

# Influence of phosphate and iron ions in selective uptake of arsenic species by water fern (*Salvinia natans* L.)

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1 **Influence of Phosphate and Iron Ions in Selective Uptake of**  
2 **Arsenic Species by Water Fern (*Salvinia natans* L.)**

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27 **Abstract:**

28 In the present study, the effect of phosphate ion and iron hydroxides (Fe-plaques) on the  
29 selective uptake of arsenic species by water fern (*Salvinia natans* L.) was investigated. The  
30 plants were grown for 5 days in aqueous Murashige and Skoog (MS) culture media modified in  
31 arsenic and phosphate concentrations. Arsenic accumulations in *Salvinia natans* L. increased  
32 with the increase of arsenate and DMAA concentrations in the culture solutions. Compared to  
33 the control treatment, *Salvinia natans* L. accumulated significantly higher amount of arsenic  
34 from phosphate deficient solutions, when the source was arsenate. However, arsenic uptake was  
35 not affected significantly by phosphate, when the source was dimethylarsinic acid (DMAA).  
36 From solutions modified in 100  $\mu\text{M}$  of phosphate and 4.0  $\mu\text{M}$  of either arsenate or DMAA, the  
37 *Salvinia natans* L. accumulated  $0.14 \pm 0.02$  and  $0.02 \pm 0.00$   $\mu\text{mol (g dry weight)}^{-1}$  of arsenic,  
38 respectively. In contrast, plants accumulated  $0.24 \pm 0.06$  and  $0.03 \pm 0.00$   $\mu\text{mol (g dry weight)}^{-1}$  of  
39 arsenic from solutions containing 4.0  $\mu\text{M}$  of either arsenate or DMAA in the absence of  
40 phosphate, respectively. Thus, it is reasonable to state that increasing phosphate concentration in  
41 culture solutions decreases the arsenic uptake into the water fern significantly, when the source  
42 was arsenate. Moreover, arsenic and phosphate content in plant tissue correlated significantly ( $r$   
43 = -0.66;  $p < 0.05$ ), when initial source was arsenate while there were no correlation between  
44 arsenic and phosphate, when initial source was DMAA ( $r = -0.077$ ;  $p > 0.05$ ). Similarly,  
45 significant correlation was observed between arsenic and iron content in plant tissues ( $r = 0.66$ ;  
46  $p < 0.05$ ), when initial source was arsenate while the correlation was not significant ( $r = 0.23$ ;  
47  $p < 0.05$ ), when initial source was DMAA. The results indicate the adsorption of arsenate on Fe-  
48 plaques of aquatic plant surfaces. Further, the study demonstrates that the DMAA uptake  
49 mechanisms into the water fern are deferent from those of arsenate.

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53 **Keywords:** Arsenate; DMAA; Uptake; Physico-chemical Adsorption; Water Fern (*Salvinia*  
54 *natans* L.); Phosphate; Phytofiltration.

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## 58 **Introduction:**

59 Arsenic is one of the toxic environmental pollutants which have recently attracted attention  
60 because of its chronic and epidemic effects to the human health through widespread water and  
61 crop contamination. Natural release of arsenic from aquifer rocks has been reported in  
62 Bangladesh [1-4], West Bengal, India [5, 6]. Geogenic contamination of arsenic in aquifer rocks  
63 has also been reported in Thailand [7], Vietnam, inner Mongolia, Greece, Hungary, USA, Ghana,  
64 Chile, Argentina and Mexico [8, 9]. Beside the large-scale arsenic pollution in soils, water  
65 pollution by geogenic arsenic has been a great health problem in many countries [2, 4, 6].

66 Phytoremediation, a plant based green technology, becomes promising to remediate the  
67 environmental pollution due to some unavoidable limitations of traditional technologies. It is  
68 relatively inexpensive, eco-friendly and proven effective in few cases [10]. Although the arsenic  
69 uptake into the plants occurs primarily through the root system, it is not readily translocated to  
70 the shoots and the edible parts of all plants. Few terrestrial plant species, such as *Agrostis*  
71 *castellana*, *Agrostis delicatula* [11], *Bidens cynapiifolia* [12], Chinese brake fern (*Pteris vittata*  
72 L.) [13] and silver fern (*Pityrogramma calomelanos* L.) [14] accumulate high concentration of  
73 arsenic in their shoots and edible parts even though the background concentration in soil is low  
74 [13]. In particular, Chinese brake fern removes a significant amount of arsenic from soil [14, 15],  
75 and stores in the fronds [14, 16]. Arsenic accumulation in aquatic plants, such as *Spirodella*  
76 *polyrhiza* L. [5], *Lemna gibba* L. [17, 18], *Hydrilla verticillata* [19], *Lepidium sativum* [20] has  
77 also been reported in literatures.

