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Arsenic uptake, accumulation and phytofiltration by duckweed (*Spirodela polyrhiza* L.)

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Abstract

This study investigates arsenic (As) accumulation and tolerance of duckweed *Spirodela polyrhiza* L. and its potential for As phytofiltration. *S. polyrhiza* was able to survive in high concentration of As(V) solution. The EC_{50} values (± SE) based on the external As(V) were (181.66 ± 20.12) μmol/L. It accumulated (999 ± 95) mg As/kg dw when exposed in 320 μmol/L As(V) solution for one week, and was able to take up appropriately 400 mg As/kg dw in tissues without a significant biomass loss. The EC_{50} values (the effective concentration of As(V) in the nutrient solution that caused a 50% inhibition on biomass production) was (866 ± 68) mg/kg dw for the tissues, indicating that *S. polyrhiza* had a high capability of As accumulation and tolerance. The uptake kinetic parameters V_{max} was (55.33 ± 2.24) nmol/(g dw·min) and K_m was (0.144 ± 0.011) mmol/L. Within 72 hr, *S. polyrhiza* decreased As concentration in the solution from 190 to 113 ng/mL with a removal rate of 41%. The study suggested that this floating aquatic plant has some potential for As phytofiltration in contaminated water bodies or paddy soils.

Key words: arsenic; duckweed; phytofiltration; *Spirodela polyrhiza* L.

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Introduction

Arsenic (As) is a highly toxic and widespread environmental contaminant that poses hazards to humans. Groundwater contamination with As has led to serious health problems in many regions of the world such as Bangladesh, India and China (Mandal et al., 1997; Nickson et al., 1998; Chowdhury et al., 1999), in which millions of people suffer from As toxicity by drinking As-contaminated groundwater (Nordstrom, 2002). Contaminated groundwater is not only used for drinking purpose but also extensively used for irrigation. Long-term use of As-contaminated groundwater for irrigation has resulted in As elevation in paddy soil-rice system (Meharg and Rahman, 2003), which is posing a great health threat to rice subsistence populations globally via the food chain (Williams et al., 2007; Zhu et al., 2008).

Remediation methods are needed to mitigate As contamination in the rice production system. A potential remediation method should exploit for As accumulation by aquatic macrophytes which usually grow in water of paddy fields and ponds, and ultimately reduce As transfer to rice crop. To understand how macrophytes take up and metabolize As is important for the development of a new approach to mitigate the negative impact of As contamination. Recent studies have found that some species of aquatic macrophytes have moderate levels of As accumulation and tolerance. Zhang et al. (2008) have screened 50 strains of *Azolla* and found a large variation in As accumulation. Other studies have shown that some species of macrophytes could accumulate more than 1000 mg As/kg dry weight by different mechanisms, including the New Zealand watercress (*Lepidium sativum*) (Robinson et al., 2003), *Lemna gibba* (duckweed) in tailing waters from two abandoned uranium mining sites (Mkandawire and Dudel, 2005), *Egeria densa* and *Ceratophyllum demersum* growing in the Waikato River system (Robinson et al., 1995) and a rootless duckweed *Wolffia globosa* (Zhang et al., 2009). Given the substantial capacity of macrophytes for As accumulation and tolerance, it may be possible to adopt these aquatic plants to reduce As concentration in water and paddy soil as a phytofiltration strategy. Mkandawire et al. (2004b) have conducted an experiment using synthetic tailing water and deduced that the potential extractions from surface waters with duckweed *L. gibba* was 751.9 kg As/(ha·yr) after desorption of surface complexed As with EDTA. Some other aquatic plants such as water hyacinth (*Eichhornia crassipes*), lesser duckweed (*Lemna minor*) (Alvarado et al., 2008) and a rootless duckweed (Zhang

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et al., 2009) have also been considered as promising candidates for phytofiltration in As contaminated water.

