Arsenic uptake and speciation in the rootless duckweed *Wolffia globosa*

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Summary

- Duckweeds are a common macrophyte in paddy and aquatic environments. Here, we investigated arsenic (As) accumulation, speciation and tolerance of the rootless duckweed *Wolffia globosa* and its potential for As phytofiltration.
- When grown with 1 μM arsenate, *W. globosa* accumulated two to 10 times more As than four other duckweed or *Azolla* species tested. *W. globosa* was able to accumulate > 1000 mg As kg^{-1} in frond dry weight (DW), and tolerate up to 400 mg As kg^{-1} DW. At the low concentration range, uptake rate was similar for arsenate and arsenite, but at the high concentration range, arsenite was taken up at a faster rate.
- Arsenite was the predominant As species (*c*. 90% of the total extractable As) in both arsenate- and arsenite-exposed duckweed. *W. globosa* was more resistant to external arsenate than arsenite, but showed a similar degree of tolerance internally. *W. globosa* decreased arsenate in solution rapidly, but also effluxed arsenite.
- *Wolffia globosa* is a strong As accumulator and an interesting model plant to study As uptake and metabolism because of the lack of a root-to-frond translocation barrier.

Introduction

Arsenic (As) is highly toxic and poses a serious threat to the environment and human health. Arsenic contamination in drinking water has been recognized as a serious global problem, threatening the health of millions of people, for example, in Bangladesh and West Bengal, India (Nordstrom, 2002). Recent studies have shown that apart from drinking water, As also enters the food chain through crop uptake from soils contaminated by irrigation with As-tainted water or mining activities (Williams *et al.*, 2006; Zhu *et al.*, 2008).

Mitigating environmental As contamination is an urgent requirement in many parts of the world. A potential solution is to exploit the potential of As accumulation by plants to remove As from water or soil. *Pteris vittata* was reported as the first As hyperaccumulator (Ma *et al.*, 2001). A number of other fern species in the *Pteridaceae* family have also been identified as As hyperaccumulators (Visoottiviseth *et al.*, 2002; Zhao *et al.*, 2002; Srivastava *et al.*, 2006; Wang *et al.*, 2007). Arsenic hyperaccumulation appears to involve enhanced arsenate uptake by the phosphate transporters (Poynton *et al.*, 2004; Caille *et al.*, 2005), much decreased arsenite-phytochelatin complexation in roots (Zhao *et al.*, 2003; Raab *et al.*, 2004), markedly enhanced root-to-frond translocation (Tu & Ma, 2002; Poynton *et al.*, 2004; Caille *et al.*, 2005), mainly in the form of arsenite (Su *et al.*, 2008), and sequestration of inorganic arsenite in the vacuoles of fronds (Lombi *et al.*, 2002; Pickering *et al.*, 2006). Huang *et al.* (2004) showed that *P. vittata* removed As from water efficiently and thus possesses a good potential for As phytofiltration.

Arsenic metabolism has been studied extensively in terrestrial plants. Arsenate and arsenite are taken up via phosphate transporters and aquaglycerolporin channels, respectively (Meharg & Hartley-Whitaker, 2002). Reduction of arsenate and complexation of arsenite by thiol peptides are considered to be the main mechanisms of As detoxification in As-nonhyperaccumulating plants (Ha *et al.*, 1999; Pickering *et al.*, 2000; Schmöger *et al.*, 2000; Dhankher *et al.*, 2002; Schat *et al.*, 2002; Bleeker *et al.*, 2006). A recent study by Xu *et al.* (2007) showed that plant roots rapidly reduce arsenate to arsenite and efflux arsenite to the external medium. Arsenic accumulation in shoots is limited by the restricted root-to-shoot translocation, except in As hyperaccumulators (Raab *et al.*, 2007).

Relatively little is known about As accumulation and metabolism in aquatic macrophytes. Some species have been found to accumulate substantial amounts of As, although the accumulation may be attributed primarily to physiochemical adsorption rather than physiological absorption (Mkandawire & Dudel, 2005; Robinson *et al.*, 2006). Recent studies have shown that the duckweed *Spirodela polyrhiza* (Rahman *et al.*, 2007), *Hydrilla verticillata* (Srivastava *et al.*, 2007) and several *Azolla* species (Zhang *et al.*, 2008) have moderate amounts of As accumulation and tolerance.

