

Arsenic Accumulation in Duckweed (*Spirodela polyrhiza* L.): A Good Option for Phytoremediation

M. Azizur Rahman^{*,1}; Hiroshi Hasegawa^{*,1}; Kazumasa Ueda¹; Teruya Maki¹; Chikako Okumura¹; M. Mahfuzur Rahman²

¹Graduate School of Natural Science & Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan; ²Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

*Corresponding author

E-mail: arahman@stu.kanazawa-u.ac.jp

hhiroshi@t.kanazawa-u.ac.jp

Tel/Fax: 81-76-234-4792

Abstract

Some unavoidable drawbacks of traditional technologies have made phytoremediation a promising alternative for removal of arsenic from contaminated soil and water. In the present study, the potential of an aquatic macrophyte *Spirodela polyrhiza* L. for phytofiltration of arsenic, and the mechanism of the arsenic uptake were investigated. The *S. polyrhiza* L. were grown in three test concentrations of arsenate and dimethylarsinic acid (DMAA) (i.e. 1.0, 2.0

and 4.0 μM) with 0 (control), 100 or 500 μM of phosphate. One control treatment was also set for each test concentrations of arsenic. The PO_4^{3-} concentration in control treatment was 0.02 μM . When *S. polyrhiza* L. was cultivated hydroponically for 6 d in culture solution containing 0.02 μM phosphate and 4.0 μM arsenate or DMAA, the arsenic uptake was $0.353 \pm 0.003 \mu\text{mol g}^{-1}$ and $7.65 \pm 0.27 \text{ nmol g}^{-1}$, respectively. Arsenic uptake into *S. polyrhiza* L. was negatively ($p < 0.05$) correlated with phosphate uptake when arsenate was applied to the culture solutions owing to similar in the sorption mechanism between AsO_4^{3-} and PO_4^{3-} , and positively ($p < 0.05$) correlated with iron uptake due to adsorption of AsO_4^{3-} onto iron oxides. Thus, the *S. polyrhiza* L. accumulates arsenic by physico-chemical adsorption and via the phosphate uptake pathway when arsenate was added to the solutions. These results indicate that *S. polyrhiza* L. would be a good arsenic phytofiltrator. In contrast, DMAA accumulation into *S. polyrhiza* L. was neither affected by the phosphate concentration in the culture nor correlated ($p > 0.05$) with iron accumulation in plant tissues, which indicates that *S. polyrhiza* L. uses different mechanisms for DMAA uptake.

Keywords: Arsenate, DMAA, Duckweed (*Spirodela polyrhiza* L.), Mechanism, Uptake, Physico-chemical adsorption.

Introduction

Arsenic has recently drawn attention due to its chronic and epidemic toxic effects to humans through widespread contamination of water and food crops through natural release of the element from aquifer rocks in Bangladesh (Fazal et al., 2000; Smith et al., 2000; Hopenhayn, 2006) and West Bengal, India (Chowdhury et al., 2000). Geogenic arsenic contamination in aquifer rocks has also been reported in Thailand (Visoottiviseth et al., 2002), Vietnam, Inner Mongolia, Greece, Hungary, U.S.A., Ghana, Chile, Argentina and Mexico (O'Neill, 1995; Smedley and Kinniburgh, 2002).

Some unavoidable limitations of the traditional chemical and physical methods have made phytoremediation, a plant-based green technology, a viable alternative to remediate environmental pollution (Cunningham and Berti, 1993; Raskin et al., 1994b; Raskin et al.,

1994a; Salt et al., 1998). Its relative inexpensiveness and eco-friendliness have made it an attractive method for water and soil remediation (Raskin et al., 1994a). Microorganisms have the potential to degrade environmental pollutants (Ahmann et al., 1997), while some plants have the ability to accumulate toxic metals at high concentrations (McGrath et al., 1998). Arsenic, accumulated into plants primarily through their root system, is not readily translocated to the shoots (Raskin et al., 1994a; Komar et al., 1995). Brooks et al. (1977) first used the term “hyperaccumulators” to describe those plants that uptake and accumulate metals more than 1000 $\mu\text{g metal g}^{-1}$ dry mass (Visoottiviset et al., 2002) which is still in common use (Reeves and Baker, 2000). *Agrostis castellana*; *Agrostis delicatula* (De Koe, 1994), *Bidens cynapiifolia* (Bech et al., 1997), Chinese brake fern (*Pteris vittata* L.) (Ma et al., 2001) and silver fern (*Pityrogramma calomelanos* L.) (Gulz et al., 2005) have been reported to be arsenic hyperaccumulators. In particular, Chinese brake ferns remove a formidable quantity of arsenic from soil (Komar et al., 1998; Gulz et al., 2005), and store it in the fronds (Tu et al., 2002; Gulz et al., 2005).