78 Arsenate; As (V) and arsenite; As (III) are the inorganic forms in the oxic aquatic systems.  
79 Arsenate predominates and arsenite is oxidized to arsenate in the oxic aquatic systems [21]. The  
80 use of aquatic macrophytes or other floating plants in phytoremediation technology is commonly  
81 known as phytoextraction. This clean up process involves biosorption and accumulation of  
82 pollutants. Recently, aquatic macrophytes and some other small floating plants have been  
83 investigated for the remediation of wastewater contaminated with Cu, Cd(II) and Hg(II) [22, 23,

84 [24]. The encouraging results of metal uptake capacity by aquatic plants [22-28] gained the  
85 attention of researchers and scientists to use them in phytoremediation technology.

86 Water fern (*Salvinia natans* L.) is a free floating freshwater macrophyte, which grows rapidly in  
87 ponds, lakes, ditches, and wastewater bodies mostly in southern Asian countries affected by  
88 arsenic especially in Bangladesh, West Bengal, India. Previously, the *Salvinia natans* L. was  
89 tested for Hg (II) [24] and Cu (II) [28] removal. In the present study, the authors investigated the  
90 effect of phosphate concentrations on arsenate and DMAA uptake and biosorption by *Salvinia*  
91 *natans* L. from aqueous culture solution. The arsenate was selected because it is the predominant  
92 inorganic species in oxic aquatic systems [21]. An organic species (DMAA) was also selected to  
93 compare the response of the plant to both organic (DMAA) and inorganic (arsenate) species  
94 uptake and biosorption in the plant.

95

## 96 **Materials and Methods:**

### 97 **Plant Cultivation**

98 The *Salvinia natans* L. were collected from rice field of Manikgonj of Dhaka, Bangladesh and  
99 stock-cultured in a green house for two weeks. The experiment was conducted in an incubator  
100 for a 5 days period with the conditions being set as 14/10 h light/dark schedule, 100-125  $\mu\text{E m}^{-2}$   
101  $\text{s}^{-1}$  light intensity, 75% humidity, 22 and 20 ( $\pm 2$ )  $^{\circ}\text{C}$  temperatures for day and night, respectively.  
102 Plants in the incubator were grown on modified murashige and skoog (MS) culture media where  
103 modifications were in phosphorus and arsenic concentrations (Table 1). The modified culture  
104 solutions had either 50 or 100  $\mu\text{M}$  of  $\text{PO}_4^{3-}$ . Either arsenate or DMAA were added to the  
105 modified solutions at the rate of 1.0, 2.0 and 4.0  $\mu\text{M}$  prepared from  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$  and  
106  $(\text{CH}_3)_2\text{AsO}_2\text{Na} \cdot 3\text{H}_2\text{O}$ , respectively. The control solution contains neither arsenic nor  $\text{PO}_4^{3-}$ .

107

### 108 **Inoculation Procedure**

109 Before inoculation, *Salvinia natans* L. strains from stock-culture were washed three times with  
110 DI water. 200-ml polystyrene test vessels (118 X 86 X 60 mm) were used for the experiments.  
111 About 10 individual plants were inoculated in each of 200-ml test vessels containing 100 ml of  
112 test solution. The pH during the experiments was maintained at 5.5 through adjustment with the  
113 addition of either 0.1 M HCl or 0.1 M NaOH. Changes in volume of culture solutions during the  
114 experiment from evaporation and accumulation were compensated by adding DI water  
115 equivalent to the volume difference in every 2 days throughout the experiment.