Spirodela polyrhiza L. is an aquatic plant floating in the ponds, lakes, ditches and rice fields in South Asian countries where As contaminated groundwater is used for irrigation during dry season. Owing to its wide distribution, high multiplication rates, short life span and easy to grow in the variable environments (Lemon et al., 2001), *S. polyrhiza* is regarded as an ideal biological material to investigate As metabolism and phytofiltration potential in macrophytes. Recent studies showed that As uptake in *S. polyrhiza* occurred through the phosphate uptake pathway and by physico-chemical adsorption on Fe-plaques of root surfaces as well (Rahman et al., 2008). The objectives of the present study were to investigate As accumulation and tolerance in *S. polyrhiza*, to determine the kinetics of As(V) uptake and to evaluate its potential for As phytofiltration.

1 Materials and methods

1.1 Preparation of plant materials

Duckweed *S. polyrhiza* was collected from ponds in Nanchang, Jiangxi Province, China. Plants were grown in hydroponic culture solution for 3 weeks before being used for experiments. The composition of the nutrient solution was as follows: 1 mmol/L $CaSO_4$, 1.6 mmol/L $MgSO_4$, 0.3 mmol/L KH_2PO_4, 0.3 mmol/L KCl, 0.7 mmol/L $NaNO_3$, 10 μmol/L $FeNa_2$-EDTA, 20 μmol/L H_3BO_3, and 7.7 μmol/L 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 14-hr light/10-hr dark cycles. Temperature was kept at 25 and 20°C during day and night, respectively. Light intensity and relative humidity were maintained around 280 μmol/(m^2·sec) and 70%, respectively.

1.2 Kinetic of As(V) uptake

Fresh *S. polyrhiza* plants were washed with de-ionized water and blotted dry. Four replicates of one gram of the duckweed were incubated in a test solution (500 mL) containing 5.0 mmol/L 2-(N-morpholin) ethansulfonic acid (MES) and 0.5 mmol/L $Ca(NO_3)_2$ (pH 5), with different concentrations of As(V) (0, 20, 40, 80, 160, 320 and 640 μmol/L). The solutions were shaken gently at 60 r/min. After 20 min, duckweed was collected and rinsed in an ice-cold phosphate buffer solution (1 mmol/L K_2HPO_4, 5 mmol/L MES and 0.5 mmol/L $Ca(NO_3)_2$) for 10 min to remove apoplastic As (Abedin et al., 2002). The tissues were then oven-dried for 48 hr at 70°C, sub-samples were digested and As concentrations were determined.

1.3 Arsenate accumulation and tolerance

After pre-cultured for one week, three grams of *S. polyrhiza* were cultured in a 500 mL conical flask containing 300 mL nutrient solution, covered with a membrane with small holes to minimize evaporation. The composition of other nutrients was the same as in the preculture, except that the phosphate concentration was decreased to 0.1 mmol/L. Seven concentrations of As(V) were used in this study (0, 10, 20, 40, 80, 160 and 320 μmol/L), each with four replicates. The conical flask were arranged randomly inside the growth chamber and re-arranged every day. The nutrient solution was renewed every three days. After seven days the plants were harvested, washed carefully with de-ionized water, blotted dry and fresh weight (fw) recorded. The samples were frozen in liquid nitrogen and freeze dried. The concentrations of total As and P were determined.

1.4 Arsenic phytofiltration by *S. polyrhiza*

After a 3-week preculture in hydroponics, *S. polyrhiza* was transferred to 5-L plastic containers filled with 0.1 mmol/L $CaCl_2$ solution for 12 hr. Three replicates (each of 10 g fw of *S. polyrhiza*) were then transferred to 1 L conical flask filled with 200 mL of 0.1 mmol/L $CaCl_2$ and 190 μg/L (2.53 μmol/L) As(V). A control without duckweed was included. The flasks were covered with a membrane with small holes to minimize evaporation. From each flask 2 mL solution was taken at 1, 6, 12, 24, 48 and 72 hr, and replaced with fresh 190 μg/L As(V) solution. Total As concentration in the solution samples was determined.