Besides contamination of the soil, contamination of water by geogenic arsenic has caused severe direct or indirect human health effects. Effective remediation of such water is currently an urgent necessity. Aquatic macrophytes can be a good remediation option, because a few species have already been reported to accumulate arsenic from water (Robinson et al., 2003; Mkandawire and Dudel, 2005). Arsenate is the predominant species in the oxic water and arsenate and arsenite are bioavailable forms to the plants in the aquatic systems (Sizova et al., 2002). The dynamics of arsenate exchange between water and adsorbing colloids are analogous to those of phosphate, though the competition for exchange sites favors phosphate over arsenate (Mkandawire et al., 2004).

The *Lemna gibba* L. and the *Lemna minor* L. are the most studied species of *Lemnaceae* family in phytoremediation and ecotoxicology (Mkandawire et al., 2004; Mkandawire and Dudel, 2005). Great duckweed (*S. polyrhiza* L.) belonging to the member of *Lemnaceae* family under the group monocotyledons was selected for the present study because of its fast growth, wide distribution, short life span and stability to environmental changes (Landolt and Kandeler, 1987; Lemon et al., 2001; Khondker, 2003). Moreover, the great duckweed (*Spirodela polyrhiza* L.) may surpass the know results of lesser duckweed (*Lemna* spp.) and

thus, we looked for a less known area. Inorganic arsenic species have been studied extensively in terms of uptake and accumulation by aquatic macrophytes (Robinson et al., 2003; Mkandawire and Dudel, 2005). Little is done using other arsenic species. Moreover, arsenate and DMAA are the major species in the oxic aquatic system. Hence, the accumulation and mechanisms of arsenate (inorganic species) and DMAA (organic species) by *S. polyrhiza* L. were investigated in the present study.

Materials and Methods

Plant Cultivation

The *S. polyrhiza* L., collected from a rice field in Manikgonj of Dhaka, Bangladesh, was stock-cultured in a green house for 2 wk using standard Murashige and Skoog (MS) culture solution (Table 1). The experiment was conducted for 6 d with the conditions being set as 14:10 h light/dark schedule, 100-125 $\mu\text{E m}^{-2} \text{s}^{-1}$ light intensity, 75% humidity, 22 °C and 20 °C (± 2 °C) temperatures for day and night, respectively. The *S. polyrhiza* L. were exposed to three test concentrations (i.e. 1.0, 2.0 and 4.0 μM) of arsenate and DMAA with 0.02 (control), 100 or 500 μM of phosphate. One control treatment was also set for each test concentrations of arsenic.

Inoculation Procedure

Before incubation, the *S. polyrhiza* L. plants from the stock-culture were rinsed three times superficially with deionized (DI) water to remove particles attached to the plant surfaces. An amount of 100 mL culture solution was prepared in each of the 200-mL polystyrene test vessels (118 x 86 x 60 mm) and about 120 individual plants were incubated in each of the test vessels. Three were three replicates for each treatment and the experiment was arranged following randomized design (RD) with a total of 36 vessels. Stock solutions of arsenate and DMAA were made from $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ and $(\text{CH}_3)_2\text{AsO}_2\text{Na} \cdot 3\text{H}_2\text{O}$, respectively. Arsenic stock solutions were added to the cultures before inoculation. The plants were grown for 6 d. Changes in the volume of cultures from evaporation and accumulation were compensated by adding DI water in every 2 d throughout the experiment.

