

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

メタデータ	言語: eng 出版者: 公開日: 2017-10-03 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	<a href="http://hdl.handle.net/2297/11729">http://hdl.handle.net/2297/11729</a>

1 **Arsenic Uptake by Aquatic Macrophyte *Spirodela polyrhiza* L.:**  
2 **Interactions with Phosphate and Iron**

3  
4  
5  
6 **M. Azizur Rahman<sup>1</sup>; H. Hasegawa<sup>\*,1</sup>; K. Ueda<sup>1</sup>; T. Maki<sup>1</sup>; M. Mahfuzur Rahman<sup>2</sup>**

7  
8  
9  
10  
11 <sup>1</sup>Graduate School of Natural Science & Technology, Kanazawa University, Kakuma,  
12 Kanazawa 920-1192, Japan; <sup>2</sup>Department of Botany, Faculty of Biological Sciences,  
13 Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

14  
15  
16  
17  
18  
19  
20 \*Corresponding author

21 E-mail: [hhiroshi@t.kanazawa-u.ac.jp](mailto:hhiroshi@t.kanazawa-u.ac.jp)

22 Tel/Fax: 81-76-234-4792

23  
24  
25

26 **Abstract**

27 The uptake of arsenate (As(V)) and dimethylarsinic acid (DMAA) by aquatic macrophyte  
28 *Spirodela polyrhiza* L. was investigated to determine the influence of arsenic interaction  
29 with  $\text{PO}_4^{3-}$  and Fe ions. Plants were grown hydroponically on standard Murashige and  
30 Skoog (MS) culture solutions. Arsenic concentrations in Fe-oxide (Fe-plaque) on plant  
31 surfaces were determined by citrate-bicarbonate-ethylenediaminetetraacetic acid (CBE)  
32 technique. *Spirodela polyrhiza* L. accumulated 51-fold arsenic from arsenate solution  
33 compared to that from DMAA solution with initial concentrations of 4.0 and 0.02  $\mu\text{M}$  of  
34 arsenic and phosphate, respectively. The arsenate uptake was negatively ( $p < 0.001$ )  
35 correlated with phosphate uptake and positively ( $p < 0.05$ ) correlated with iron uptake.  
36 About 56% of the total arsenic was accumulated into the plant tissues while 44% was  
37 adsorbed on Fe plaque (CBE-extract), when the plants were grown on arsenate solution.  
38 The DMAA uptake into the plant was neither affected by the phosphate concentrations nor  
39 correlated ( $p > 0.05$ ) with iron accumulation. The results suggest that adsorption of  
40 arsenate on Fe plaque of the surface of *Spirodela polyrhiza* L. contributes to the arsenic  
41 uptake significantly. Thus, arsenate uptake in *Spirodela polyrhiza* L. occurred through the  
42 phosphate uptake pathway and by physico-chemical adsorption on Fe-plaques of plant  
43 surfaces as well. The *Spirodela polyrhiza* L. uses different mechanisms for DMAA uptake.

44

45

46

47

48 **Keywords:** Arsenate, DMAA, Uptake, Interactions, Physico-chemical adsorption, Fe-  
49 plaque, *Spirodela polyrhiza* L.

50

51

## 52 **1. Introduction**

53 Arsenic is an important environmental and health concern due to its known chronic and  
54 epidemic toxicity. The main arsenic exposures to humans are through water pathway and  
55 food contamination, for instance in Bangladesh [1-3] and West Bengal, India [4] where  
56 most of the contaminations originate from natural release from rocks in the aquifer.  
57 Geogenic arsenic contamination from aquifer rocks has also been reported in Thailand [5],  
58 Vietnam, Inner Mongolia, Greece, Hungary, U.S.A., Ghana, Chile, Argentina and Mexico  
59 [6, 7]. Unfortunately, the traditional chemical and physical remediation techniques are  
60 limited due to the pattern of discharge. Hence, Phytoremediation, a plant-based green  
61 technology, is proposed as a viable alternative. Its relative inexpensiveness and eco-  
62 friendliness have made it an attractive method for water and soil remediation [8]. Some  
63 terrestrial plant species such as *Agrostis castellana*; *Agrostis delicatula* [9], *Bidens*  
64 *cynapiifolia* [10], Chinese brake fern (*Pteris vittata* L.) [11] and silver fern (*Pityrogramma*  
65 *calomelanos* L.) [12] have been reported to accumulate significant fractions of arsenic  
66 from soil. In particular, Chinese brake fern accumulates a formidable quantity of arsenic  
67 from soil [12, 13] and stores in the fronds [12, 14]. The arsenic hyperaccumulating  
68 terrestrial plants are considered for soil remediation. However, restoration of contaminated  
69 waters of ponds, lacks, ditches as well as irrigation water remains unresolved. Aquatic  
70 macrophytes could be a good tool for the environmentally sound and effective remediation  
71 of arsenic contaminated waters [15, 16]. Hence, we investigated the possible use of  
72 duckweed in aquatic phytoremediation.

73

74 In the present study, duckweed (*Spirodela polyrhiza* L.) was selected because of its fast  
75 growth, wide distribution, short life span and stability to the large scale environmental  
76 changes [17, 18]. The plant commonly grows in inland small water bodies such as ponds,

77 lacks, ditches in Bangladesh and West Bengal, India into which arsenic contaminated  
78 water from hand tube wells (used for household necessity) and shallow tube wells (used  
79 for irrigation) is drained. Moreover, duckweed (*Spirodela polyrhiza* L.) grows in the rice  
80 fields of south Asian countries where arsenic contaminated groundwater is the main  
81 source of irrigation during dry season. The plant is also beneficial to rice cultivation as it  
82 suppressed or reduce weed growth in the rice field.