1.5 Plant tissue analysis

Approximately 0.05 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 at room temperature. Samples were randomized and then heated in a microwave-accelerated reaction system (CEM Microwave Technology Ltd., USA). The temperature was gently raised, first to 55°C and then to 75°C with holding time of 10 min. Finally the digest was heated at 95°C for 30 min before reaching the room temperature. The digests were made up to a volume of 25 mL with ultrapure water (18.2 MΩ). The concentrations of P in the digests were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin-Elmer, USA). Arsenic concentration was determined by atomic fluorescence spectrometry (AFS, AF-610A, Beijing Haiguang Analytical Instrument Co., 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 (Zhu et al., 2008).

1.6 Data analysis

All data were subjected to analysis of variance (ANOVA) using windows-based SPSS 13.0. The data of As(V) influx were fitted to a Michaelis-Menten equation using the SigmaPlot software (version 10) to estimate the maximum influx velocity (V_{max}) and K_m (Abedin et al., 2002).

2 Results

2.1 As(V) influx kinetics

As(V) influx into duckweed *S. polyrhiza* exhibited a

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hyperbolic pattern in relation to the increasing As concentrations in the incubation solutions (Fig. 1). As(V) influx kinetics was adequately described by the Michaelis-Menten equation with R^2 value of 0.995 (n = 4). The kinetic parameters V_{max} (± SE) was (55.33 ± 2.24) nmol/(g dw·min) and K_m (± SE) was (0.144 ± 0.011) mmol/L.

2.2 Arsenic accumulation and tolerance

Increasing As(V) concentration in the nutrient solution decreased ($P < 0.005$) the growth of duckweed *S. polyrhiza* (Fig. 2). The dose-response data could be described satisfactorily by a log-logistic equation with R^2 value of 0.98. Based on the fitted equations, the effective concentration of As(V) in the nutrient solution that caused a 50% inhibition on biomass production (EC_{50}) could be estimated. The EC_{50} values (± SE) were (181.66 ± 20.12) μmol/L.

Tissue As concentration significantly increased ($P < 0.001$) and exhibited a hyperbolic pattern in relation to the increasing concentration of As(V) in the incubation solutions. *S. polyrhiza* accumulated (999 ± 95) mg As/kg dw in the 320 μmol/L As(V) treatment after one-week As(V) exposure (Fig. 3). To determine the EC_{50} values based on tissue As concentrations, the biomass data were plotted against tissue As concentration and the relationship was fitted with a log-logistic equation (Fig. 4; R^2 = 0.75). The EC_{50} values (± SE) were (865.72 ± 68.19) mg/kg dw.

2.3 Phosphorus accumulation

Tissue P concentration significantly decreased ($P < 0.001$) and exhibited a hyperbolic pattern in relation to the increasing concentration of As(V) in the incubation solutions (Fig. 5). Regression analysis showed a negative correlation between tissue As and P concentrations with R^2 value of 0.77 (Fig. 6).

2.4 Arsenic phytofiltration

Within 72 hr, *S. polyrhiza* decreased total As concentration in the solution from 190 to 113 ng/mL (Fig. 7). The total As concentration in the solution exhibited a hyperbolic pattern within time. After that the duckweed became yellow and atrophied and there was no further decrease in

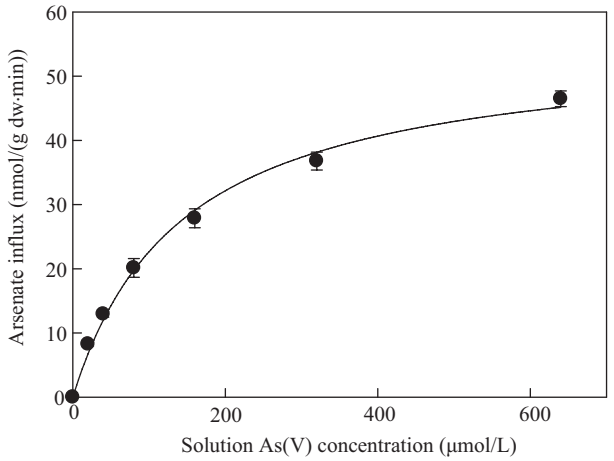

Fig. 1 Concentration-dependent kinetics for As(V) uptake by duckweed *S. polyrhiza*. Each point is represented as mean ± SE (n = 4).

