The Effects of Phosphorous on the Accumulation of Arsenic in Water Fern (Azolla pinnata L.)

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The water fern (Azolla pinnata L.) was grown in culture solution with arsenate (As(V)) and dimethylarsinic acid (DMAA). Arsenic concentrations were measured in tissues of the plant after harvest. Plant of control and solution having lower phosphate concentration accumulated significantly higher amount of arsenate compared to that of higher phosphate solution. The Azolla pinnata L. accumulated about 7 folds arsenate than that of DMAA when the plants were grown in solution containing 4.0 μM of arsenate and no phosphate. With the increase of phosphate concentration in culture solution the arsenate uptake into the plant decreased significantly (p < 0.001). The arsenate accumulation in Azolla pinnata L. decreased by 40% for the addition of 100 μM of phosphate in the solution. However, the Azolla pinnata L. accumulated 0.034±0.005 μmol g⁻¹ dry weight of DMAA from solution having 4.0 μM of DMAA and no phosphate. The phosphate concentration did not affect DMAA uptake into the plant significantly. Arsenate uptake was clearly correlated with iron and phosphate uptake into the water fern though DMAA uptake was not. The results suggest that arsenate uptake in the macrophytes might be dependent on phosphate and iron uptake while DMAA was independent thereof.

Key Words: Arsenate; DMAA, Water Fern (Azolla pinnata L.), Uptake, Physico-chemical adsorption, Phytoremediation, Fe-plaque.

1. Introduction

Arsenic is one of the toxic environmental pollutants which have recently attracted attention because of its chronic and epidemic effects on human health through widespread water and crop contamination due to its natural release from aquifer rocks in Bangladesh1 and West Bengal, India2. Effective remediation of this water is urgent necessity.

Phytoremediation is a plant based green technology, which refers to the phytoextraction, phytostabilization and phytofiltration or detoxification of metals and organic pollutants from the environment. Due to some unavoidable limitations of traditional methods, phytoremediation becomes promising to remediate the environmental pollutants. It is relatively inexpensive and eco-friendly that has been proven effective in some cases3. Hyperaccumulating plants can accumulate very high level of arsenic in their shoots and edible parts4. In particular, Chinese brake fern (Pteris vitata L.) removes a formidable quantity of arsenic from soil and store it in the fronds5.

Recently, the encouraging results of the metal uptake potential by aquatic plants and the uptake mechanisms gained the attention of researchers to use them in phytoremediation technology. Arsenate predominates in the aquatic system because it is oxidized to arsenate easily under oxic conditions6. The metabolism of arsenic by aquatic organisms results in the occurrence of thermodynamically unstable methylarsenic compounds in natural waters7,8 and the DMAA predominate among the methylarsenic compounds7,8. Although phosphate has been reported to inhibit arsenic uptake in terrestrial plant species, its influences on arsenic uptake in aquatic plants have not been studied intensively. Moreover, information about the uptake mechanisms of arsenic in aquatic macrophyte is limited, which is important for the implementation of aquatic plants in phytoremediation technology.

Azolla pinnata L. is a free-floating freshwater macrophyte commonly known as water fern. In the present study, we investigated the accumulation of arsenate and DMAA into this aquatic macrophyte. The objectives of the study were to evaluate the effects of phosphate on arsenic uptake (arsenate and DMAA) in aquatic macrophyte (Azolla pinnata L.) and to assess the uptake mechanisms of arsenic into this plant. The ultimate goal of the present research was to justify the usefulness of Azolla pinnata L. in phytoremediation technology.
2. Materials and Methods

2.1. Plant Cultivation

Plants were grown in MS solution (Table 1) for 5 days with the conditions being set as 14/10 h light/dark schedule, 100-125 µE m⁻² s⁻¹ light intensity, 75% humidity, 22 and 20 (±2) °C temperatures. The phosphate concentration in the solution was modified as 50 and 100 µM. Either arsenate or DMAA was added to the solutions at 1.0, 2.0 and 4.0 µM. One control treatment of arsenic and phosphate was also run to compare the results.