116

### 117 **Sample Preparation and Chemical Analysis**

118 The plants (in whole) were harvested after 5 days of inoculation. After rinsing with DI water for  
119 four times, plants were taken on clean absorbent paper to remove water from plant surfaces. The  
120 samples were then placed into a drying oven at 65 °C until they reached a constant weight. Dried  
121 samples were weighed and 0.10-0.20-g samples were digested in 50-ml polyethylene tubes  
122 (*DigiTubes*, SCP Science, Canada). Five ml of 65% HNO<sub>3</sub> were added and the samples were  
123 kept under a fume hood for 12 hours. Then the samples were heated to 95 °C for 2 hours on a  
124 heating block (*DigiPREP*, SCP Science, Canada). After cooling to room temperature, 3 ml of  
125 30% hydrogen peroxide were added to the digests and the samples were heated again to 105 °C  
126 for 20 min and then diluted to 10 ml using DI water and stored in 15-ml polythene bottles  
127 (HDPE, NALGENE<sup>®</sup>, Nalge Nunc International, Rochester, NY).

128 The concentrations of arsenic and iron were analyzed using a graphite-furnace atomic absorption  
129 spectrometer (GF-AAS, Z-8100, Hitachi, Japan). For the determination of arsenic, 5 µL of 0.05  
130 M nickel nitrate was added to a 10-µL sample as matrix modifier in the cuvette. The accuracy of  
131 the analysis was checked by the analysis of certified standard reference material 1573a tomato  
132 leaf (NIST, USA). The arsenic concentration in certified reference material was 0.112±0.004 µg  
133 g<sup>-1</sup> while the measured arsenic concentration was 0.123±0.009 µg g<sup>-1</sup>. The concentrations

134 detected in all samples were above the instrumental limits of detection ( $\geq 0.01 \mu\text{M}$  in samples in  
135 water). Total phosphate was determined spectrophotometrically [29].

136 Chemical reagents used in this experiment were of analytical grade. All glass wares used were  
137 washed with detergent solution, 3 M HCl and finally with DI water for eight times before use. In  
138 each analytical batch at least two reagent blanks and three replicate samples were included.

139

#### 140 **Data Analysis**

141 The experimental data were statistically analyzed for mean separation of different arsenic  
142 treatments according to the least significant difference (LSD) at 5% level by IRRI-STAT 4.0 for  
143 windows (developed by the Biometrics unit, IRRI, Philippines) and the Pearson correlation  
144 coefficient ( $r$ ) was calculated by SPSS<sup>®</sup> statistical package (version 10.0 for windows).

145

#### 146 **Results and Discussions:**

##### 147 **Uptake of Arsenic Species by *Salvinia natans* L. From Culture Solution**

148 The arsenic uptake by water fern (*Salvinia natans* L.) at different phosphate concentrations are  
149 shown in Fig. 1. After 5 days of incubation, the water fern accumulated a maximum of  
150  $0.24 \pm 0.02 \mu\text{mol (g dry weight)}^{-1}$  of arsenic from phosphate deficient solution ( $P = 0 \mu\text{M}$ ) and a  
151 minimum of  $0.14 \pm 0.02 \mu\text{mol (g dry weight)}^{-1}$  from phosphate-rich solution ( $P = 100 \mu\text{M}$ ) when  
152 the MS culture solutions were modified with  $4.0 \mu\text{M}$  of arsenate. The results imply that arsenate  
153 uptake into the water fern was significantly higher in phosphate deficient solutions than the  
154 phosphate-rich solutions and the increase of phosphate concentration in culture solution  
155 decreases arsenate uptake. However, arsenic accumulation by the plants was highest ( $0.03 \pm 0.00$   
156  $\mu\text{mol g}^{-1}$  dry weight) in phosphate sufficient solution ( $P = 100 \mu\text{M}$ ) when the initial  
157 concentrations of DMAA in growth medium was  $4.0 \mu\text{M}$ . This concentration of arsenic in plant  
158 tissue did not differ significantly with the concentration ( $0.02 \pm 0.00 \mu\text{mol g}^{-1}$  dry weight), when  
159 the plants were grown in phosphate deficient growth medium ( $P = 0 \mu\text{M}$ ). This might be because

160 the DMAA uptake in the aquatic macrophyte was not affected by the initial phosphate  
161 concentrations in the solution.