83

84 Arsenate and arsenite are bioavailable inorganic forms of arsenic in aquatic systems [19].  
85 The dynamics of arsenate exchange between water and adsorbing colloids are analogous  
86 to those of phosphate, though the competition for exchange sites favors phosphate over  
87 arsenate [20]. Arsenate and DMAA are the major species of arsenic in oxic aquatic  
88 systems [21]. Uptake behavior of these two arsenic species could reflect the influence of  
89 inorganic and organic arsenic species and their interactions with  $\text{PO}_4^{3-}$  and Fe ions. The  
90 comparison between inorganic (arsenate) and organic (DMAA) arsenic species uptake is  
91 important because of their limit of toxicity too.

92

93 In nature, wetland plants form dense root networks in upper wetland sediments and, under  
94 flooded conditions, pump oxygen to their roots for respiration [22]. Thus, oxygenation of  
95 the rhizosphere by wetland plants leads to precipitation of iron (oxyhydro)-oxides in the  
96 rhizosphere and on the roots of plants [23]. Precipitation of iron (oxyhydro)-oxides on  
97 roots of aquatic plants has also been reported in literatures [24]. Due to the high adsorptive  
98 affinity of arsenic for iron hydroxides, Fe plaque formation on root surface of aquatic  
99 plants might be significant in the uptake of arsenic by the plants. In the present study we  
100 reported the uptake of arsenate and DMAA in duckweed (*Spirodela polyrhiza* L.) and their  
101 interactions with  $\text{PO}_4^{3-}$  and Fe ions. The contribution of Fe-plaque formation on plant's  
102 surfaces in the arsenic uptake has also been discussed.

103

## 104 **2. Materials and Methods**

### 105 **2.1. Conditions for plant cultivation**

106 The *Spirodela polyrhiza* L., collected from a rice field in Manikgonj of Dhaka,  
107 Bangladesh, was stock-cultured in green house for 2 weeks. Then, the plants were rinsed  
108 three times with deionized (DI) water and transferred to growth chamber. In the growth  
109 chamber, the experiment was conducted with the conditions being set as 14:10 h light/dark  
110 schedule, 100-125  $\mu\text{E m}^{-2} \text{s}^{-1}$  light intensity, 75% humidity, 22 °C and 20( $\pm$ 2) °C  
111 temperatures for day and night, respectively.

112

113 Modified standard Murashige and Skoog (MS) culture solution was used as growth  
114 medium in the experiment (Table 1). The control culture solution contained 0.02  $\mu\text{M PO}_4^{3-}$   
115 and other culture solutions were prepared by modifying the  $\text{PO}_4^{3-}$  concentration to 100 or  
116 500  $\mu\text{M}$ . Three test concentrations (1.0, 2.0 and 4.0  $\mu\text{M}$ ) of either arsenate or DMAA were  
117 added to the modified MS culture solutions. The pH of the solution was adjusted to 6.0.

118

119 Before inoculation, *Spirodela polyrhiza* L. from the stock-culture were rinsed for three  
120 times with deionized (DI) water. About 100 ml of culture solution was taken into 200-ml  
121 polystyrene test vessels (118 x 86 x 60 mm). About 120 individual plants were inoculated  
122 in each of the test vessels. The experiment was arranged following the randomized design  
123 (RD) with three replicates. Stock solutions of arsenate and DMAA were made by  
124 dissolving  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$  and  $(\text{CH}_3)_2\text{AsO}_2\text{Na} \cdot 3\text{H}_2\text{O}$  in DI water, respectively. Arsenic  
125 stock solutions were added to the cultures before inoculation. The plants were grown for  
126 12 days. Changes in the volume of cultures from evaporation and accumulation were  
127 compensated by adding DI water every 2 days throughout the experiment.

128

129 **2.2. Iron plaque induction**

130 A separate experiment was conducted to investigate the role of iron plaque on arsenic  
131 uptake in *Spirodela polyrhiza* L. Plants were grown in 1.5 L of DI water for 24 h before  
132 iron induction to minimize interferences from other elements with iron. They were then,  
133 transferred into 1 L of the MS solution containing 0.36 mM of iron as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and  
134 grown for 2 days. The pH of solution was adjusted to 6.0 using either 0.1 M KOH or 0.1  
135 M HCl. The specified standard concentration of phosphate for MS culture solution was not  
136 modified. After 2 days in high iron medium, plants were inoculated into MS culture  
137 solution for 12 days as described in the previous section, with 6.0  $\mu\text{M}$  of either arsenate or  
138 DMAA.

139

140 **2.3. CBE-extraction of Fe-plaques**

141 Iron plaques from plant surfaces were extracted using citrate-bicarbonate-  
142 ethylenediaminetetraacetate (CBE)-technique, a modification of dithionite-citrate-  
143 bicarbonate (DCB)-extraction method of [Taylor and Crowder \[25\]](#) and [Otte et al. \[26\]](#). The  
144 CBE solution was prepared from 0.03, 0.125 and 0.050 M of sodium citrate, sodium  
145 bicarbonate and EDTA, respectively. Plants were treated with 30 ml of CBE solution for  
146 60 min. at room temperature. The plants were then, rinsed with DI water for 3 times, and  
147 the rinsed water was added to the CBE-extracts to make a total volume of 50 ml.

148

149 **2.4. Sample preparation and chemical analysis**

150 All plants were harvested after 12 days of inoculation. After rinsing with DI water for four  
151 times, the plant samples were kept on clean absorbent paper to remove the water from the  
152 plant surfaces. The samples were dried at 65 °C until they reached a constant weight. Then,  
153 0.10-0.20 g of dried samples was taken into 50-ml polyethylene tubes (*DigiTubes*, SCP  
154 Science, Canada) for digestion. Five ml of 65%  $\text{HNO}_3$  were added to the sample and then,

155 left to incubate for 12 hours. The samples were heated on a heating block (*DigiPREP*, SCP  
156 Science, Canada) at 95 °C for 2 hours. After cooling to room temperature, 3 ml of 30%  
157 hydrogen peroxide were added and the samples were heated again at 105 °C for 20 min.  
158 Then, the digests were diluted to 10 ml with DI water and taken into 15-ml polyethylene  
159 bottles (HDPE, NALGENE<sup>®</sup>, Nalge Nunc International, Rochester, NY) in readiness for  
160 analysis.