2.2. Inoculation procedure

Before inoculation, strains of Azolla pinnata L. from stock-culture were rinsed for three times with deionized (DI) water. The pH was adjusted to 5.5 and 100 ml of culture solution was taken into 200-ml volumetric polystyrene test vessels (118 X 86 X 60 mm). About 20-30 individual Azolla pinnata L. were inoculated in the test vessels separately. Three replicates of each treatment were arranged following randomized design (RD). Stock solutions of arsenate and DMAA were prepared from Na₂H₂AsO₄·7H₂O and (CH₂)₃AsO₂Na·3H₂O, respectively. Change in the volume of culture solutions from evaporation and accumulation was compensated by adding DI water in every 2 days throughout the experiment.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Concentrations (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>1900</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>1650</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>440</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>370</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>Modified a</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>27.80</td>
</tr>
<tr>
<td>MnSO₄·5H₂O</td>
<td>22.30</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>8.60</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>6.20</td>
</tr>
<tr>
<td>KI</td>
<td>0.13</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.25</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.025</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.025</td>
</tr>
<tr>
<td>Na₂-EDTA</td>
<td>37.30</td>
</tr>
</tbody>
</table>

The modifications in phosphate concentration were 50 and 100 µM.

Plants were harvested separately after 5 days of incubation. Samples were digested and analyzed for arsenic, phosphate and iron following the procedure described elsewhere.

2.3. Data Analysis

The experimental data were statistically analyzed for mean separation of treatments according to the least significant difference (LSD) at 5% level by IRRI-STAT 4.0 for windows (developed by the biometrics unit, IRRI, Philippines) and the Pearson correlation coefficient (r) was calculated by SPSS statistical package (version 10.1 for windows).

3. Results and Discussion

3.1. Arsenic accumulation in A. pinnata L.

The accumulation of arsenate and DMAA in Azolla pinnata L. was increased significantly (p < 0.001) with the increase of their concentrations in culture solution (Fig. 1). It was estimated that an average of 11 folds arsenate was accumulated in Azolla pinnata L. than those of DMAA, when phosphate was not added to the culture solutions. However, when 100 µM of phosphate was added to the solutions, the arsenate accumulation in the plant was about 2 folds than that of DMAA.

![Fig. 1 Arsenic uptake in Azolla pinnata L. exposed to different concentrations of arsenate (A) and DMAA (B).](image)

In a study with tumbleweed (Salsola kali), a wetland plant, De La Rosa, et al.⁹ found that the plant accumulated higher concentration of arsenic into its tissues when exposed to As(III). However, Aldrich¹⁰ found a higher concentration of arsenic in tissues of Prosopis spp., a terrestrial plant species, when the plants were grown in agar media containing As(V). In another study, Liu et al.¹² reported higher accumulation of arsenic in roots and shoots of rice (Oryza sativa L.) grown in culture solution containing As(V) compared to those plants grown in As(III), though the concentrations of As(V) and As(III) in shoots did not differ significantly (p > 0.05).

After a comprehensive greenhouse study with an aquatic floating macrophyte Lemma gibba L., Mkandawire et al.¹³ observed that the arsenic concentration was higher in tissues of plants exposed to As(V) than those plants exposed to As(III). All these studies reported on the accumulation of
inorganic arsenic in plant tissues. Little is known about the accumulation of organic arsenic species from previous studies. The present data indicate that whatever the phosphate concentrations were in the solutions, arsenate accumulation in aquatic macrophytes *Azolla pinnata* L. was significantly higher than those of DMAA. This might be because of higher bioavailability of inorganic arsenic species to the aquatic plants compared to organic arsenic species.

Fig. 1 shows that the *Azolla pinnata* L. accumulated 0.18±0.04 µmol g⁻¹ dry weight of arsenic when the plants were grown in solutions containing 4.0 µM of arsenate without phosphate. The arsenate accumulation in *Azolla pinnata* L. decreased by 42% when the phosphate concentration in culture solution was increased from 0 to 100 µM. The results imply that arsenate accumulations in *Azolla pinnata* L. decreased significantly (r = -0.750; p = 0.005) (Table 2) with the increase of phosphate concentrations in culture solution.

### Table 2 Pearson correlations coefficient (r) between arsenic and phosphate; arsenic and iron concentrations in tissues of *Azolla pinnata* L.