In the present study, we screened a number of duckweed and *Azolla* species and identified the duckweed *Wolffia globosa* as a strong accumulator of As. *W. globosa* is one of the smallest flowering plants consisting of small rootless spherical fronds. This duckweed species, owing to its rootless characteristic and no translocation barrier from roots to fronds, offers an interesting and simple model for studying As metabolism in macrophytes and its potential for phytofiltration. It has been documented that *W. globosa* has the potential to accumulate Cd and Cr (Garg & Chandra, 1994; Boonyapookana *et al.*, 2002). However, there are no reports regarding the ability of this duckweed to accumulate As. Thus, the objectives of this study were to investigate As accumulation and tolerance in *W. globosa*, to determine the kinetics of arsenate and arsenite uptake and the As speciation in the frond tissues, and to evaluate its potential for As phytofiltration.

Materials and Methods

Plant culture

Three species of duckweed (*Spirodela polyrhiza* (L.) Schleid., *Lemna minor* L. and *Wolffia globosa* L.) and two species of *Azolla* (*Azolla filiculoides* Lam. and *Azolla caroliniana* Willd.) were collected from ponds in Nanchang, Jiangxi province, and Wuhan, Hubei province, China. Plants were grown in hydroponic culture for 3 wk before being used in experiments. The composition of the nutrient solution was as follows: 1 mM $CaSO_4$, 1.6 mM $MgSO_4$, 0.3 mM KH_2PO_4, 0.3 mM KCl, 0.7 mM $NaNO_3$, 10 μM $FeNa_2$-EDTA, 20 μM H_3BO_3, and 7.7 μM Na_2MoO_4 (pH adjusted to 6.0 with KOH or HCl solutions). Nutrient solution was renewed twice every week. Experiments were carried out in a controlled-environment growth chamber with the following conditions: 14 h light period d^{-1} with a light intensity of *c.* 280 μmol m^{-2} s^{-1}, 25 : 20°C day : night temperatures, and 70% relative humidity.

Comparison of As accumulation by duckweed and *Azolla* species

One gram (fresh weight) each of the three species of duckweed and two species of *Azolla* were pre-cultured in 1 l of the normal nutrient solution for 1 wk. Thereafter, plants were exposed to 1 μM arsenate (Na_3AsO_4) for 5 d, with three replicates for each species. The nutrient solution was changed every day. After 5 d, the fronds were washed with deionized water, blotted dry and then oven-dried at 70°C for 48 h before As analysis. This experiment identified *W. globosa* as the highest As-accumulating species, which was chosen for further studies described in the following sections.

Kinetics of arsenate and arsenite uptake

Fresh *W. globosa* plants were washed with deionized water and blotted dry. Four replicates of 1.5 g of the duckweed were incubated in 500 ml uptake solution containing 5.0 mM MES (pH = 5), 0.5 mM $Ca(NO_3)_2$ and 0, 1, 2, 5, 10 or 20 μM arsenate (Na_3AsO_4) or arsenite ($NaAsO_2$), with or without 0.1 mM phosphate. The bottles were shaken gently at 60 rpm. After 30 min, plants were collected and rinsed with an ice-cold phosphate buffer solution (1 mM K_2HPO_4, 5 mM MES and 0.5 mM $Ca(NO_3)_2$) for 10 min to remove apoplastic As (Abedin *et al.*, 2002). The fronds were then oven-dried at 70°C for 48 h. A second experiment was carried out as before but with higher concentrations (0, 20, 40, 80, 160 and 320 μM) of arsenite or arsenate, both without 0.1 mM phosphate.

Arsenic accumulation and tolerance

Four replicates of 3 g *W. globosa* were cultured in a jar (10 cm diameter and 10 cm depth) containing 350 ml nutrient solution and different concentrations of arsenate (0, 1, 5, 10, 30, 50, 100 μM) or arsenite (0, 1, 5, 10, 15, 50 μM). The nutrient solution was renewed every day. After 7 d, the plants were harvested, washed carefully with deionized water, blotted dry and their fresh weight (FW) recorded. The samples were frozen in liquid nitrogen and freeze-dried. The concentrations of total As and As species were determined.

Potential of *W. globosa* for phytofiltration of arsenic

After pre-culture in nutrient solution for 3 wk, *W. globosa* was transferred to 5 l plastic containers filled with 0.1 mM $CaCl_2$ solution for 12 h. Three replicates each of 10 g FW of *W. globosa* were then transferred to a 1 l conical flask filled with 200 ml of 0.1 mM $CaCl_2$ and 200 μg l^{-1} (2.67 μM) arsenate. A control treatment without *W. globosa* was included. The flasks were covered with a membrane with small holes to minimize evaporation. At 1, 6, 12, 24, 48 and 72 h, 2 ml solution was taken from each flask, and replaced with fresh 200 μg l^{-1} arsenate solution. Arsenic species and total As concentration in the solution samples were determined. At 72 h, *W. globosa* was collected, cleaned with deionized water, blotted dry and then freeze-dried. Arsenic speciation and total As concentration in the fronds were determined.