Sample Preparation and Chemical Analysis

All plants (about 120 individuals) were harvested after 6 d of incubation. After rinsing with DI water for four times, plants were kept on clean absorbent paper to remove the water from plant surfaces. Then the samples were taken into small ceramic cups and covered with ceramic cover and placed into a drying oven. The samples were dried for 48 h at 65 °C until they reached a constant weight. Dried samples were weighted and 0.10-0.20 g samples were taken into 50-mL polyethylene tubes (*DigiTubes*, SCP Science, Canada). Five mL of 65% HNO₃ were added and the samples were kept under fume hood for 12 h. The samples were then heated on a heating block (*DigiPREP*, SCP Science, Canada) at 95 °C for 2 h. After cooling to room temperature, 3 mL of 30% hydrogen peroxide were added to the digests and the samples were heated again at 105 °C for 20 min and then diluted to 10 mL using DI water and stored in 15-mL polythene bottles (HDPE, NALGENE[®], Nalge Nunc International, Rochester, NY). [Williams et al. \(2005\)](#) also digested rice samples at 120 °C and evaporated to dryness at 160 °C for arsenic speciation analysis.

The concentrations of arsenic and iron were analyzed by graphite-furnace atomic absorption spectrometer (GF-AAS, Z-8100, Hitachi, Japan). For the determination of arsenic, 5 µL of 0.05 M nickel nitrate was added to a 10-µL sample as matrix solution in the cuvette. The accuracy of the analysis was checked by the analysis of certified standard reference material 1573a tomato leaf (NIST, USA). The arsenic concentration in certified tomato leaf was 0.112±0.004 µg g⁻¹ dry weight while the measured arsenic concentration was 0.123±0.009 µg g⁻¹. The concentrations detected in all samples were above the instrumental limits of detection (≥ 0.01 µM samples in water). Total phosphate and nitrate were determined spectrophotometrically ([APHA, 1998](#)).

Chemicals used in this experiment were of analytical grade. All glassware used was washed with detergent solution, 3 M HCl and finally with DI water for eight times before use. In each analytical batch, at least two reagent blanks and three replicate samples were included.

Data Analysis

Bioaccumulation values (φ) for arsenic in *S. polyrhiza* were defined as $\varphi = g_u/g_L$, where g_u is the mass of arsenic in plant material and g_L is the dry biomass ([Mkandawire et al., 2004](#)). The experimental data were statistically analyzed for mean separation of treatments according to

the least significant difference (LSD) at 5% level by IRRISTAT 4.0 for windows (developed by the biometrics unit, IRRI, Philippines) and the Pearson correlation coefficient (r) was calculated by SPSS[®] statistical package (version 10.0 for windows).

Results and Discussion

Arsenic Accumulation in *S. polyrhiza* L.

On exposure to 4.0 μM arsenate and 0.02 μM PO_4^{3-} (control), *S. polyrhiza* accumulated $0.353 \pm 0.003 \mu\text{mol g}^{-1}$ dry weight of arsenic after 6 d of incubation (Fig. 1a). In contrast, the arsenic accumulation was only $7.65 \pm 0.27 \text{ nmol g}^{-1}$ dry weight when the plants were exposed to DMAA (Fig. 1b). Thus, *S. polyrhiza* L. accumulated 39 folds more arsenic when exposed to arsenate than those plants exposed to DMAA. The increase of arsenic concentrations in tissues of *S. polyrhiza* L. showed a strong positive correlation with its concentrations in culture solutions ($r = 0.752$ and 0.801 for arsenate and DMAA, respectively; $p < 0.01$).

When the concentration of PO_4^{3-} in the culture solution was increased from 0.02 (control) to 500 μM with a constant arsenate concentration (4.0 μM), a 3.18 folds decrease in arsenic accumulation in *S. polyrhiza* L. was observed. In contrast, a very small decrease in the accumulation of arsenic was found with the increase of phosphate concentration when DMAA was applied to the culture solutions. The results imply that the arsenic uptake into *S. polyrhiza* L. is related to the arsenic species and phosphate concentration in the culture solution. Arsenic uptake is depended on phosphate status when the plants were exposed to arsenate while it is independent of phosphate when exposed to DMAA.

