be achieved. Doubling time of less than 4 days has been observed in the natural field of Northern Thailand for *W. globosa* (Bhanthum and McGarry 1971). In general, sufficient Cd accumulation and rapid growth make *Wolffia* species potential Cd phyto-remediators in some fresh aquatic environments, such as paddy soils. The extracting efficiency would probably be optimal at low concentrations of Cd. In addition, *Wolffia* is a floating macrophyte with fronds sizes from 1 to 3 mm (Hillman 1961). The size and growth habit facilitate its harvest (Hillman and Culley 1978). Furthermore, Lemnaceae are a family distributed worldwide, of which *Wolffia* can be found in the tropics of America, Africa, and Asia (Hillman 1961). The origins of *W. globosa*, *W. australiana*, *W. cylindracea*, *W. columbiana*, and *W. arrhiza* used in the present study were China, Australia, Zimbabwe, Mexico, and Italy, respectively. The vast geographic distribution could avoid the risk of plant intrusion when conducting the application, which further makes the utilizing practice more environmentally friendly.

CONCLUSION

Our results showed that *W. globosa* has great accumulation ability when exposed to low-levels of Cd with moderate tolerance (EC_{50} on biomass at $4.80 \mu\text{M}$). Most of the accumulated Cd by the fresh plant was due to passive adsorption by the apoplast. Arsenic had no significant effect on the short-term uptake or the phytofiltration ability of Cd by the fronds. In addition, the substantial accumulation abilities of both As and Cd suggested the potential of using *W. globosa* in environmental remediation. Moreover, Cd accumulation

ability seemed to be a genus-specific characteristic, which further enlarges the geographic range of Cd phytoremediation by *Wolffia*.

SUPPORTING INFORMATION

Additional information is available on changes in Cd concentrations in the solutions with 10 μM As(V) incubated with fresh or dry *W. globosa* for 3 days (Figure S1), Cd accumulations by 5 species of *Wolffia* and the biomass (Figure S2), and kinetic parameters for Cd (0–2 μM) influx into *W. globosa* (Table S1).

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