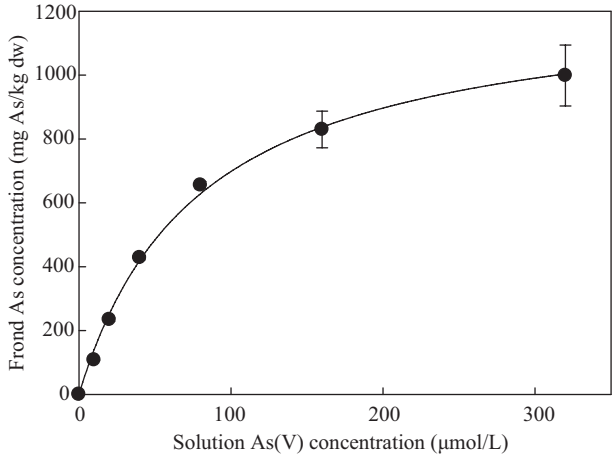

Fig. 3 Changes in tissue As concentration in duckweed *S. polyrhiza* grown in nutrient solutions with different As(V) concentrations for one week. Each point is represented as mean ± SE (n = 4).

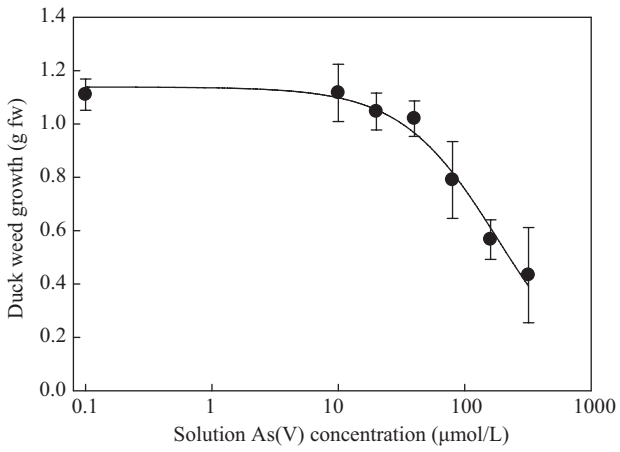

Fig. 2 Effect of As(V) exposure for one week on growth of duckweed *S. polyrhiza*. Data are mean ± SE (n = 4). Lines are the fitted log-logistic curves. To allow log transformation, a small value (0.1) was added to the zero As concentration in the control.

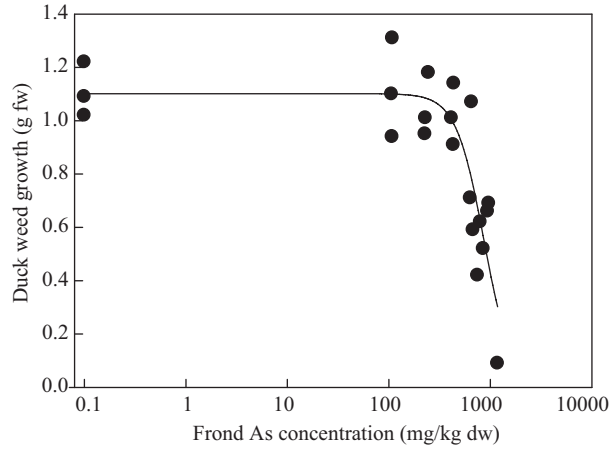

Fig. 4 Relationship between growth of duckweed *S. polyrhiza* and tissue As concentration after one-week exposure to different concentrations of As(V). Data are individual replicates. Lines are the fitted log-logistic curves. To allow log transformation, a small value (0.1) was added to the zero As concentration in the control.

