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Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation

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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 ± 0.003 μmol g^{-1} and 7.65 ± 0.27 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

1. 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 (Smith et al., 2000; Fazal et al., 2001; 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, USA, 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 Betri, 1993; Raskin et al., 1994a,b; 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,

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accumulated into plants primarily through their root system, is not readily translocated to the shoots (Raskin et al., 1994a; Kumar et al., 1995). Brooks et al. (1977) first used the term "hyperaccumulators" to describe those plants that uptake and accumulate metals more than 1000 μg metal g^{-1} dry mass (Visoottiviseth 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 (*Spirodela 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 (*S. 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.

2. Materials and methods

2.1. 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 week 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 $\mu E\ m^{-2}\ s^{-1}$ 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.

Table 1
Modified Murashige and Skoog (MS) nutrients for *S. polyrhiza* L. hydroponic culture medium[a]

Nutrient	Concentration ($mg\ l^{-1}$)
KNO_3	1900.00
NH_4NO_3	1650.00
$CaCl_2 \cdot 2H_2O$	440.00
$MgSO_4 \cdot 7H_2O$	370.00
K_2HPO_4	Modified[a]
$FeSO_4 \cdot 7H_2O$	27.80
$MnSO_4 \cdot 5H_2O$	22.30
$ZnSO_4 \cdot 7H_2O$	8.60
H_3BO_3	6.20
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
Na_2–EDTA	37.30

[a] The PO_4^{3-} concentration in control treatment was 0.02 μM and the other concentrations of PO_4^{3-} in the solutions were 100 and 500 μM.

2.2. 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 polystylene test vessels (118 × 86 × 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 $Na_2HAsO_4 \cdot 7H_2O$ and $(CH_3)_2AsO_2Na \cdot 3H_2O$, 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.

2.3. 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