162 Phosphate added to the growth medium plays two important roles: i) it enhances arsenate  
163 availability in the solution; and, ii) it competes with arsenate for uptake carriers in the  
164 plasmalemma due to the similar chemical behavior of arsenate and phosphate [30, 31]. The fact  
165 that arsenate and phosphate concentrations in tissues of *Salvinia natans* L. were significantly  
166 negatively correlated ( $r = -0.662$ ,  $p < 0.05$ ) (Table 2) suggests that the competition for uptake,  
167 indeed, occurred (Fig. 2A). Mkandawire and Dudel [18] also reported that the arsenate uptake in  
168 *Lemna gibba* L. occurs through the phosphate uptake pathway due to similar chemical behavior  
169 of arsenate and phosphate.

170 In contrast, DMAA and phosphate concentrations in tissues of *Salvinia natans* L. did not  
171 correlate significantly ( $r = -0.076$ ,  $p > 0.05$ ) (Fig. 2B). This is because DMAA does not compete  
172 with phosphate for plant uptake due to their dissimilar chemical behavior.

173

#### 174 **Effect of Arsenic Species on Phosphate Uptake by *Salvinia natans* L.**

175 Arsenate in the culture solutions significantly ( $p < 0.05$ ) reduced phosphate uptake in tissues of  
176 *Salvinia natans* L. However, the DMAA did not affect phosphate uptake into the plant  
177 significantly ( $p > 0.05$ ). The Pearson correlation analysis (Table 2) revealed a significant  
178 negative relationship between arsenate and phosphate concentrations in tissues of *Salvinia*  
179 *natans* L. (Fig. 2A). No significant correlation was observed between DMAA and phosphate  
180 concentrations in tissues of *Salvinia natans* L. (Fig. 2B). Reduction of phosphate uptake in  
181 plants exposed to arsenate has also been reported in literatures [31, 32]. This is because the  
182 arsenate uptake occurs through the phosphate uptake pathway even replacing the phosphate from  
183 sorption site [33]. The DMAA may be accumulated in *Salvinia natans* L. through different  
184 mechanisms.

185



### 186 **Arsenic Removal Efficiency of *Salvinia natans* L.**

187 After 5 days of exposure to culture solutions containing different concentrations of arsenate, the  
188 *Salvinia natans* L. removed a significant amount of arsenic (Fig. 3). Regardless of phosphate  
189 concentrations in solution, between 32-65% arsenate was removed from the solution by *Salvinia*  
190 *natans* L. within the five days for a plant dry biomass of 0.15 g. On the other hand, DMAA  
191 removal was negligible (about 0.7-3.2%). The results indicate that removal of arsenic were  
192 increased with the increase of arsenate concentrations and decreased with the increase of  
193 phosphate concentrations in the solution. Mukherjee et al. [34] reported a 74.8% removal of  
194 arsenic by the same plant within 120 hrs of exposure when the initial source of arsenic was  
195 arsenate (As(V)).

196

### 197 **Influence of Phosphate and Iron on Arsenic Uptake in *Salvinia natans* L.**

198 Fig. 4 shows the correlation between arsenic and iron concentrations in *Salvinia natans* L.  
199 Arsenate was found to be significantly positively correlated ( $r = 0.662$ ;  $p < 0.05$ ) with iron while  
200 DMAA was independent of iron concentration ( $r = 0.233$ ;  $p > 0.05$ ) (Table 2). Robinson et al.  
201 [33] also found a positive correlation between arsenic and iron in native aquatic ferns  
202 (*Asplenium bulbiferum*, *Blechnum discolor*, *Histiopteris incisa*, *Pneumatopteris penningera* and  
203 *Polystichum vestitum*) as well as watercress (*Rorippa nasturium-aquaticum*). This might be due  
204 to the physico-chemical adsorption of arsenate on iron oxides on plant surfaces. Robinson et al.  
205 [33] discussed the physico-chemical as an alternative mechanism of arsenic accumulation in  
206 aquatic plants. In this mechanism, iron oxides (iron plaques) on the plant surfaces adsorb and  
207 accumulate arsenic. Although arsenic adsorption on iron oxide plaques on the surface of aquatic  
208 plants has been reported by Robinson et al. [33], which species of arsenic predominated in such  
209 adsorption was not clear from their studies. However, Blute et al. [35] reported arsenate to be  
210 positively correlated with iron plaques on roots of *Typha latifolia* (cattail) grown in arsenic-  
211 contaminated wetland sediments. According to Blute et al. [35], the ferric plaques were

212 predominantly Fe(III) oxyhydroxide and 80% of the arsenic in it were arsenate. The present  
213 study demonstrates that arsenic adsorbed on the iron plaques of aquatic plant surfaces is mainly  
214 arsenate, as it was adsorbed on iron plaques of wetland plant *Typha latifolia* (cattail).

