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

M. Azizur Rahman*[, 1]; H. Hasegawa*[, 1]; K. Ueda[1]; T. Maki[1]; M. Mahfuzur Rahman[2]

[1]Graduate School of Natural Science & Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan; [2]Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

*Corresponding author

E-mail: hhiroshi@t.kanazawa-u.ac.jp (H. Hasegawa)
arahman@stu.kanazawa-u.ac.jp (M. A. Rahman)
Tel/Fax: 81-76-234-4792

Abstract:

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

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

Introduction:

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

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

Arsenate; As (V) and arsenite; As (III) are the inorganic forms in the oxic aquatic systems. Arsenate predominates and arsenite is oxidized to arsenate in the oxic aquatic systems [21]. The use of aquatic macrophytes or other floating plants in phytoremediation technology is commonly known as phytoextraction. This clean up process involves biosorption and accumulation of pollutants. Recently, aquatic macrophytes and some other small floating plants have been investigated for the remediation of wastewater contaminated with Cu, Cd(II) and Hg(II) [22, 23, 24]. The encouraging results of metal uptake capacity by aquatic plants [22-28] gained the attention of researchers and scientists to use them in phytoremediation technology.

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

Materials and Methods:

Plant Cultivation

The *Salvinia natans* L. were collected from rice field of Manikgonj of Dhaka, Bangladesh and stock-cultured in a green house for two weeks. The experiment was conducted in an incubator for a 5 days period 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) ºC temperatures for day and night, respectively. Plants in the incubator were grown on modified murashige and skoog (MS) culture media where modifications were in phosphorus and arsenic concentrations (Table 1). The modified culture solutions had either 50 or 100 µM of PO_4^{3-}. Either arsenate or DMAA were added to the modified solutions at the rate of 1.0, 2.0 and 4.0 µM prepared from $Na_2HAsO_4 \cdot 7H_2O$ and $(CH_3)_2AsO_2Na \cdot 3H_2O$, respectively. The control solution contains neither arsenic nor PO_4^{3-}.

Inoculation Procedure

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

Sample Preparation and Chemical Analysis

The plants (in whole) were harvested after 5 days of inoculation. After rinsing with DI water for four times, plants were taken on clean absorbent paper to remove water from plant surfaces. The samples were then placed into a drying oven at 65 ºC until they reached a constant weight. Dried samples were weighed and 0.10-0.20-g samples were digested in 50-ml polyethylene tubes (*Digi*Tubes, SCP Science, Canada). Five ml of 65% HNO_3 were added and the samples were kept under a fume hood for 12 hours. Then the samples were heated to 95 ºC for 2 hours on a heating block (*Digi*PREP, SCP Science, Canada). After cooling to room temperature, 3 ml of 30% hydrogen peroxide were added to the digests and the samples were heated again to 105 ºC for 20 min and then diluted to 10 ml using DI water and stored in 15-ml polythene bottles (HDPE, NALGENE®, Nalge Nunc International, Rochester, NY).

The concentrations of arsenic and iron were analyzed using a 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 as matrix modifier in the cuvette. The accuracy of the analysis was checked by the analysis of certified standard reference material 1573a tomato leaf (NIST, USA). The arsenic concentration in certified reference material was 0.112±0.004 µg g^{-1} while the measured arsenic concentration was 0.123±0.009 µg g^{-1}. The concentrations detected in all samples were above the instrumental limits of detection ($\geq$ 0.01 µM in samples in water). Total phosphate was determined spectrophotometrically [29].

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

Data Analysis

The experimental data were statistically analyzed for mean separation of different arsenic 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 10.0 for windows).

Results and Discussions:

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

The arsenic uptake by water fern (*Salvinia natans* L.) at different phosphate concentrations are shown in Fig. 1. After 5 days of incubation, the water fern accumulated a maximum of 0.24±0.02 µmol (g dry weight)$^{-1}$ of arsenic from phosphate deficient solution (P = 0 µM) and a minimum of 0.14±0.02 µmol (g dry weight)$^{-1}$ from phosphate-rich solution (P = 100 µM) when the MS culture solutions were modified with 4.0 µM of arsenate. The results imply that arsenate uptake into the water fern was significantly higher in phosphate deficient solutions than the phosphate-rich solutions and the increase of phosphate concentration in culture solution decreases arsenate uptake. However, arsenic accumulation by the plants was highest (0.03±0.00 µmol g^{-1} dry weight) in phosphate sufficient solution (P = 100 µM) when the initial concentrations of DMAA in growth medium was 4.0 µM. This concentration of arsenic in plant tissue did not differ significantly with the concentration (0.02±00 µmol g^{-1} dry weight), when the plants were grown in phosphate deficient growth medium (P = 0 µM). This might be because the DMAA uptake in the aquatic macrophyte was not affected by the initial phosphate concentrations in the solution.

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

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

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

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

Arsenic Removal Efficiency of *Salvinia natans* L.

