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Arsenic uptake by aquatic macrophyte *Spirodela polyrhiza* L.: Interactions with phosphate and iron

M. Azizur Rahman[a], H. Hasegawa[a,*], K. Ueda[a], T. Maki[a], M. Mahfuzur Rahman[b]

[a] *Graduate School of Natural Science & Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan*
[b] *Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh*

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ABSTRACT

The uptake of arsenate (As(V)) and dimethylarsinic acid (DMAA) by aquatic macrophyte *Spirodela polyrhiza* L. was investigated to determine the influence of arsenic interaction with PO_4^{3-} and Fe ions. Plants were grown hydroponically on standard Murashige and Skoog (MS) culture solutions. Arsenic concentrations in Fe-oxide (Fe-plaque) on plant surfaces were determined by citrate–bicarbonate–ethylenediaminetetraacetic acid (CBE) technique. *S. polyrhiza* L. accumulated 51-fold arsenic from arsenate solution compared to that from DMAA solution with initial concentrations of 4.0 and 0.02 μM of arsenic and phosphate, respectively. The arsenate uptake was negatively ($p < 0.001$) correlated with phosphate uptake and positively ($p < 0.05$) correlated with iron uptake. About 56% of the total arsenic was accumulated into the plant tissues while 44% was adsorbed on Fe-plaque (CBE-extract), when the plants were grown on arsenate solution. The DMAA uptake into the plant was neither affected by the phosphate concentrations nor correlated ($p > 0.05$) with iron accumulation. The results suggest that adsorption of arsenate on Fe-plaque of the surface of *S. polyrhiza* L. contributes to the arsenic uptake significantly. Thus, arsenate uptake in *S. polyrhiza* L. occurred through the phosphate uptake pathway and by physico-chemical adsorption on Fe-plaques of plant surfaces as well. The *S. polyrhiza* L. uses different mechanisms for DMAA uptake.

1. Introduction

Arsenic is an important environmental and health concern due to its known chronic and epidemic toxicity. The main arsenic exposures to humans are through water pathway and food contamination, for instance in Bangladesh [1–3] and West Bengal, India [4] where most of the contaminations originate from natural release from rocks in the aquifer. Geogenic arsenic contamination from aquifer rocks has also been reported in Thailand [5], Vietnam, Inner Mongolia, Greece, Hungary, U.S.A., Ghana, Chile, Argentina and Mexico [6,7]. Unfortunately, the traditional chemical and physical remediation techniques are limited due to the pattern of discharge. Hence, Phytoremediation, a plant-based green technology, is proposed as a viable alternative. Its relative inexpensiveness and eco-friendliness have made it an attractive method for water and soil remediation [8]. Some terrestrial plant species such as *Agrostis castellana*; *Agrostis delicatula* [9], *Bidens cynapiifolia* [10], Chinese brake fern (*Pteris vittata* L.) [11] and silver fern (*Pityrogramma calomelanos* L.) [12] have been reported to accumulate significant fractions of arsenic from soil. In particular, Chinese brake fern accumulates a formidable quantity of arsenic from soil [12,13] and stores in the fronds [12,14]. The arsenic hyperaccumulating terrestrial plants are considered for soil remediation. However, restoration of contaminated waters of ponds, lacks and ditches as well as irrigation water remains unresolved. Aquatic macrophytes could be a good tool for the environmentally sound and effective remediation of arsenic contaminated waters [15,16]. Hence, we investigated the possible use of duckweed in aquatic phytoremediation.

In the present study, duckweed (*Spirodela polyrhiza* L.) was selected because of its fast growth, wide distribution, short life span and stability to the large-scale environmental changes [17,18]. The plant commonly grows in inland small water bodies such as ponds, lacks, ditches in Bangladesh and West Bengal, India into which arsenic contaminated water from hand tube wells (used for household necessity) and shallow tube wells (used for irrigation) is drained. Moreover, duckweed (*S. polyrhiza* L.) grows in the rice fields of South Asian countries where arsenic contaminated groundwater is the main source of irrigation during dry season. The plant is also beneficial to rice cultivation as it suppressed or reduce weed growth in the rice field.

Arsenate and arsenite are bioavailable inorganic forms of arsenic in aquatic systems [19]. The dynamics of arsenate exchange between water and adsorbing colloids are analogous to those

* Corresponding author. Tel.: +81 76 234 4792; fax: +81 76 234 4792.
E-mail address: hhiroshi@t.kanazawa-u.ac.jp (H. Hasegawa).

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of phosphate, though the competition for exchange sites favors phosphate over arsenate [20]. Arsenate and dimethylarsinic acid (DMAA) are the major species of arsenic in toxic aquatic systems [21]. Uptake behavior of these two arsenic species could reflect the influence of inorganic and organic arsenic species and their interactions with PO_4^{3-} and Fe ions. The comparison between inorganic (arsenate) and organic (DMAA) arsenic species uptake is important because of their limit of toxicity too.

In nature, wetland plants form dense root networks in upper wetland sediments and, under flooded conditions, pump oxygen to their roots for respiration [22]. Thus, oxygenation of the rhizosphere by wetland plants leads to precipitation of iron (oxyhydro)-oxides in the rhizosphere and on the roots of plants [23]. Precipitation of iron (oxyhydro)-oxides on roots of aquatic plants has also been reported in literatures [24]. Due to the high adsorptive affinity of arsenate for iron hydroxides, Fe-plaque formation on root surface of aquatic plants might be significant in the uptake of arsenic by the plants. In the present study we reported the uptake of arsenate and DMAA in duckweed (*S. polyrhiza* L.) and their interactions with PO_4^{3-} and Fe ions. The contribution of Fe-plaque formation on plant's surfaces in the arsenic uptake has also been discussed.