Mkandawire and Dudel (2005) reported 0.02 and 106 mg kg^{-1} dry weight of arsenic accumulation in fronds of *Lemna gibba* L. (lesser duckweed) when the PO_4^{3-} concentrations in culture solutions were 40 and 0.014 mg L^{-1} . In another study of *L. gibba* L., Mkandawire et al. (2004) found 1.39 and 1.45 folds decrease in arsenic accumulations when the plants were exposed to 1000 $\mu\text{g L}^{-1}$ arsenate and arsenite, respectively and the PO_4^{3-} concentration in the culture solution was increased from 0.014 to 40 mg L^{-1} . The magnitude of arsenic accumulation in *S. polyrhiza* L. in relation to PO_4^{3-} concentration is comparable with that of *L.*

gibba. Arsenic uptake in *S. polyrhiza* L. decreased by 3.18 folds when the plants were exposed to 4 μM arsenate and the PO_4^{3-} concentration in the culture solution was increased from 0.02 to 500 μM .

Phosphate Uptake in Relation to Arsenic Species

Phosphate uptake in *S. polyrhiza* L. was reduced significantly ($p < 0.05$) by arsenate, while DMAA had no significant effect ($p > 0.05$) on its uptake (Table 2). Pearson correlation analysis revealed a strong negative correlation between the arsenate concentrations in culture solutions and phosphate concentrations in plant tissues ($r = -0.613$; $p < 0.05$). On the other hand, for DMAA, the correlation was not significant ($r = -0.203$; $p > 0.05$).

Figure 2 shows the relationship between arsenic and total phosphate content in *S. polyrhiza* L. exposed to either arsenate (Fig. 2a) or DMAA (Fig. 2b). The strong negative correlation between arsenic and phosphate concentrations in *S. polyrhiza* L. treated with arsenate indicates that the phosphate uptake in this aquatic macrophyte was inhibited by arsenate. [De La Rosa et al. \(2006\)](#) reported the reduction of phosphate uptake into tumbleweed (*Salsola kali*) when the plant was treated with arsenate. Other researchers ([Wang et al., 2002](#); [Patra et al., 2004](#)) reported the inhibition of arsenate uptake by phosphate, which demonstrates that arsenate is accumulated into plants through the phosphate uptake mechanism, even replacing phosphorus from the biomolecules containing phosphate groups.

Most of the experiments demonstrating the suppression of arsenic uptake by phosphate were conducted with terrestrial plants ([Carbonell-Barrachina et al., 1998](#); [Cao et al., 2003](#); [Gulz et al., 2005](#)). However, [Mkandawire et al. \(2004\)](#) reported the preference of *L. gibba* to accumulate more As(III) than As(V) and suppression of these two inorganic arsenic species uptake by phosphate. They also demonstrated that the effect of phosphate on arsenic uptake seems to differ depending on the plant species as well as the growth conditions. The present study revealed that not only the plant species and growth conditions but also the arsenic species are related to the effect of phosphate on the arsenic uptake into aquatic macrophytes.

Influence of PO_4^{3-} and Fe on Arsenic Uptake by Duckweed

The accumulation of arsenic in *S. polyrhiza* L. from solutions containing arsenate decreased significantly ($p < 0.05$) with the increase of the phosphate concentration in the culture solution

for all three concentrations tested (Fig. 1). AsO_4^{3-} is a sorption analog of PO_4^{3-} and competes with it for uptake carriers in the plasmalemma (Mkandawire et al., 2004); hence, more arsenate is expected to be desorbed in the solution with increasing phosphate (Smith and Read, 1997). The arsenate uptake in *Lemna gibba* L. occurs through the phosphate uptake pathway (Mkandawire and Dudel, 2005) due to similar chemical behavior of AsO_4^{3-} and phosphate and the present findings suggest the same for *S. polyrhiza* L.