Plant tissue analysis

Approximately 0.02 g dried plant material were weighed into 50 ml polypropylene digest tubes and steeped in 2 ml of high-purity nitric acid. The mixture was allowed to stand overnight. The tubes were then heated in a microwave-accelerated reaction system (CEM Microwave Technology Ltd, Matthews, NC, USA). The temperature was gently raised, first to 55°C and then to 75°C, with holding times of 10 min. Finally the digest was heated at 95°C for 30 min before cooling. The digests were made up to a volume of 25 ml with ultrapure water (18.2 MΩ). Arsenic concentration was determined by atomic fluorescence spectrometry (AFS, AF-610A, Beijing Haiguang Analytical Instrument Co., Beijing, China). A reagent blank and a certified reference material (bush twigs and leaves, GBW07603 from the National Research Center for Standard Materials in China) were included for quality assurance. Repeated analysis of the reference material gave 1.20 ± 0.03 mg As kg^{-1} DW, which agrees well with the certified value of 1.25 ± 0.15 mg As kg^{-1} DW.

Determination of As species

Freeze-dried samples were extracted with 10 ml of 1% nitric acid in a microwave-accelerated reaction system (Zhu *et al.*, 2008). The temperature was gently raised, first to 55°C and then to 75°C, with holding times of 10 min. Finally the digest was heated at 95°C for 30 min before cooling. The certified reference material GBW 10010 Chinese rice flour was used to validate the analytical procedure. Spikes of both arsenite and arsenate (0.5 ml of 1000 μg As ml^{-1}) and blanks were run with each extraction batch. The extract solutions were centrifuged and passed through a 0.45 μm nylon filter. To minimize potential transformation of As species, samples were kept on ice and in the dark and analyzed within a few hours after extraction. Arsenic speciation was assayed by high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) (7500a Agilent Technologies, Palo Alto, CA, USA). Chromatographic columns consisted of a Hamilton precolumn (11.2 mm, 12–20 mm) and a Hamilton PRP-X100 10 μm anion-exchange column (240 × 4.1 mm). The mobile phase consisted of 6.67 mM ammonium di-hydrogen phosphate ($NH_4H_2PO_4$) and 6.67 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using ammonia. Arsenic species in the samples were identified by comparing their retention times with those of the standards, including arsenite, arsenate, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA), and quantified by external calibration curves with peak areas.

Data analysis

All data were subjected to analysis of variance (ANOVA) using windows-based SPSS 13.0.

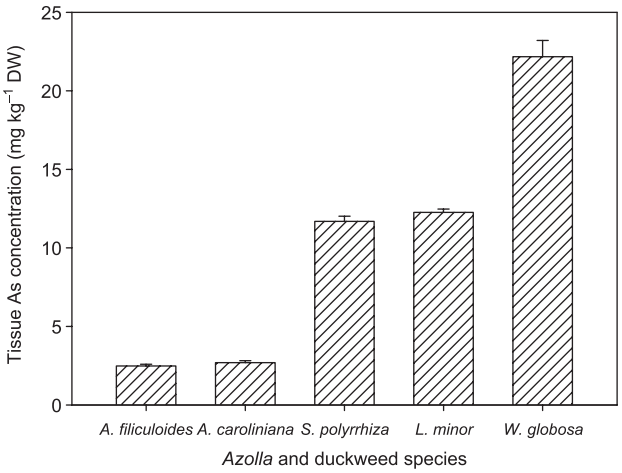

Fig. 1 Arsenic concentration in three species of duckweed (*Spirodela polyrhiza*, *Lemna minor* and *Wolffsia globosa*) and two strains of *Azolla* grown in nutrient solutions with 1 μM arsenate for 5 d. Data are means ± SE ($n = 3$).

Results

Comparison of As accumulation by duckweed and *Azolla* species

After incubation in 1 μM arsenate for 5 d, *W. globosa* accumulated the highest concentration of As among the five species of macrophytes tested (Fig. 1). The concentration of As in the fronds of *W. globosa* was approx. twice that of the other two species of duckweed (*S. polyrhiza* and *L. minor*) and 10 times higher than that of the two *Azolla* species. No toxicity symptoms were observed in any plant species, as the concentration of arsenate used in this experiment was low and environmentally more relevant. Biomass growth was greatest in *W. globosa* and smallest in *S. polyrhiza* (data not shown).