2. Materials and methods

2.1. Conditions for plant cultivation

The *S. polyrhiza* L., collected from a rice field in Manikgonj of Dhaka, Bangladesh, was bring to Japan and stock-cultured in green house for 2 weeks. Then, the plants were rinsed three times with deionized (DI) water and transferred to growth chamber. In the growth chamber, the experiment was conducted 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(±2) °C temperatures for day and night, respectively.

Modified standard Murashige and Skoog (MS) culture solution was used as growth medium in the experiment (Table 1). The control culture solution contained 0.02 μM PO_4^{3-} and other culture solutions were prepared by modifying the PO_4^{3-} concentration to 100 or 500 μM. Three test concentrations (1.0, 2.0 and 4.0 μM) of either arsenate or DMAA were added to the modified MS culture solutions. The pH of the solution was adjusted to 6.0 using 0.1 M KOH or 0.1 M HCl.

Before inoculation, *S. polyrhiza* L. from the stock-culture were rinsed for three times with DI water. About 100 ml of culture solution was taken into 200-ml polystyrene test vessels (118 mm × 86 mm × 60 mm). About 120 individual plants were inoculated in each of the test vessels. The experiment was arranged following the randomized design (RD) with three replicates. Stock solutions of arsenate and DMAA were made by dissolving $Na_2HAsO_4{\cdot}7H_2O$ and $(CH_3)_2AsO_2Na{\cdot}3H_2O$ in DI water, respectively. Arsenic stock solutions were added to the cultures before inoculation. The plants were grown for 12 days. Changes in the volume of cultures from evaporation and accumulation were compensated by adding DI water every 2 days throughout the experiment.

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

Nutrients	Concentration ($mg\,l^{-1}$)
KNO_3	1900
NH_4NO_3	1650
$CaCl_2{\cdot}2H_2O$	440
$MgSO_4{\cdot}7H_2O$	370
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 control solution contained 0.02 μM PO_4^{3-} and the modifications of the solutions were 100 and 500 μM of PO_4^{3-}. The pH of the solution was adjusted to 6.0.

2.2. Iron plaque induction

A separate experiment was conducted to investigate the role of iron plaque on arsenic uptake in *S. polyrhiza* L. Plants were grown in 1.5 l of DI water for 24 h before iron plaque induction to minimize interferences from other elements with iron. They were then, transferred into 1 l of the MS solution containing 0.36 mM of iron as $FeSO_4{\cdot}7H_2O$ and grown for 2 days. The pH of solution was adjusted to 6.0 using either 0.1 M KOH or 0.1 M HCl. The specified standard concentration of phosphate for MS culture solution was not modified. After 2 days in high iron medium, plants were inoculated into MS culture solution for 12 days as described in the previous section, with 6.0 μM of either arsenate or DMAA.

2.3. CBE-extraction of Fe-plaques

Iron plaques from plant surfaces were extracted using citrate–bicarbonate–ethylenediaminetetraacetate (CBE)-technique, a modification of dithionite–citrate–bicarbonate (DCB)-extraction method of Taylor and Crowder [25] and Otte et al. [26]. The CBE solution was prepared from 0.03, 0.125 and 0.050 M of sodium citrate, sodium bicarbonate and EDTA, respectively. Plants were treated with 30 ml of CBE solution for 60 min at room temperature. The plants were then, rinsed with DI water for three times, and the rinsed water was added to the CBE-extracts to make a total volume of 50 ml.

2.4. Sample preparation and chemical analysis

All plants were harvested after 12 days of inoculation. After rinsing with DI water for four times, the plant samples were kept on clean absorbent paper to remove the water from the plant surfaces. The samples were dried at 65 °C until they reached a constant weight. Then, 0.10–0.20 g of dried samples was taken into 50-ml polyethylene tubes (*Digi*Tubes, SCP Science, Canada) for digestion. Five milliliters of 65% HNO_3 were added to the sample and then, left to incubate for 12 h. The samples were heated on a heating block (*Digi*PREP, SCP Science, Canada) at 95 °C for 2 h. After cooling to room temperature, 3 ml of 30% hydrogen peroxide were added and the samples were heated again at 105 °C for 20 min. Then, the digests were diluted to 10 ml with DI water and taken into 15-ml polyethylene bottles (HDPE, NALGENE®, Nalge Nunc International, Rochester, NY) in readiness for analysis.

Arsenic and iron were analyzed using 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 into the cuvette as matrix modifier. Certified standard reference material 1573a (tomato leaf from NIST, USA) was used to check the accuracy of analysis. Arsenic concentration in certified reference material was $0.112 \pm 0.004\,\mu g\,g^{-1}$ while the measured arsenic concentration was $0.123 \pm 0.009\,\mu g\,g^{-1}$. The concentrations detected in all samples were above the instrumental limits of detection (≥0.01 μM in samples in water). Total phosphate was determined spectrophotometrically [27].

All chemical reagents used in this experiment were of analytical grade. Glassware and dishes were washed with detergent solution,