161

162 Arsenic and iron were analyzed using graphite-furnace atomic absorption spectrometer  
163 (GF-AAS, Z-8100, Hitachi, Japan). For the determination of arsenic, 5 µL of 0.05 M  
164 nickel nitrate was added to a 10-µL sample into the cuvette as matrix modifier. Certified  
165 standard reference material 1573a (tomato leaf from NIST, USA) was used to check the  
166 accuracy of analysis. Arsenic concentration in certified reference material was  
167  $0.112 \pm 0.004 \mu\text{g g}^{-1}$  while the measured arsenic concentration was  $0.123 \pm 0.009 \mu\text{g g}^{-1}$ . The  
168 concentrations detected in all samples were above the instrumental limits of detection ( $\geq$   
169  $0.01 \mu\text{M}$  in samples in water). Total phosphate was determined spectrophotometrically  
170 [27].

171

172 All chemical reagents used in this experiment were of analytical grade. Glassware and  
173 dishes were washed with detergent solution, 3 M HCl and finally rinsed with DI water for  
174 eight times before use. In each analytical batch, at least two reagent blanks and three  
175 replicate samples were included.

176

## 177 **2.5. Data analysis**

178 Bioaccumulation of arsenic by *Spirodela polyrhiza* L. was determined on dry weight basis  
179 [18]. The experimental data were statistically analyzed for mean separation of different  
180 arsenic treatments according to the least significant difference (LSD) at 5% level by IRRI-



181 STAT 4.0 for windows (Developed by the Biometrics unit, IRRI, Philippines) and the  
182 Pearson correlation coefficient (r) was calculated by SPSS<sup>®</sup> statistical package.

183

### 184 **3. Results and Discussion**

#### 185 **3.1. Accumulation of As species in *S. polyrhiza* L.**

186 The accumulation of arsenic in *Spirodela polyrhiza* L. from arsenate treatment is presented  
187 in Fig. 1., where as the accumulation from DMAA treatment is presented in Fig. 2. The  
188 results show that *Spirodela polyrhiza* L. accumulated about 51-fold arsenic, when the  
189 plants were inoculated in arsenate solution compared to that in DMAA solution. Arsenic  
190 contents in tissues had a strong positive correlation with the initial concentrations of  
191 arsenate in culture solutions ( $r = 0.979$ ;  $p < 0.001$  at 95% confidence interval).

192

#### 193 **3.2. Influence $\text{PO}_4^{3-}$ on As uptake**

194 The accumulation of arsenic in *Spirodela polyrhiza* L. decreased significantly with the  
195 increase of the phosphate concentration in the culture solutions for all three arsenate  
196 concentrations (Fig. 1). When the concentration of  $\text{PO}_4^{3-}$  in the culture solution was  
197 increased from 0.02 to 500  $\mu\text{M}$  with a constant arsenate concentration (4.0  $\mu\text{M}$ ), arsenic  
198 accumulation into the *Spirodela polyrhiza* L. decreased by 68%. The result implies the  
199 suppression of arsenic uptake in *Spirodela polyrhiza* L. by phosphate from arsenate  
200 solution.

201

202 [Mkandawire and Dudel \[15\]](#) reported 0.26 and 1.45  $\mu\text{mol g}^{-1}$  dry weight of arsenic  
203 accumulation in fronds of *Lemna gibba* L. (lesser duckweed), when the  $\text{PO}_4^{3-}$   
204 concentrations in arsenate treated culture solution were 421 and 0.014  $\mu\text{M}$ , respectively. In  
205 another study, [Mkandawire et al. \[18\]](#) observed that arsenic accumulation decreased by  
206 28-32%, when  $\text{PO}_4^{3-}$  concentration in arsenate treated culture solution was increased from

207 0.014 to 421  $\mu\text{M}$ . The impact of increasing phosphate concentration in culture solutions  
208 was similar to that of present experiment. Thus, the magnitude of arsenic accumulation in  
209 *Spirodela polyrhiza* L. in relation to  $\text{PO}_4^{3-}$  concentrations in culture solution with arsenate  
210 is comparable with that in *Lemna gibba* L. This might be because  $\text{AsO}_4^{3-}$  is a sorption  
211 analog of  $\text{PO}_4^{3-}$  and competes with it for uptake carriers in the plasmalemma [18].  
212 [Mkandawire and Dudel \[15\]](#) proposed the arsenate uptake in *Lemna gibba* L. might occur  
213 through the phosphate uptake pathway due to similar chemical behavior of  $\text{AsO}_4^{3-}$  and  
214  $\text{PO}_4^{3-}$ . The present findings suggest the same for *Spirodela polyrhiza* L.

215

216 In contrast, arsenic accumulation was not affected with the increase of phosphate  
217 concentration in DMAA solution ([Fig. 2](#)). The results imply that the arsenate uptake into  
218 the aquatic macrophyte is related to the phosphate concentration in the culture solution,  
219 while DMAA uptake was not.

220

### 221 **3.3. Effect of As species on $\text{PO}_4^{3-}$ uptake**

222 Phosphorus uptake in *Spirodela polyrhiza* L. decreased significantly ( $p < 0.001$ ) with the  
223 increase of arsenate concentrations in culture solutions, while DMAA had no significant  
224 effect ( $p > 0.05$ ) on its uptake. Pearson correlation analysis revealed a strong negative  
225 relationship between the arsenate concentration in culture solutions and phosphate  
226 concentration in plant tissues ( $r = -0.994$ ;  $p < 0.001$  at 95% confidence interval). On the  
227 other hand, the correlation was not significant ( $r = -0.220$ ;  $p > 0.05$  at 95% confidence  
228 interval) for DMAA. [De La Rosa et al. \[28\]](#) reported the reduction of phosphate uptake  
229 into tumbleweed (*Salsola kali*), when the plant was exposed to arsenate.