Arsenic influx kinetics

In the low-concentration range (0–20 μM), short-term (30 min) uptake of both arsenate and arsenite by *W. globosa* was linear in relation to the external concentration (Fig. 2a, Table 1). In the absence of phosphate, arsenate uptake was slightly higher than arsenite uptake. The presence of 0.1 mM phosphate suppressed arsenate uptake markedly, but had little effect on arsenite uptake. With added phosphate, the slope of arsenite uptake was nearly threefold that of arsenate uptake (Table 1).

In the high-concentration range (0–320 μM), both arsenate and arsenite uptake exhibited a hyperbolic pattern in relation to the external concentration (Fig. 2b). The uptake kinetics can be described satisfactorily by the Michaelis–Menten equation using nonlinear curve fitting (Table 1). Addition of a linear component to the Michaelis–Menten equation did

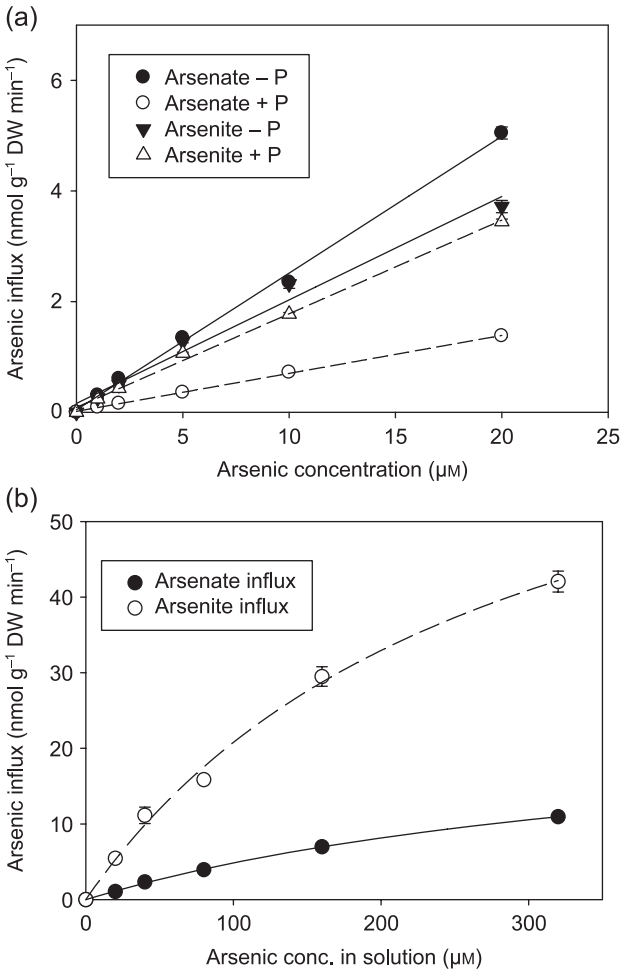

Fig. 2 (a) Concentration-dependent kinetics for arsenate and arsenite (0–20 μM) uptake with or without 0.1 mM phosphate by *Wolffia globosa*. Each point is presented as mean ± SE (n = 4). (b) Concentration-dependent kinetics for arsenate and arsenite (0–320 μM) uptake without phosphate by *W. globosa*. Each point is presented as mean ± SE (n = 4).

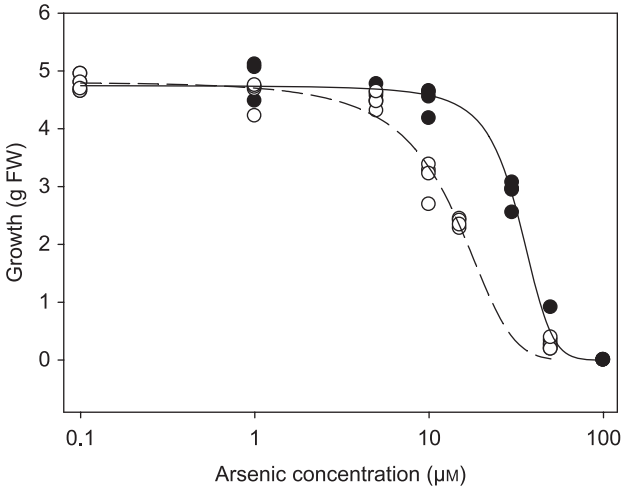

Fig. 3 Effect of 1 wk arsenic (As) exposure (closed circles, arsenate; open circles, arsenite) on growth of *Wolffia globosa*. Data are individual replicates. Lines are the fitted log-logistic curves. To allow log transformation, a small value (0.1) was added to the zero As concentration in the control treatment.