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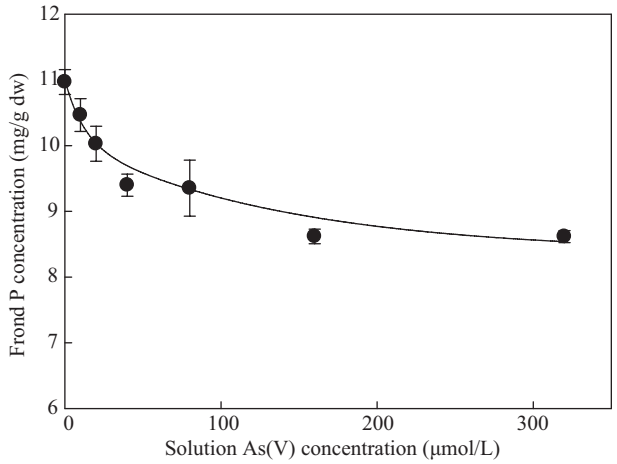

Fig. 5 Changes in P concentration in duckweed *S. polyrhiza* under different As(V) concentrations after plants had been supplied with As(V) for a week. Each point is represented as mean ± SE (n = 4).

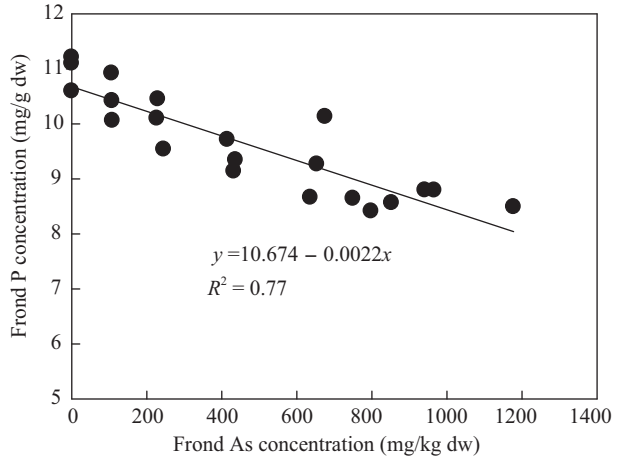

Fig. 6 Relationship between tissue As and P concentrations in duckweed *S. polyrhiza* after one-week exposure to different As(V) concentrations.

As concentration. While for the control without duckweed As concentration in the solution remained stable.

3 Discussion

In the short-term influx kinetic study, As(V) influx into *S. polyrhiza* followed the Michaelis–Menten equation. With regard to the kinetic parameters, the V_{max} value was the same as rice (Chen et al., 2005) and aquatic fern *Azolla* (Zhang et al., 2008), while the K_m value was 1–2 orders of magnitude higher than those reported for rice (Abedin et al., 2002; Chen et al., 2005), *Azolla* (Zhang et al., 2008) and white lupin (Esteban et al., 2003). The K_m values could be comparable to those for a rootless duckweed *W. globosa* (Zhang et al., 2009) and for the arsenate-tolerant plant *Holcus lanatus* (Meharg and Macnair, 1992).

It has been documented that arsenate is taken up by plants via phosphate transporter systems because arsenate acts as a phosphate analog (Dixon, 1997). Most experiments demonstrated that arsenate inhibits phosphate uptake in yeast (Rothstein and Donovan, 1963), terrestrial plants such as wheat (Geng et al., 2006), the As hyperaccumulator, Chinese brake fern *Pteris vittata* (Wang et al., 2002), and macrophytes such as *Azolla* (Zhang et al., 2008), duckweed *L. gibba* (Mkandawire et al., 2004a). In our experiment, the negative correlation between tissue As and P concentrations in *S. polyrhiza* also indicated that the P uptake in this aquatic macrophyte was inhibited by As(V).