230

231 [Figure 3](#) shows the relationship between arsenic and phosphate concentrations in  
232 *Spirodela polyrhiza* L. The correlation between arsenic and phosphate concentrations ( $r =$

233 -0.982;  $p < 0.001$  at 95% confidence interval) in *Spirodela polyrhiza* L. was stronger and  
234 negative, when the plants were exposed to arsenate solution (Fig. 3a). On the other hand,  
235 the correlation was very poor ( $r = -0.281$ ;  $p > 0.05$  at 95% confidence interval), when the  
236 plants were exposed to DMAA solution (Fig. 3b). The results suggest that the phosphate  
237 uptake into the aquatic macrophyte might be inhibited by arsenate while its uptake was not  
238 influenced by DMAA. The reduction of phosphate uptake might be due to the desorption  
239 of arsenate from iron plaque of plant surfaces. Barrow (29) investigated As(V) and P  
240 competitive adsorption in soil and found that, though As(V) desorbed some previously  
241 adsorbed P, a substantial portion of the bound P was not displaced by As(V).

242

#### 243 **3.4. Influence of Fe on As species uptake**

244 Iron concentrations were positively correlated with those of arsenic ( $r = 0.662$ ;  $p = 0.019$   
245 at 95% confidence interval) in *Spirodela polyrhiza* L. exposed to arsenate solution. On the  
246 other hand, iron concentrations did not correlate with those of arsenic ( $r = 0.031$ ;  $p = 0.923$   
247 at 95% confidence interval) in plants exposed to DMAA solution. Robinson et al. [30] also  
248 reported positive correlation between arsenic and iron concentrations in aquatic plants  
249 because arsenic could be adsorbed by iron oxides on plant surfaces. However, which  
250 species of arsenic predominated in such adsorption was not clear from their study. The  
251 present study suggest that inorganic arsenic species are more likely to be adsorbed on Fe  
252 plaques on *Spirodela polyrhiza* L. Blute et al. [31] reported that arsenate correlated  
253 positively with iron in plaque and negatively with iron adsorbed on the roots of *Typha*  
254 *latifolia* (cattail) growing on arsenic contaminated wetland sediments. According to Blute  
255 et al. [31], the ferric plaque was predominantly Fe(III) oxyhydroxide, and arsenate  
256 accounted for 80% of the total adsorbed arsenic. Adsorption of arsenic on ferric iron  
257 inhibited the mobility of arsenic into the roots. Another report [32] suggested the same  
258 mechanism for arsenic retention by rice root.

259

### 260 **3.5. Influence of $\text{PO}_4^{3-}$ on As adsorption on Fe plaque of plant surfaces**

261 Arsenic and iron concentrations in plants grown in solution with arsenate and lower  
262 phosphate were highly correlated ( $r = 0.994$ ;  $p < 0.001$  at 95% confidence interval) (Fig.  
263 4a). But they were not significantly correlated when the plants were grown in solution  
264 with higher phosphate ( $r = -0.220$  and  $-0.461$  for 100 and 500  $\mu\text{M}$  of  $\text{PO}_4^{3-}$  in solutions,  
265 respectively;  $p > 0.05$ ) and the same arsenic species (Fig. 4b, 4c). This might attribute to  
266 the adsorption of arsenate on iron plaques of plant surfaces in lower phosphate solution,  
267 which was desorbed by phosphate in higher phosphate solution.

268

269 The adsorption of phosphate on iron plaque has been reported by Zhang et al. [33]. They  
270 demonstrated that the amounts of phosphorus accumulated in iron plaque were correlated  
271 positively to the amount of iron plaque on roots. Therefore, iron plaque on roots might act  
272 as a phosphorus pool. Beside this, there are contradictory reports on the effects of iron  
273 plaque on phosphorus uptake by plant [26, 34, 35]. The reasons for such opposite results  
274 that iron plaque affect phosphorus uptake may be due to the different plant species and the  
275 amount of iron plaque, especially to the latter. Zhang et al. [33] reported that the  
276 phosphorus concentration in shoots of rice increased by 72% with the increase of iron  
277 plaque from 0.22 to 24.5  $\text{g}^{-\text{kg}}$  dry root weight. But higher plaque deposition (28.3  $\text{g}^{-\text{kg}}$  dry  
278 root weight) on rice root surface decreased phosphate concentration.

279

280 Though Zhang et al. [33] demonstrated the adsorption of phosphate on Fe plaques of  
281 plant's root surface the role of phosphate is not clear from their study. The present study  
282 suggests that arsenate adsorbed on iron plaques of plant surfaces might be desorbed by  
283 phosphate at higher concentration.

284

### 285 3.6. Comparison between internalized and surface adsorbed As

286 Physico-chemical adsorption, a different mechanism for arsenic accumulation into aquatic  
287 plants, has been proposed in the literature (Robinson et al. [30]). In this mechanism,  
288 suspended oxides of iron (Fe plaques) on the root and lower surface of the fronds of  
289 aquatic plants adsorb arsenic.

290

291 To understand the arsenate adsorption on iron plaques, iron plaques were induced on  
292 *Spirodela polyrhiza* L. surfaces before expose them to the arsenic species. Arsenic  
293 concentrations in plant tissues and iron plaques (CBE-extracts) were determined  
294 separately. Results showed that when *Spirodela polyrhiza* L. was exposed to 6.0  $\mu\text{M}$   
295 arsenate,  $0.86 \pm 0.06 \mu\text{mol g}^{-1}$  dry weight of arsenic was adsorbed on iron plaques of plant  
296 surfaces. On the other hand, arsenic concentration was  $1.08 \pm 0.12 \mu\text{mol g}^{-1}$  dry weight into  
297 the plant tissues (Table 2). The result shows that about 56% of the total arsenic is  
298 distributed into the plant tissues compared to 44% in Fe-plaques. However, significantly  
299 higher concentration of iron ( $547 \pm 5 \mu\text{M g}^{-1}$  dry weight) in CBE-extracts compared with  
300 plant tissues ( $69.3 \pm 1.0 \mu\text{M g}^{-1}$  dry weight) (Table 2) confirms the formation of iron  
301 plaques on plant surfaces. The current results imply that adsorption of arsenate on Fe  
302 plaque of the surface of *Spirodela polyrhiza* L. contributes to arsenate uptake significantly.