215 Arsenate and iron concentrations in *Salvinia natans* L. were highly positively correlated ( $p <$   
216  $0.01$ ) when the plants were grown in phosphate-deficient solution while their correlation was not  
217 significant ( $p > 0.05$ ), when the plants were grown in phosphate-sufficient solution. The result  
218 suggests that phosphate is adsorbed on iron oxides (Fe-plaques) of aquatic plant surfaces and  
219 displace arsenate from the sorption sites on iron oxides. It is well established that iron  
220 (hydr)oxides are important phosphate adsorbents in soils [36-39] oxic sediments [40]. The use of  
221 Fe oxides to adsorb phosphate on-site and reduce its concentrations in runoff and leachates is a  
222 proven approach to potentially lowering phosphate loadings of water bodies [41-43]. Numerous  
223 laboratory studies have also been directed at the sorption of phosphate on Fe oxides [44-47].  
224 Some studies have attempted to quantify differences in phosphate adsorption associated with  
225 variations in mineral properties such as surface area, morphology, and chemical composition [47,  
226 48]. Ferrihydrite is perhaps the most effective of these minerals in terms of phosphate adsorption  
227 in soils due to its small particle size, high surface area, and gel-like form. In nature, ferrihydrite  
228 is formed by the rapid oxidation of Fe(II) in Fe-rich waters [49]. Thus, the phosphate provably  
229 not only compete with arsenate for uptake carriers in plasmalemma [17] but also compete for  
230 adsorption on iron oxides of roots or plant surfaces as the phosphate and arsenate are analogous  
231 in chemical properties. The competition between arsenate and phosphate for the adsorption on  
232 iron oxides of plant surfaces results in the reduction of physico-chemical adsorption of arsenate  
233 in aquatic plants.

234

### 235 **Conclusion:**

236 Phosphate and iron are two important nutrient elements affecting the arsenic uptake in water fern  
237 *Salvinia natans* L. The *Salvinia natans* L. uptake arsenate probably through symplastic or

238 apoplastic pathway and compete with phosphate for uptake carriers in plasmalemma. But  
239 stronger binding affinity of phosphate with the uptake carriers inhibits arsenate uptake in aquatic  
240 plants. However, physicochemical adsorption would be an alternative and potential mechanism  
241 for arsenic uptake in aquatic plants. In this mechanism, arsenate is adsorbed by iron oxides on  
242 plant surfaces.

243 Although the present study reveals the physicochemical uptake of arsenate in water fern, the  
244 individual concentrations of arsenic in plant tissue and iron plaques were not measured.  
245 Therefore, it is difficult to interpret how much arsenic and iron was taken up in the plant tissues.  
246 It needs microanalysis of the tissues to make the fact clear. But as iron (hydr)oxides are  
247 important phosphate adsorbents and the phosphate has stronger binding affinity to the uptake  
248 carriers in plasmalemma, low correlation coefficient between arsenate and iron in plants of  
249 phosphate-sufficient solution suggest that most of the arsenate might be bound to the outer cell  
250 wall rather than entering into the plant tissues. Nevertheless, this does not decrease the  
251 importance of aquatic macrophytes in arsenic phytoremediation.

252

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387 **Table 1:** Modified<sup>a</sup> Murashige and Skoog (MS) culture solution used for *Salvinia natans* L.  
 388 cultivation.

| Nutrients   | Concentrations (mg l <sup>-1</sup> ) |
|---|--------------------------------------|
| KNO <sub>3</sub>                                    | 1900                                 |
| NH <sub>4</sub> NO <sub>3</sub>                     | 1650                                 |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O                | 440                                  |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O                | 370                                  |
| K <sub>2</sub> HPO <sub>4</sub>                     | Modified <sup>a</sup>                |
| FeSO <sub>4</sub> ·7H <sub>2</sub> O                | 27.80                                |
| MnSO <sub>4</sub> ·5H <sub>2</sub> O                | 22.30                                |
| ZnSO <sub>4</sub> ·7H <sub>2</sub> O                | 8.60                                 |
| H <sub>3</sub> BO <sub>3</sub>                      | 6.20                                 |
| KI  | 0.83                                 |
| Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O | 0.25                                 |
| CuSO <sub>4</sub> ·5H <sub>2</sub> O                | 0.025                                |
| CoCl <sub>2</sub> ·6H <sub>2</sub> O                | 0.025                                |
| Na <sub>2</sub> -EDTA                               | 37.30                                |

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 390 <sup>a</sup> The control culture solution did not contain phosphate. The other solutions were modified  
 391 either with 50 or 100 μM of phosphate.