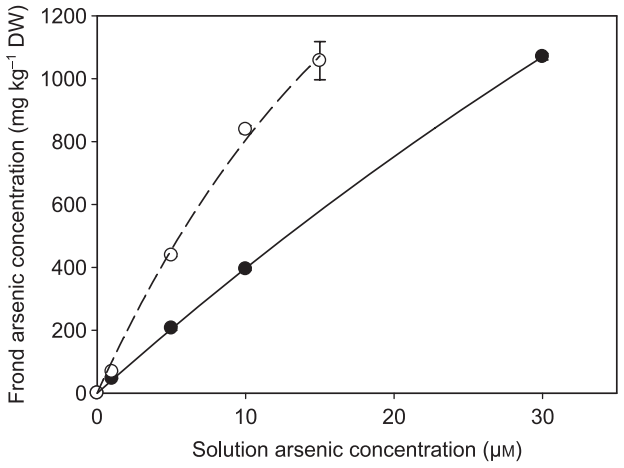

Fig. 4 Arsenic (As) concentration in *Wolffia globosa* exposed to different concentrations of arsenate (closed circles) and arsenite (open circles) for 1 wk. Each point is presented as mean ± SE (n = 4).

not improve the fit; also the slope obtained for the linear component was negligible. The V_{max} for arsenite uptake was *c.* three times higher than that of arsenate uptake, whereas the K_m for arsenite uptake was about a third lower than that of arsenate uptake.

Arsenic accumulation and tolerance

Growth of *W. globosa* was significantly ($P < 0.001$) inhibited by arsenate at ≥ 30 μM or by arsenite at ≥ 10 μM (Fig. 3). The growth was more severely inhibited by arsenite than by arsenate, but note that 0.1 mM phosphate was added in the nutrient solution. The dose–response data could be described satisfactorily by a log-logistic equation with R^2 values of 0.979 and 0.990 for arsenite and arsenate, respectively. Based on the fitted equations, the effective concentration of arsenite or arsenate in the nutrient solution that caused a 50% inhibition on growth (EC_{50}) could be estimated. The EC_{50} values (± SE) were 12.8 ± 1.3 μM arsenite and 32.9 ± 0.7 μM arsenate, respectively.

Tissue As concentration increased significantly ($P < 0.001$) with increasing concentration of arsenate or arsenite in the nutrient solution (Fig. 4). Arsenic accumulation was much greater in the duckweed exposed to arsenite than that exposed to arsenate at each same external concentration. After 7 d incubation, *W. globosa* accumulated 1057 ± 61 mg As kg^{-1} DW in the 15 μM arsenite treatment and 1070 ± 10 mg As kg^{-1} DW in the 30 μM arsenate treatment. In these two

Table 1 Kinetic parameters for arsenate and arsenite influx into *Wolffia globosa*

	Linear regression model (low-As concentration)		
Arsenic species with or without P	*a* (slope)	*B* (intercept)	R^2
Arsenite + P	0.17 ± 0.00	0.09 ± 0.05	0.997
Arsenite – P	0.19 ± 0.10	0.16 ± 0.12	0.982
Arsenate + P	0.07 ± 0.00	0.02 ± 0.01	0.999
Arsenate – P	0.25 ± 0.01	0.04 ± 0.06	0.998
Arsenic species without P	Michaelis–Menten function (high-As concentration)		
	V_{max} (nmol g^{-1} DW min^{-1})	K_m (mM)	R^2
Arsenite – P	79.30 ± 7.45	0.28 ± 0.05	0.996
Arsenate – P	25.69 ± 1.26	0.43 ± 0.03	1.000

Kinetic parameters were calculated from mean As influx (n = 4) using linear regression model (As treatment is 0–20 μM) and Michaelis–Menten function (As treatment is 0–320 μM).

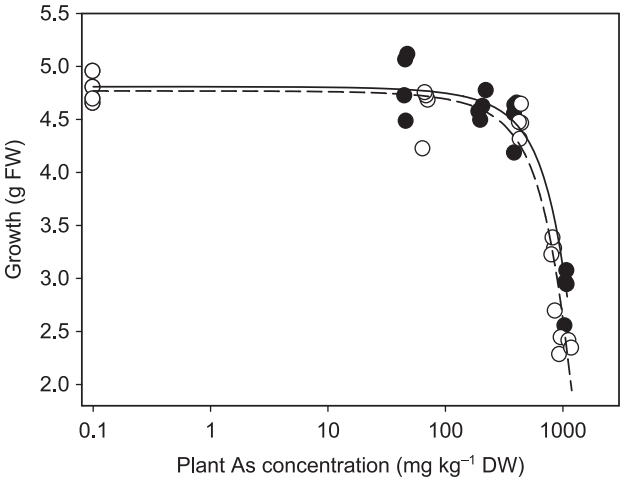

Fig. 5 Relationship between growth of *Wolffia globosa* and plant arsenic (As) concentration after 1 wk exposure to different concentrations of arsenate (closed circles) or arsenite (open circles). Data are individual replicates. Lines are the fitted log-logistic curves. To allow log transformation, a small value (0.1) was added to the zero As concentration in the control treatment.

treatments, the bioconcentration factors (BCF, the ratio of tissue As concentration to solution As concentration) were 940 and 476, respectively.