303

304 There was no significant correlation between DMAA and phosphate concentrations in  
305 *Spirodela polyrhiza* L. (Fig. 3b). Moreover, DMAA and iron concentrations in plants did  
306 not correlate significantly ( $p > 0.05$ ) in neither low nor high phosphate solutions (Fig. 4A,  
307 4B and 4C). It suggests that the accumulation of DMAA might not correlate with  
308 phosphate accumulation. Arsenic concentrations in Fe-plaques and plant tissues were low  
309 and did not differ significantly, when the plants were exposed to DMAA (Table 2). The

310 results imply that DMAA less adsorbed to Fe-plaques on the plant surface and Fe has  
311 more effect on As uptake from inorganic arsenic sources.

312

#### 313 **4. Conclusion:**

314 The results of the present study show that not only internalized, but also surface adsorbed  
315 arsenic (mostly arsenate) contributes significantly to the total amount of arsenic uptake in  
316 aquatic macrophyte *Spirodela polyrhiza* L. Thus, it could be suggest that arsenic uptake in  
317 *Spirodela polyrhiza* L. occurred through the phosphate uptake pathway as well as by  
318 physico-chemical adsorption on Fe-plaques of plant's surfaces. The arsenate uptake in the  
319 plant is related to the Fe ion and phosphate concentrations in culture medium while  
320 DMAA was not. It is well reported in many previous studies that arsenate compete with  
321 phosphate for uptake carriers in the plasmalemma, which is also consistent to the present  
322 study. But the current study reports that higher phosphate concentration in the culture  
323 medium might desorbs arsenate from iron plaques of plant surfaces.

324

#### 325 **5. Acknowledgements:**

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

329

#### 330 **6. References:**

- 331 [1] M.A. Fazal, T. Kawachi, E. Ichio, Validity of the latest research findings on causes of  
332 groundwater arsenic contamination in Bangladesh. *Water Inter.* 26 (2001) 380-389.
- 333 [2] A.H. Smith, E.O. Lingas, M. Rahman, Contamination of drinking water by arsenic in  
334 Bangladesh: a public health emergency. *Bull. World Health Organ.* 78 (2000) 1093-  
335 1103.

- 336 [3] C. Hopenhayn, Arsenic in drinking water: Impact on human health. *Elements* 2 (2006)  
337 103-107.
- 338 [4] U.K. Chowdhury, B.K. Biswas, T.R. Chowdhury, G. Samanta, B.K. Mandal, G.C.  
339 Basu, C.R. Chanda, D. Lodh, K.C. Saha, S.K. Mukherjee, S. Roy, S. Kabir, Q.  
340 Quamruzzaman, D. Chakraborti, Groundwater arsenic contamination in Bangladesh  
341 and West Bengal, India. *Environ. Health Perspect.* 108 (2000) 393-397.
- 342 [5] P. Visoottiviset, K. Francesconi, W. Sridokchan, The potential of Thai indigenous  
343 plant species for the phytoremediation of arsenic contaminated land. *Environ. Pollut.*  
344 118 (2002) 453-461.
- 345 [6] P. O'Neill, Arsenic. In: Alloway BJ, ed. *Heavy Metals in Soils*. Springer, (1995) 105–  
346 121.
- 347 [7] P.L. Smedley, D.G. Kinniburgh, A review of the source, behaviour and distribution of  
348 arsenic in natural waters. *Appl. Geochem.* 17 (2002) 517–568.
- 349 [8] I. Raskin, P.B.A. Nanda-Kumar, S. Dushenkov, D.E. Salt, B.D. Ensley, Removal of  
350 radionuclides and heavy metals from water and soil by plants. *OECD Document on*  
351 *Bioremediation*, (1994) 345-354.
- 352 [9] T. De Koe, *Agrostis castellana* and *Agrostis delicatula* on heavy metal and arsenic  
353 enriched sites in NE Portugal. *Sci. Total Environ.* 145 (1994) 103–109.
- 354 [10] J. Bech, C. Poschenrieder, M. Llugany, J. Barcelo, P. Tume, F.J. Toloias, As and  
355 heavy metal contamination of soil and vegetation around a copper mine in Northern  
356 Peru. *Sci. Total Environ.* 203 (1997) 83–91.
- 357 [11] L.Q. Ma, K.M. Komar, C. Tu, W. Zhang, Y. Cai, E.D. Kennelley, A fern that  
358 hyperaccumulates arsenic. *Nature* (2001) 409, 579.
- 359 [12] P.A. Gulz, S.K. Gupta, R. Schulin, Arsenic accumulation of common plants from  
360 contaminated soils. *Plant soil* 272 (2005) 337-347.