<table>
<thead>
<tr>
<th>Correlation objects</th>
<th>Pearson correlation coefficient (r)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As(V) &amp; P</td>
<td>-0.750**</td>
<td>0.005</td>
</tr>
<tr>
<td>DMAA &amp; P</td>
<td>0.316</td>
<td>0.317</td>
</tr>
<tr>
<td>As(V) &amp; Fe</td>
<td>0.641*</td>
<td>0.025</td>
</tr>
<tr>
<td>DMAA &amp; Fe</td>
<td>0.126</td>
<td>0.696</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level  
** Correlation is significant at the 0.01 level

On the other hand, arsenic accumulations in *Azolla pinnata* L. was found to be 0.034±0.003 µmol g⁻¹ dry weight when the plant was grown in solution containing 4.0 µM of DMAA and without phosphate. The phosphate concentration in the solution did not affect the arsenic accumulation into the tissues of the plant grown in DMAA significantly (r = 0.316; p > 0.05) (Table 2).

Mkandawire et al. [14] reported 0.02 and 106 µg g⁻¹ dry weight of arsenate accumulation in fronds of *Lemma gibba* L. (lesser duckweed) when the phosphate concentrations in culture solution were 40 and 0.0136 mg L⁻¹. In another study with *Lemma gibba* L., Mkandawire et al. [13] found 1.39 to 1.47 folds decrease in arsenate accumulation when the phosphate concentration in the culture solution was increased from 0.0136 to 40.0 mg L⁻¹. Although the pattern of arsenic accumulation in *Azolla pinnata* L. in relation to phosphate concentration in solution is comparable with that of *Lemma gibba* L., the DMAA accumulation in aquatic macrophytes in relation to the phosphate concentration in solution was not clear from previous studies. Present study reports that DMAA accumulation in aquatic macrophytes is independent of phosphate concentrations in the solutions.

### 3.2. Correlation between arsenic and phosphate uptake

Pearson correlation analysis revealed a significant negative relationship between arsenate and phosphate concentrations in tissues of *Azolla pinnata* L. On the other hand, the correlation between DMAA and phosphate concentrations in tissues of the plant was not significant (Fig. 2). De La Rosa et al. [10] reported the reduction of phosphate uptake into tumbleweed (*Salsola kali*) when the plant was exposed to arsenate. Wang et al. [15] and Patra et al. [16] also reported the inhibition of arsenate uptake by phosphate. The arsenate is a sorption analog of phosphate and competes with it for uptake carriers in the plasmalemma [13]. The inhibition of arsenate by phosphate might be because phosphate has stronger affinity to bind with uptake carrier than that of arsenate. The results of the present study are in agreement with those of previous studies. In addition, present study suggests that the phosphate might not suppress DMAA uptake in aquatic macrophytes because of dissimilar chemical behavior.

### 3.3. Correlation between arsenic and iron uptake

Fig. 3 shows the correlation between arsenic and iron concentrations in tissues of *Azolla pinnata* L. Among these two arsenic species, arsenate was found to be significantly positively correlated (r = 0.641; p < 0.05) with iron concentration while DMAA was independent thereof (r = 0.126; p > 0.05) (Table 2). Robinson et al. [13] found a positive correlation between arsenic and iron concentrations in aquatic plants and concluded that arsenic was adsorbed on iron oxides of
the plant surfaces. But the predominant species of arsenic adsorbed on iron oxides was not clear from their study. The present study demonstrates that arsenate is the predominant species in such incorporation.


4. Conclusion

Arsenate is the predominant species of arsenic in oxic aquatic system. In the present study, the *Azolla pinnata* L. was found to accumulate a significant amount of arsenic from solution having arsenate. The accumulation of arsenic in *Azolla pinnata* L. is interesting in term of mechanisms and efficiency. Arsenate uptake was inhibited by phosphate though DMAA was not. The inhibition of arsenate uptake by phosphate was due to the stronger binding affinity of phosphate to the uptake carrier of plasmalemma. Arsenate is predominantly adsorbed on iron oxides of the aquatic plant surfaces.

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References


Fig. 3 Relationship between arsenic and iron content in *Azolla pinnata* L. after being exposed to arsenate (A) and DMAA (B).