To determine the EC_{50} values based on tissue As concentration, the growth data were plotted against tissue As concentration and the relationship was fitted with a log-logistic equation (Fig. 5; $R^2 = 0.92$ for both treatments). Similar EC_{50} values (± SE) were obtained for the two forms of As: 1186 ± 41 and 1031 ± 41 mg kg^{-1} DW for the arsenate and arsenite treatments, respectively.

Arsenic speciation in *W. globosa*

Regardless of whether arsenate or arsenite was supplied to the plants, arsenite was the predominant species of As in the duckweed, accounting for 87–90% and 86–91% of the total As in the plants exposed to arsenate and arsenite, respectively (Fig. 6). The concentrations of both arsenite and arsenate in the plants increased with increasing As concentration in the nutrient solution. No methylated As species were detected in the plants. Total As concentrations in the plants determined by HNO_3 digestion followed by AFS measurement were in good agreement with the sum of arsenite and arsenate in the 1% HNO_3 extracts determined by HPLC-ICP-MS, with a mean extraction recovery of 92.7 ± 10.5% (data not shown).

Phytofiltration potential

Wolffia globosa decreased total As concentration in the solution from 200 to 116 μg l^{-1} within 48 h, beyond which there was no further decrease in As concentration (Fig. 7a). For comparison, As concentration remained stable in the control treatment without duckweed. During the time course of this experiment, As speciation in the solution was monitored. Arsenate concentration decreased more rapidly than total As concentration, falling to 55 μg l^{-1} at the end of the experiment (72 h). However, arsenite was produced in the solution in the presence of the duckweed; by 72 h, 30% of the initial arsenate in the solution had been converted to arsenite (Fig. 7b). No arsenite was detected in the solution of the control treatment. At the end of the experiment, *W. globosa* contained 35.9 ± 0.8 μg As g^{-1} DW with 87.8% as arsenite.

Discussion

The present study has identified *W. globosa* as a strong accumulator of As. Arsenic hyperaccumulators usually refer to plant species capable of accumulating and tolerating > 1000 mg As kg^{-1} in the shoot/frond biomass (McGrath & Zhao, 2003). *W. globosa* was found to be able to accumulate, but not tolerate, > 1000 mg As kg^{-1} and, for this reason, should not be considered as an As hyperaccumulator.

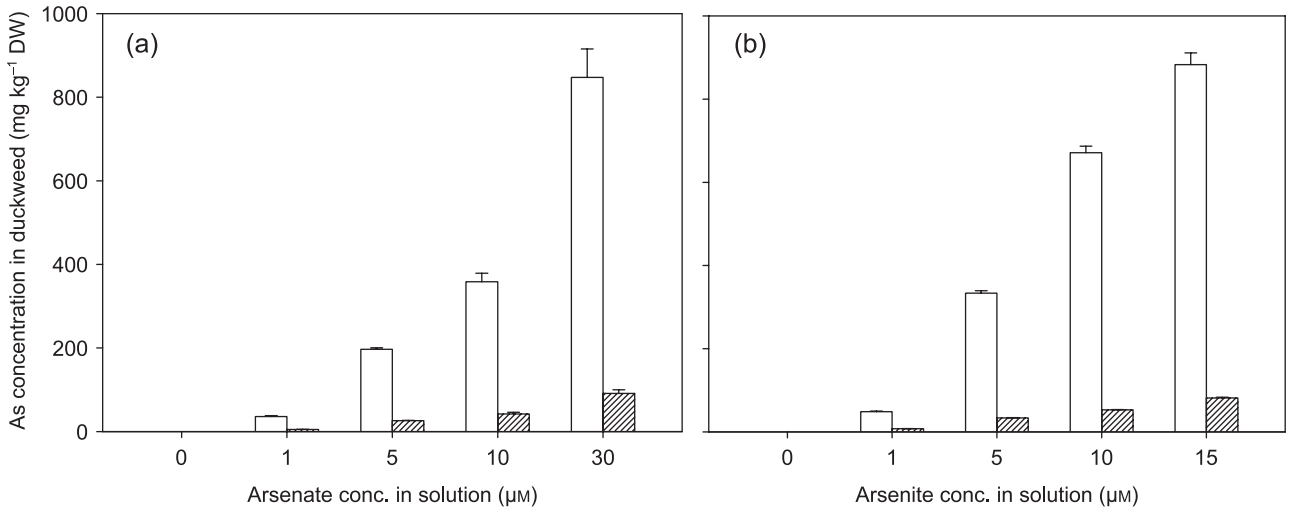

Fig. 6 Arsenic (As) speciation in *Wolffia globosa* after exposure to different arsenate (a) or arsenite (b) concentrations for 1 wk. Arsenic species detected in plants: arsenite (open bars) and arsenate (hatched bars). Data are means ± SE (n = 4).