- 361 [13] K. Komar, L.Q. Ma, D. Rockwood, A.A. Syed, Identification of arsenic tolerant and  
362 hyperaccumulating plants from arsenic contaminated soils in Florida. *Agronomy*  
363 *Abstract* (1998) 343.
- 364 [14] C. Tu, L.Q. Ma, B. Bondada, Arsenic accumulation in the hyperaccumulator Chinese  
365 brake and its utilization potential for phytoremediation. *Environ. Qual.* 31 (2002)  
366 1671–1675.
- 367 [15] M. Mkandawire, E.G. Dudel, Accumulation of arsenic in *Lemna gibba* L. (duckweed)  
368 in tailing water of two abandoned uranium mining sites in Saxony, Germany. *Sci.*  
369 *Total Environ.* 336 (2005) 81-89.
- 370 [16] B. Robinson, C. Duwig, N. Bolan, M. Kannathasan, A. Saravanan, Uptake of arsenic  
371 by New Zealand watercress (*Lepidium sativum*). *Sci. Total Environ.* 301 (2003) 67–  
372 73.
- 373 [17] E. Landolt, R. Kandeler, The family Lemnaceae: a monographic study. Volume 2.  
374 Veroeffentlichungen des geobotanisches Institutes der ETH Zurich, Stiftung Rubel,  
375 Zurich. 95 (1987) 638.
- 376 [18] G.D. Lemon, U. Posluszny, B.C. Husband, Potential and realized rates of vegetative  
377 reproduction in *Spirodela polyrhiza*, *Lemna minor*, and *Wolffia borealis*. *Aqu. Bot.*  
378 70 (2001) 79–87.
- 379 [19] O.I. Sizova, V.V. Kochetkov, S.Z. Validov, A.M. Boronin, P.V. Kosterin, Y.V.  
380 Lyubun, Arsenic-contaminated soils: genetically modified *Pseudomonas* spp. and  
381 their arsenic-phytoremediation potential. *Soils Sed.* 2 (2002) 19 –23.
- 382 [20] M. Mkandawire, Y.V. Lyubun, P.V. Kosterin, E.G. Dudel, Toxicity of arsenic species  
383 to *Lemna gibba* L. and the influence of phosphate on arsenic bioavailability. *Environ.*  
384 *Toxicol.* 19 (2004) 26-35.



- 385 [21] H. Hasegawa, M. Matsui, S. Okamura, M. Hojo, N. Iwasaki, Y. Sohrin, Arsenic  
386 speciation including 'Hidden' arsenic in natural waters. *Appl. Organometal. Chem.*  
387 13 (1999) 113-119.
- 388 [22] W. Armstrong, Aeration in higher plants. *Adv. Bot. Res.* 7 (1979) 226-332.
- 389 [23] N.K. Blute, D.J. Brabander, H.F. Hemond, S.R. Sutton, M.G. Newville, M.L. Rivers,  
390 Arsenic sequestration by ferric iron plaque on cattail roots. *Environ. Sci. Technol.* 38  
391 (2004) 6074-6077.
- 392 [24] C.M. Hansel, S. Fendorf, S. Sutton, M. Newville, Characterization of Fe plaque and  
393 associated metals on the roots of mine-waste impacted aquatic plants. *Environ. Sci.*  
394 *Technol.* 35 (2001) 3863-3868.
- 395 [25] G.J. Taylor, A. Crowder, Use of DCB technique for extraction of hydrous iron  
396 oxides from roots of wetland plants. *American J. Bot.* 70 (1983) 1254-1257.
- 397 [26] M.L. Otte, M.J. Dekkers, J. Rozema, R.A. Broekman, Uptake of arsenic by *Aster*  
398 *tripolium* in relation to rhizosphere oxidation. *Canadian J. Bot.* 69 (1991) 2670-2677.
- 399 [27] S.C. Lenore, E.G. Arnold, D.E. Andrew, Standard methods for the examination of  
400 water and wastewater, 20<sup>th</sup> edition. APHA, AWWA and WEF. USA. (1998)
- 401 [28] G. De La Rosa, J.G. Parsons, A.M. Martinez, J.R. Peralta-vida, J.L. Gardea-  
402 Torresdey, Spectroscopic study of the impact of arsenic speciation on  
403 arsenic/phosphorus uptake and plant growth in Tumbleweed (*Salsola kali*). *Environ.*  
404 *Sci. Technol.* 40 (2006) 1991-1996.
- 405 [29] N.J. Barrow, On the displacement of adsorbed anions from soil: 2. Displacement of  
406 phosphate by arsenate, *Soil Sci.* 117 (1974) 28-33.
- 407 [30] B. Robinson, N. Kim, M. Marchetti, C. Moni, L. Schroeter, C. Dijssel, G. van den  
408 Milne, B. Clothier, Arsenic hyperaccumulation by aquatic macrophytes in the Taupo  
409 Volcanic Zone, New Zealand. *Environ. Exp. Bot.* 58 (2006) 206-215.

410 [31] N.K. Blute, D.J. Brabander, H.F. Hemond, S.R. Sutton, M.G. Newville, M.L. Rivers,  
411 Arsenic sequestration by ferric iron plaque on cattail roots. *Environ. Sci. Technol.* 38  
412 (2004) 6074-6077.

413 [32] Z. Chen, Y.G. Zhu, W.J. Liu, A.A. Meharg, Direct evidence showing the effect of  
414 root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots.  
415 *New Phytol.* 165 (2005) 91-97.

416 [33] X. Zhang, F. Zhang, D. Mao, Effect of iron plaque outside roots on nutrient uptake by  
417 rice (*Oryza sativa* L.): Phosphorus uptake. *Plant Soil* 209 (1999) 187-192.

418 [34] A.A. Crowder, L. St.-Cyr, Iron oxide plaque on wetland roots. *Trends Soil Sci.* 1  
419 (1991) 315-329.

420 [35] S. Greipsson, Effect of iron plaque on roots of rice on growth of plants in excess zinc  
421 and accumulation of phosphorus in plant in excess copper or nickel. *J. Plant Nutr.* 18  
422 (1995) 1659-1665.

423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435

436 **Table 1:** Modified<sup>a</sup> murashige & skoog (MS) nutrients for *Spirodela polyrhiza* L.  
 437 hydroponic culture medium

438

Nutrients	Concentration (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

439

440 <sup>a</sup> The control solution contained 0.02 μM PO<sub>4</sub><sup>3-</sup> and the modifications of the  
 441 solutions were 100 and 500 μM of PO<sub>4</sub><sup>3-</sup>. The pH of the solution was adjusted to  
 442 6.0.