Physico-chemical adsorption, an alternative mechanism for arsenic accumulation into aquatic plants, has been proposed in the literature (Robinson et al., 2006). In this mechanism, suspended oxides of iron (iron plaque) on the aquatic plant surfaces adsorb and accumulate arsenic. The iron concentration in *S. polyrhiza* L. was positively correlated with that of arsenic ($r = 0.591$; $p < 0.05$) when the plants were exposed to arsenate and was independent ($r = 0.259$; $p > 0.05$) of arsenic when exposed to DMAA (Table 2). Robinson et al. (2006) also reported a positive correlation between arsenic and iron concentrations in aquatic plants since arsenic is adsorbed on iron oxides biosorbed on the plant surfaces. The actual species of arsenic adsorbed was not clear from their studies. The present study suggests arsenate as the predominant species in such incorporation for *S. polyrhiza* L. as the correlation between arsenic and iron concentrations in plant tissues was significantly positive (Table 2) when arsenate was added to the solutions than that of DMAA. Blute et al. (2004) reported arsenate to be correlated positively with iron in plaque and negatively with iron absorption into the roots of cattail (*Typha latifolia*) grown in arsenic-contaminated wetland sediments. According to them, the plaque was predominantly Fe(III) oxyhydroxide and 80% of the arsenic in it was arsenate and the ferric ion inhibited its mobility into the roots. Another report (Chen et al., 2005) says the same for rice root.

In the present study of *S. polyrhiza* L., however, arsenic and iron concentrations accumulated into the plants grown in solution containing arsenate and low concentration of phosphate (0.02 μM) were highly correlated ($p < 0.01$) while they were not significantly correlated ($p > 0.05$) in phosphate-sufficient solution (Fig. 2). This can be attributed to the adsorption of arsenic on iron plaque of plant surfaces in phosphate-deficient solution, which was blocked by phosphate in phosphate-sufficient solution. Thus, *S. polyrhiza* L. may accumulate arsenic onto the roots

by physico-chemical adsorption, and into the roots via the phosphate uptake pathway.

Arsenic uptake in *S. polyrhiza* L. treated with DMAA differs from those treated with arsenate in terms of mechanism and efficiency. There was no significant correlation between the concentrations of arsenic and phosphate in *S. polyrhiza* L. when the plants were exposed to DMAA (Fig. 2). Arsenate and DMAA differ significantly in their chemical behavior. The similar chemical properties of As(V) and P(V) often explain the poor bioavailability of As(V) to plants because PO_4^{3-} is stronger than arsenate in surface chemistry competition (Meharg and Hartley-Whitaker, 2002; Mkandawire et al., 2003) whereas DMAA does not compete with phosphate because of their dissimilarities in chemical properties. Thus, DMAA is not accumulated into *S. polyrhiza* L. via the phosphate uptake pathway. Pearson correlation analysis between the arsenic and iron concentrations in the plant treated with DMAA also revealed that DMAA was not accumulated via physico-chemical adsorption (Table 2). Moreover, the arsenic and iron concentrations in the plants treated with DMAA were not significantly correlated ($p > 0.05$) with phosphate, which suggests that the accumulation of DMAA was independent of phosphate (Fig. 3). The *S. polyrhiza* L., thus, appears to use a different mechanism for DMAA uptake.

Potential of *S. polyrhiza* L. for the Phytoremediation of Arsenic

The arsenic bioaccumulation in *S. polyrhiza* L. exposed to arsenate was about 79% higher than those exposed to DMAA. In the culture solutions having 4.0 μM of arsenate or DMAA and 0.02 μM of phosphate, the arsenic accumulations were $0.353 \pm 0.003 \mu\text{mol g}^{-1}$ dry weight and $7.65 \pm 0.27 \text{ nmol g}^{-1}$ dry weight, respectively after 6 d of incubation.

S. polyrhiza L. grows in rice field and prevents the growth of harmful aquatic weeds. Under natural conditions with sufficient nutrients, this plant forms a mat-like covering on the water surface of ponds, lakes and ditches. In addition, *S. polyrhiza* L. is characterized by fast growth, wide distribution and stability to the environmental changes. Under ideal conditions, its biomass doubles in 24 h (Khondker, 2003). Moreover, arsenate is the predominant species in the oxic water and arsenate and arsenite are bioavailable forms to the plants in the aquatic systems (Sizova et al., 2002). The present study showed that *S. polyrhiza* L. accumulates a good amount of arsenic when exposed to arsenate. Thus, *S. polyrhiza* L. could be a good

option for phytoremediation.

Acknowledgements:

This research was supported partly by Grants-in-Aid for Scientific Research (18510071) from the Japan Society for the Promotion of Science, and the Steel Industry Foundation for the Advancement of Environmental Protection Technology, Japan.

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