Nevertheless, its capacity for As accumulation and tolerance was considerably higher than most nonhyperaccumulator species, which usually suffer from As phytotoxicity when tissue As concentration exceeds 10–100 mg kg^{-1} (Kabata-Pendias & Pendias, 1992). In comparison, the upper limit of As tolerance in *W. globosa* without a significant growth inhibition was *c.* 400 mg kg^{-1} (Fig. 5). Moreover, the EC_{50} of frond As concentration for *W. globosa* (*c.* 1000 mg kg^{-1}) was approx. 10-fold higher than that for *Azolla filiculoides* and *A. caroliniana* (Zhang *et al.*, 2008). Field surveys of aquatic plants growing in As-contaminated environments showed that some species accumulated more than 1000 mg As kg^{-1} (Mkandawire & Dudel, 2005; Robinson *et al.*, 2006). However, this high As accumulation was thought to be primarily the result of physicochemical adsorption of arsenate to the plant's surface, facilitated by co-deposition of other adsorptive species such as hydrated Fe-oxides (Robinson *et al.*, 2006), rather than a direct uptake by the plants. In the present study, there was no evidence of Fe-oxide deposition on the plant surface of *W. globosa*. For these reasons, most of the As accumulated by the duckweed was likely to be taken up inside the cells. A reason for the high As accumulation in this duckweed may be because it is rootless, thus presenting no translocation barrier from roots to fronds/shoots as observed in nonhyperaccumulating plant species.

In the low-concentration range (≥ 10 μμ), *W. globosa* took up arsenate and arsenite at a similar rate when phosphate was absent from the solution. However, the presence of phosphate suppressed the uptake of arsenate markedly, but not of arsenite. This is consistent with studies on other aquatic plants (Ullrich-Eberius *et al.*, 1989; Rahman *et al.*, 2007; Srivastava *et al.*, 2007) and terrestrial plants (Meharg & Hartley-Whitaker, 2002), indicating that arsenate is taken up by phosphate transporters. By contrast, arsenite is likely to be taken up by a different mechanism. It has been reported recently that a number of the NIP (nodulin 26-like intrinsic protein) aquaporin channels from rice and *Arabidopsis thaliana* mediate arsenite influx, which is present predominantly as an undissociated neutral molecule at pH < 8 because of its high pK_a (9.22) (Bienert *et al.*, 2008; Isayenkov & Maathuis, 2008; Ma *et al.*, 2008). It remains to be investigated whether NIP channels are responsible for arsenite uptake in *W. globosa*. In the high-concentration range (0–320 μμ), arsenite uptake was faster than arsenate uptake even in the absence of phosphate. This is similar to the study on rice (Abedin *et al.*, 2002). The fast uptake of arsenite is consistent with an aquaporin-mediated fast flux of neutral solutes. The V_{max} and K_m values for arsenite were comparable to those for rice, pea and wheat reported by Meharg & Jardine (2003), who used a similar concentration range as the one used in of the present study (Fig. 2b). Irtelli & Navari-Izzo (2008) found that a linear model fitted the kinetics of arsenite influx to *Brassica carinata* better than the Michaelis–Menten model, both in the low and high concentration ranges. For the kinetics of arsenate influx, our V_{max} and K_m values (Table 1) were comparable to those of the arsenate-tolerant plants of *Holcus lanatus* (Meharg & Macnair, 1992). However, these K_m values were on to two orders of magnitude higher than those reported for the arsenate-nontolerant *H. lanatus* (Meharg & Macnair, 1992), rice (Abedin *et al.*, 2002) and *Brassica carinata* (Irtelli & Navari-Izzo, 2008). It should be emphasized that As uptake at the low-concentration range is environmentally and physiologically more relevant, and at the low-concentration range, the kinetics of both arsenate and arsenite influx were linear (Fig. 2a).