443

444

445

446

447 **Table-2:** Arsenic and iron concentrations into the tissues of *Spirodela polyrhiza* L. and  
 448 Fe-plaques of the plant surfaces grown for 12 days in solution containing 6.0  
 449  $\mu\text{M}$  arsenic <sup>a</sup>

As treatments in solutions	$\mu\text{mol As (g dry weight)}^{-1}$		$\mu\text{mol Fe (g dry weight)}^{-1}$	
	Plant tissues	CBE-extracts	Plant tissues	CBE-extracts
Control	0.04±0.01c	0.02±0.00c	65.2±0.2a	914±3a
Arsenate	1.08±0.12a	0.86±0.06a	69.3±1.0a	547±5b
DMAA	0.05±0.02b	0.08±0.03b	50.2±0.5b	484±5c

450

451 <sup>a</sup> Different letters indicate significant differences ( $p < 0.05$ ) between treatments  
 452 according to the least significant difference (LSD).

453

454

455

456

457

458

459

460

461

462

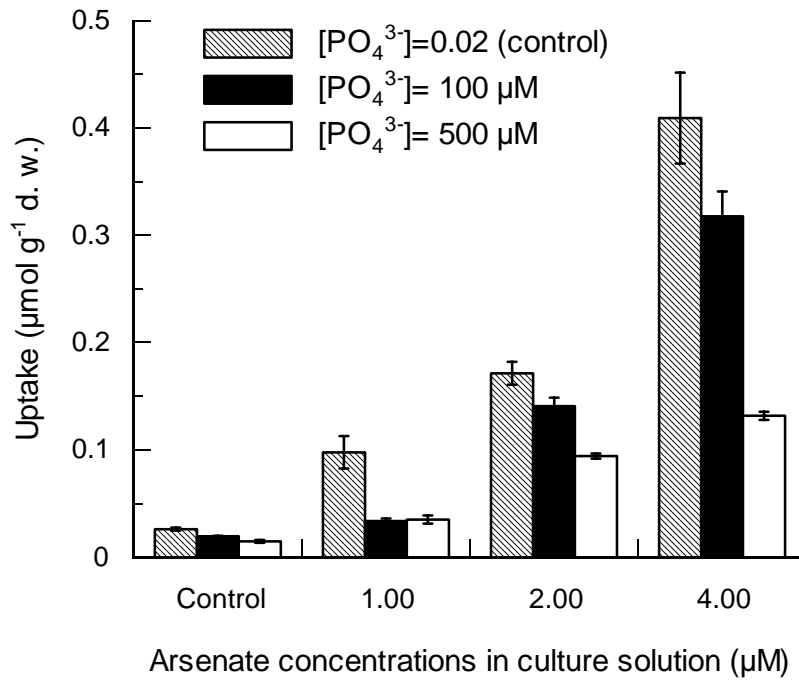
463

464

465

466

467



468

469 **Figure 1:** Arsenate uptake in *S. polyrhiza* L. affected by the PO<sub>4</sub><sup>3-</sup> concentrations in culture  
 470 solution. Each point is the average of three replicates. Error bars represent ± SD  
 471 (*n*=3).

472

473

474

475

476

477

478

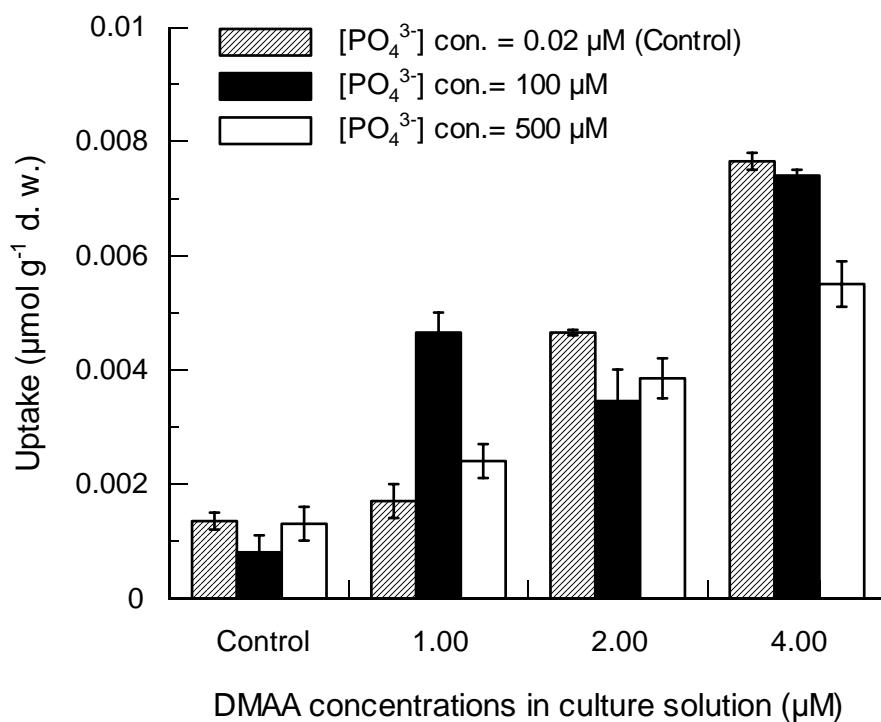
479

480

481

482

483



484

485 **Figure 2:** DMAA uptake in *S. polyrhiza* L. affected by the PO<sub>4</sub><sup>3-</sup> concentrations in culture  
 486 solution. Each point is the average of three replicates. Error bars represent ± SD  
 487 (*n*=3).