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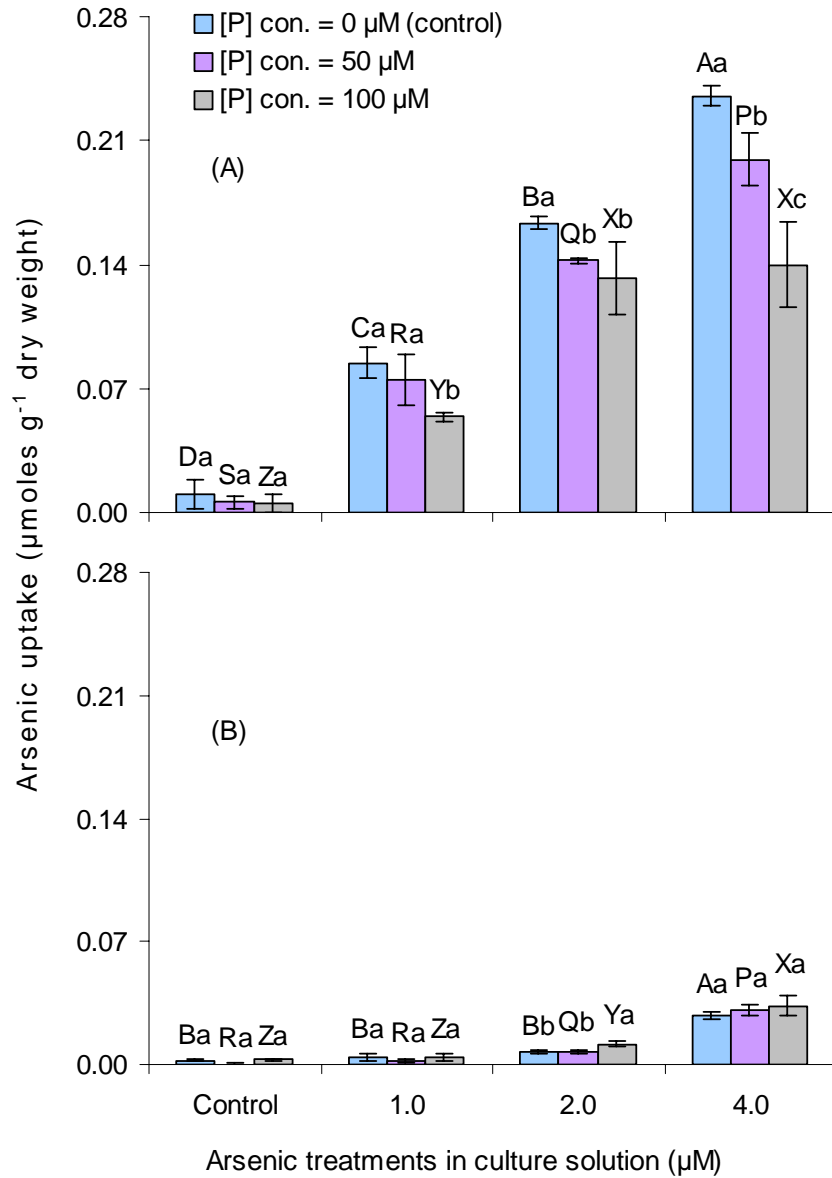
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399 **Table 2:** Pearson correlations co-efficient (r) between arsenic (arsenate and DMAA) and  
 400 phosphate; arsenic (arsenate and DMAA) and iron concentrations in *Salvinia natans* L.  
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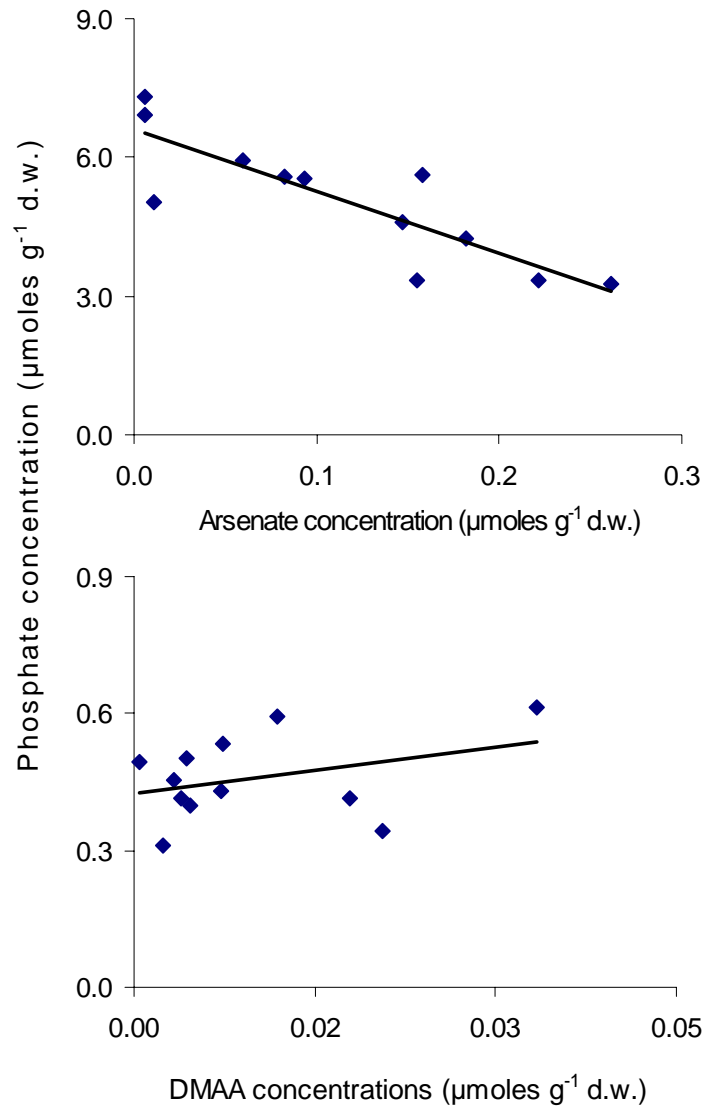
| Exposure time | Pearson Correlation (r) | Significance (p) |
|---------------|-------------------------|------------------|
| As(V) & P     | -0.662*                 | 0.019            |
| DMAA & P      | -0.076                  | 0.814            |
| As(V) & Fe    | 0.662*                  | 0.019            |
| DMAA & Fe     | 0.233                   | 0.466            |