It is often claimed that arsenite is more phytotoxic than arsenate (Carbonell-Barrachina *et al.*, 1998). This was the case for *W. globosa* only when the toxicity threshold EC_{50}

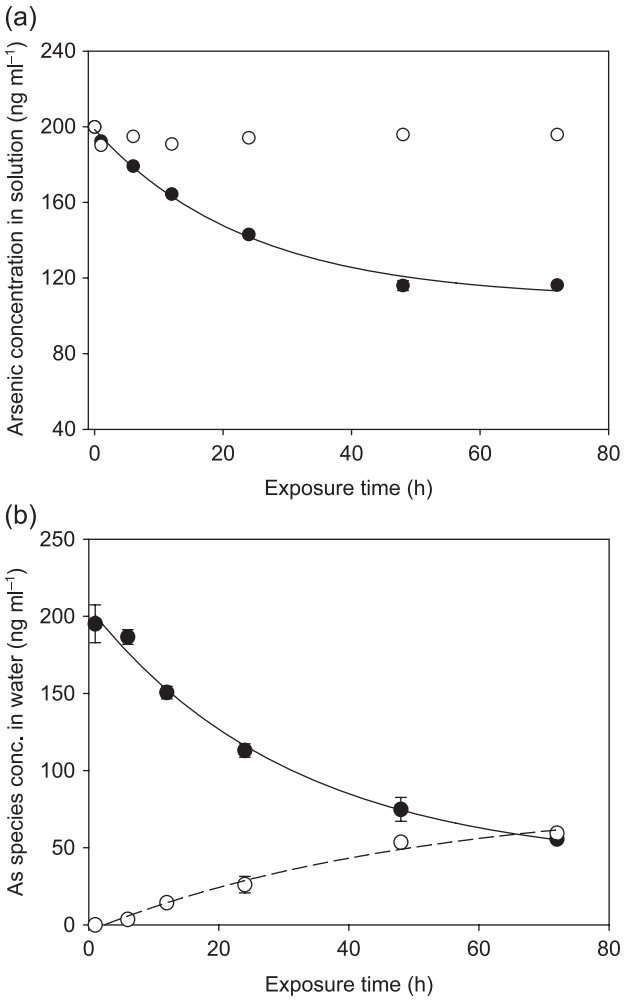

Fig. 7 (a) Phytofiltration of arsenic (As) from water by 10 g *Wolffia globosa* in 72 h (closed circles, with *W. globsa*; open circles, control, without *W. globosa*). The initial water was 200 ml of 0.1 mM $CaCl_2$ containing 200 ng g^{-1} arsenate. Data are means ± SE (n = 3). (b) Changes of As species in the phytofiltration solutions during 72 h (closed circles, arsenate; open circles, arsenite). Data are means ± SE (n = 3).

was based on the concentration in the external medium, which contained phosphate. However, when EC_{50} was based on tissue As concentration, arsenate and arsenite showed a similar degree of toxicity. There are two reasons for this apparent difference. First, arsenate uptake was smaller than arsenite uptake because of the presence of phosphate (Fig. 2a). Second, arsenate was reduced to arsenite rapidly in *W. globosa*, with the latter being largely responsible for causing cellular toxicity. Indeed, the majority (*c*. 90%) of tissue As was present as arsenite, regardless of whether the duckweed was exposed to arsenate or arsenite (Fig. 6). Because uptake of arsenate or arsenite is dependent on the experimental conditions, As toxicity should preferably be expressed in relation to the tissue As concentration, and on this criterion, the two As species appear to be similarly toxic to *W. globosa*.

Given the substantial capacity of *W. globosa* for As accumulation and tolerance, it may be possible to use this duckweed to decrease As concentration in water (e.g. in ponds or paddy fields) as a phytofiltration strategy. The results obtained in the current study demonstrate a partial success. While arsenate concentration was rapidly depleted by the duckweed, there was a concurrent production of arsenite in the medium accounting for almost a third of the arsenate uptake (Fig. 7b). Arsenite efflux appears to be the limiting factor in phytofiltration using *W. globosa*. Xu *et al*. (2007) have recently shown that the roots of rice and tomato took up and reduced arsenate rapidly, followed by efflux of arsenite to the nutrient solution. It thus appears that *W. globosa* also possesses a similar pathway of arsenite efflux. This pathway may be a mechanism of As detoxification, as has been shown for microbes (Bhattacharjee & Rosen, 2007). While arsenite extruded by *W. globosa* may be oxidized to arsenate in the aerobic medium, some arsenite may be reabsorbed by the plants through arsenite transporters. The present study thus reveals the dynamic nature of As cycling mediated by an aquatic plant that was unknown before.

Wolffia globosa appears to have some potential for As phytofiltration in contaminated water and paddy soil, and may play a significant role in the As biogeochemical cycle in paddy soils and other aquatic environments.

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