488

489

490

491

492

493

494

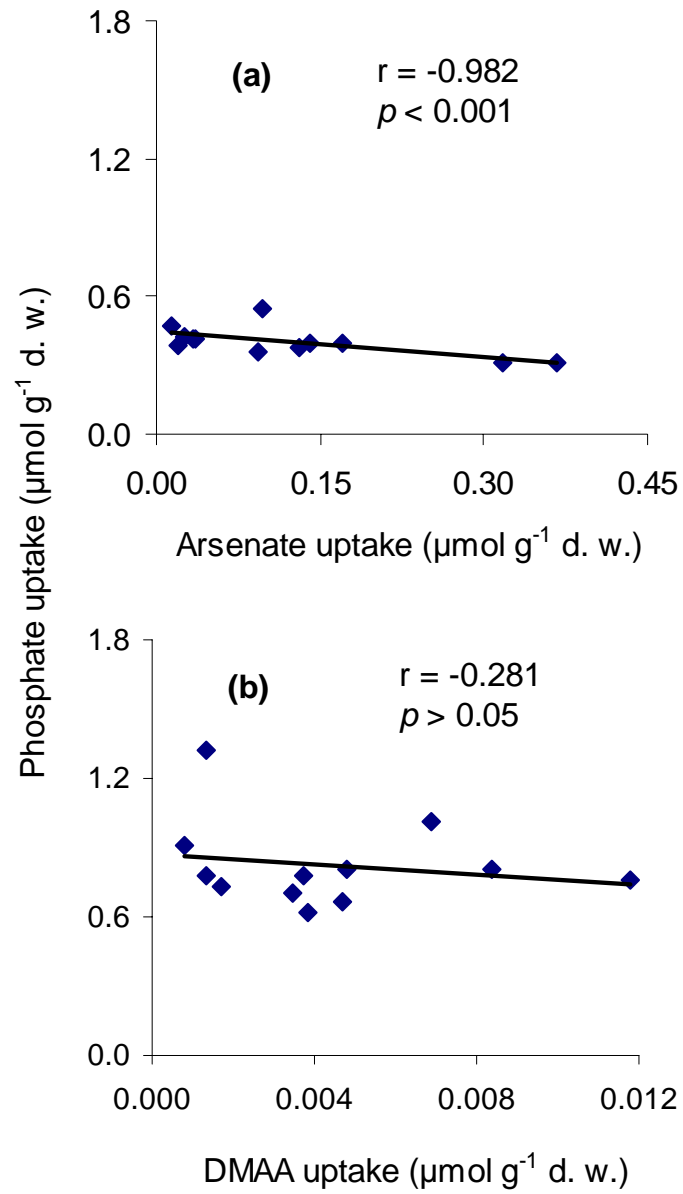
495

496

497

498

499



500

501 **Figure 3:** Relationship between arsenic and phosphate uptake in *S. polyrhiza* L. when the

502

plant was exposed to arsenate (a) and DMAA (b).

503

504

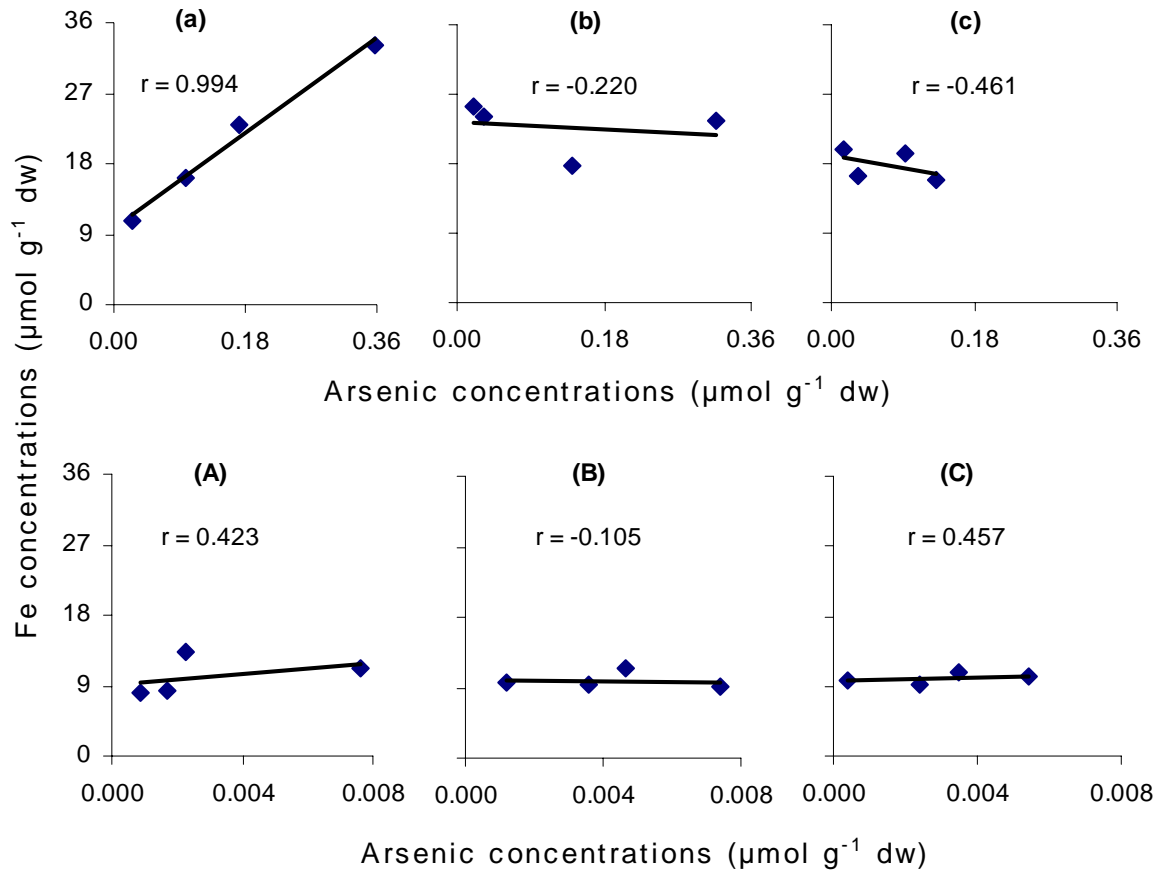
505

506

507

508

509



510

511 **Figure 4:** Correlation between arsenic and iron concentrations in *S. polyrhiza* L. when the

512 plant was exposed to arsenate (above) and DMAA (bellow).  $\text{PO}_4^{3-} = 0.02 \mu\text{M}$  (a,

513 A);  $\text{PO}_4^{3-} = 100 \mu\text{M}$  (b, B);  $\text{PO}_4^{3-} = 500 \mu\text{M}$  (c, C).