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 403 \* Correlation is significant at the 0.05 level  
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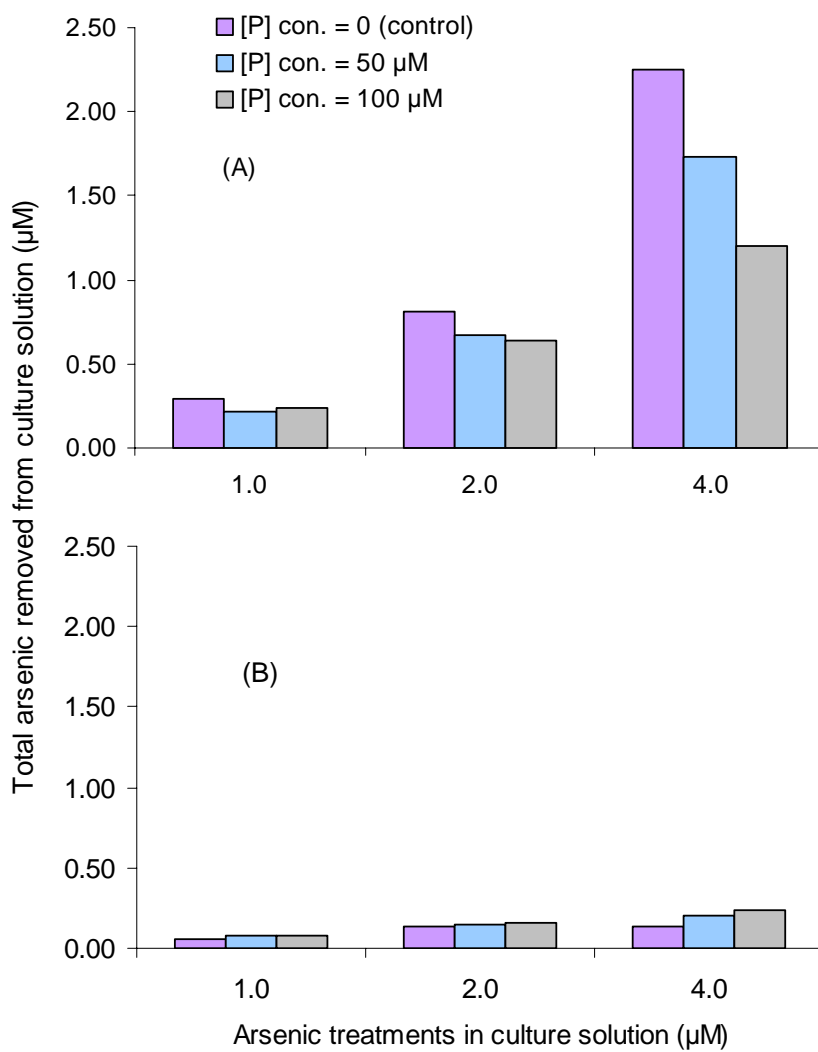
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 431 **Figure 1:** Arsenic uptake in *Salvinia natans* L. affected by the phosphate concentrations in culture  
 432 solution. Error bars represent  $\pm$ S.D. ( $n = 3$ ). Arsenate (A); DMAA (B). Different lowercase  
 433 letters indicate statistically significant differences ( $p < 0.05$ ) between phosphate treatments and different uppercase letters indicate statistically significant differences ( $p < 0.05$ ) between  
 434 different arsenic treatments.  
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**Figure 2:** Correlation between arsenic and phosphate in of *Salvinia natans* L.