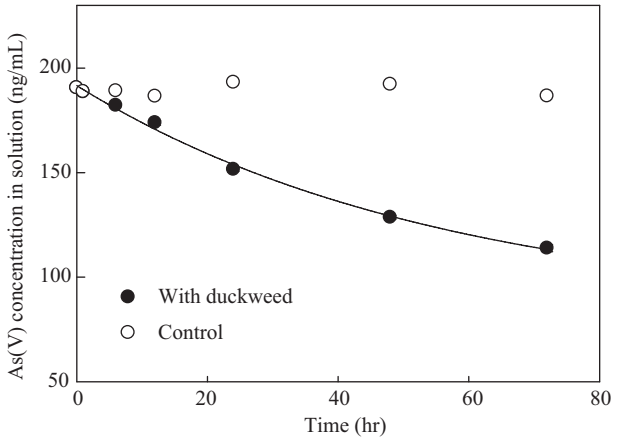

Fig. 7 Phytofiltration of As from water by 10 grams duckweed *S. polyrhiza* in 72 hr. The initial water contains 200 mL of 0.1 mmol/L $CaCl_2$ and 190 ng/mL As(V). Data are represented as mean ± SE (n = 3). The control means the same treatment without *S. polyrhiza*. Data is represented as one replicate.

S. polyrhiza was found to be able to survive in a high concentration of As(V) solution, indicating that this duckweed was tolerant to As. The EC_{50} values based on an external As(V) of *S. polyrhiza* was six times higher than that rootless duckweed *W. globosa* (Zhang et al., 2009). However, for the tissue As concentration *S. polyrhiza* accumulated (107.6 ± 0.6) mg/kg dw incubating in 10 μmol/L arsenate solution. The bioconcentration factor (BCF: the ratio of tissue As concentration to solution As concentration) was 142, which is four times lower than the rootless duckweed *W. globosa* exposed to the same As(V) concentration. It has been documented that a suppression of As accumulation is a mechanism for higher plants *H. lanatus* to achieve an enhanced resistance to external As(V), tolerant genotypes of *H. lanatus* accumulated As to a much less extent than non-tolerant plants (Meharg and Macnair, 1991). The results from the present study showed that *S. polyrhiza* enhanced resistance to external As(V) by reducing the As accumulation. The present study enriched this mechanism and proved that for macrophyte reducing As accumulation is also a way for improving tolerance to external As(V).

Unlike As non-hyperaccumulating species of higher plants, which usually appears As toxicity when tissue As concentration is above 10–100 mg/kg (Kabata-Pendias and Pendias, 1992). *S. polyrhiza* was able to accumulate appropriately 400 mg As/kg in tissue dry weight (dw) without a significant biomass loss and the EC_{50} of the tissue As is about (866 ± 68) mg/kg dw. Our results indicated that *S. polyrhiza* had a higher ability of As accumulation and tolerance, which was higher than *Azolla filiculoides* and *Azolla caroliniana* (Zhang et al., 2008) and aquatic weed *Hydrilla verticillata* (Srivastava et al., 2007). Like *S. polyrhiza*, macrophytes *L. gibba* growing in mine tailing waters could also accumulate large amounts

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of As (Mkandawire and Dudel, 2005) and some aquatic species in Taupo Volcanic Zone, New Zealand had >1000 mg As/kg (Robinson et al., 2006), which indicated that some of the macrophytes have the capacity of higher arsenic accumulation. Such property opens up the possibility of selecting these macrophytes as ideal options of As phytoremediation. Recent studies have made great effort in developing cost-effective and eco-friendly As phytoremediation by using As hyperaccumulating ferns or macrophytes. Arsenic hyperaccumultor *Pteris vittata* and *Pteris cretica* were found to be very effective in removing As from the water to a level below the guideline value of 10 μg/L (Huang et al., 2004). Rootless duckweed *W. globosa* remove almost 50% of As in a hydroponic system (Zhang et al., 2009). Alvarado et al. (2008) also found the water hyacinth (*E. crassipes*) and lesser duckweed (*L. minor*) had some potential for As bioremediation in waters. In the present study, by *S. polyrhiza* arsenic removal was 41%, suggesting that phytofiltration by *S. polyrhiza* is a promising alternative for As removal from contaminated water or paddy soil.

4 Conclusions

The present study provides insights into the underlying mechanisms of As accumulation and resistance in *S. polyrhiza*, which had a high capacity of As accumulation and tolerance. This floating aquatic plant has some potential for As phytofiltration, and could be used as an alternative for As phytoremediation in contaminated water and paddy soil.

Acknowledgments

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