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 449  
 450 **Figure 3:** Arsenic removal efficiency of *Salvinia natans* L. from culture solutions containing different  
 451 phosphate concentrations. The duration of exposure was 5 days. Arsenate (A); DMAA (B).  
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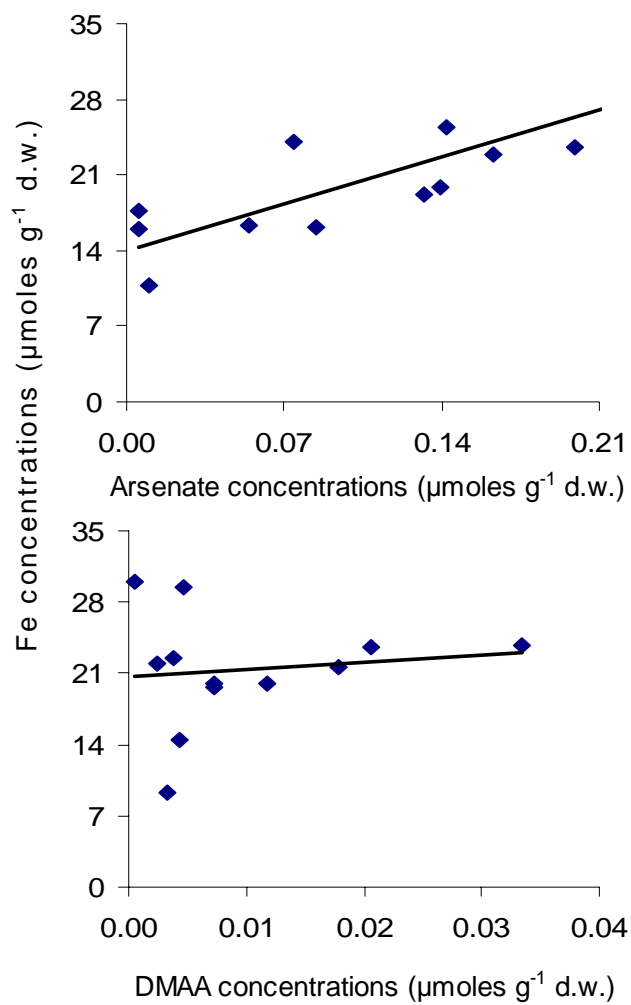
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465 **Figure 4:** Correlation between arsenic and iron in *Salvinia natans* L.

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