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

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

Fig. 4 shows the correlation between arsenic and iron concentrations in *Salvinia natans* L. Arsenate was found to be significantly positively correlated ($r = 0.662$; $p < 0.05$) with iron while DMAA was independent of iron concentration ($r = 0.233$; $p > 0.05$) (Table 2). Robinson et al. [33] also found a positive correlation between arsenic and iron in native aquatic ferns (*Asplenium bulbiferum*, *Blechum discolor*, *Histiopteris incisa*, *Pneumatopteris penningera* and *Polystichum vestitum*) as well as watercress (*Rorippa nasturium-aquaticum*). This might be due to the physico-chemical adsorption of arsenate on iron oxides on plant surfaces. Robinson et al. [33] discussed the physico-chemical as an alternative mechanism of arsenic accumulation in aquatic plants. In this mechanism, iron oxides (iron plaques) on the plant surfaces adsorb and accumulate arsenic. Although arsenic adsorption on iron oxide plaques on the surface of aquatic plants has been reported by Robinson et al. [33], which species of arsenic predominated in such adsorption was not clear from their studies. However, Blute et al. [35] reported arsenate to be positively correlated with iron plaques on roots of *Typha latifolia* (cattail) grown in arsenic-contaminated wetland sediments. According to Blute et al. [35], the ferric plaques were predominantly Fe(III) oxyhydroxide and 80% of the arsenic in it were arsenate. The present study demonstrates that arsenic adsorbed on the iron plaques of aquatic plant surfaces is mainly arsenate, as it was adsorbed on iron plaques of wetland plant *Typha latifolia* (cattail).

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

Conclusion:

Phosphate and iron are two important nutrient elements affecting the arsenic uptake in water fern *Salvinia natans* L. The *Salvinia natans* L. uptake arsenate probably through symplastic or apoplastic pathway and compete with phosphate for uptake carriers in plasmalemma. But stronger binding affinity of phosphate with the uptake carriers inhibits arsenate uptake in aquatic plants. However, physicochemical adsorption would be an alternative and potential mechanism for arsenic uptake in aquatic plants. In this mechanism, arsenate is adsorbed by iron oxides on plant surfaces.

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

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Table 1: Modified[a] Murashige and Skoog (MS) culture solution used for *Salvinia natans* L. cultivation.

Nutrients	Concentrations (mg l^{-1})
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4.7H_2O$	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 culture solution did not contain phosphate. The other solutions were modified either with 50 or 100 µM of phosphate.

Table 2: Pearson correlations co-efficient (r) between arsenic (arsenate and DMAA) and phosphate; arsenic (arsenate and DMAA) and iron concentrations in *Salvinia natans* L.

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

* Correlation is significant at the 0.05 level

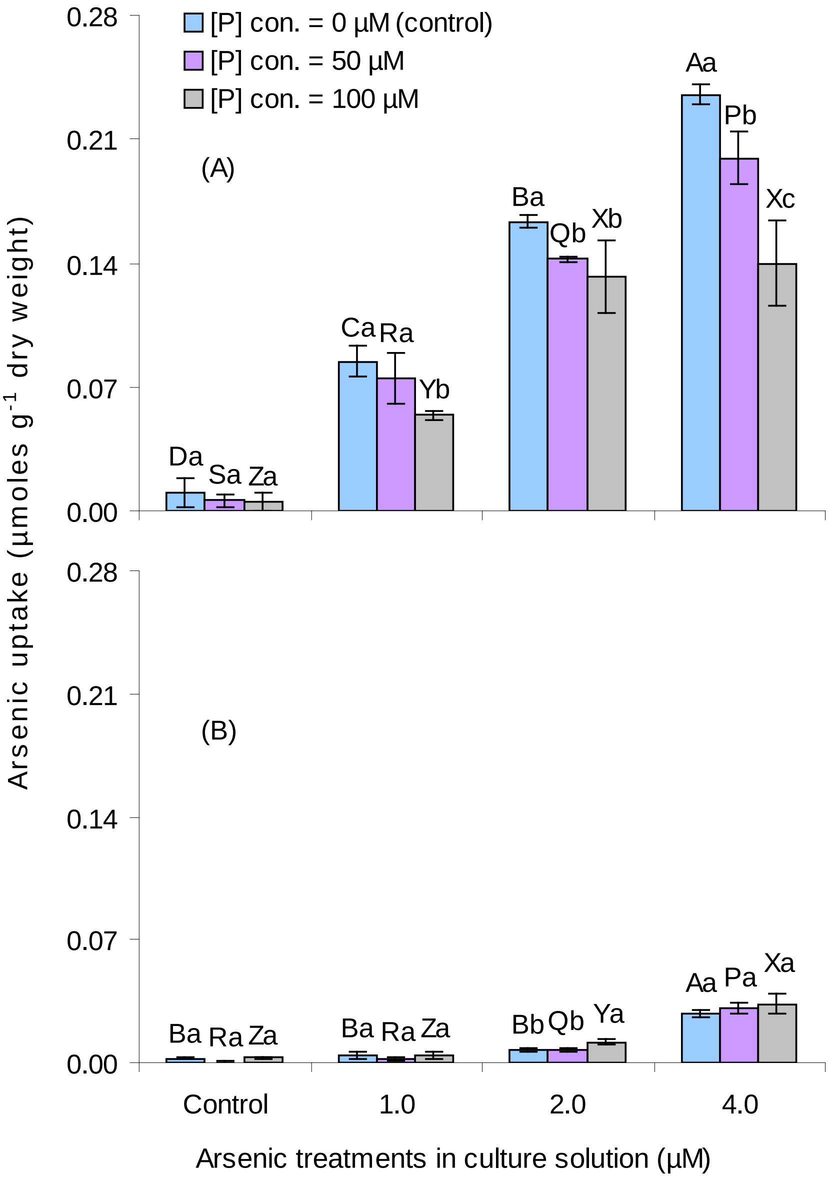

Figure 1: Arsenic uptake in *Salvinia natans* L. affected by the phosphate concentrations in culture solution. Error bars represent ±S.D. (n = 3). Arsenate (A); DMAA (B). Different lowercase letters indicate statistically significant differences ($p < 0.05$) between phosphate treatments and different uppercase letters indicate statistically significant differences ($p < 0.05$) between different arsenic treatments.

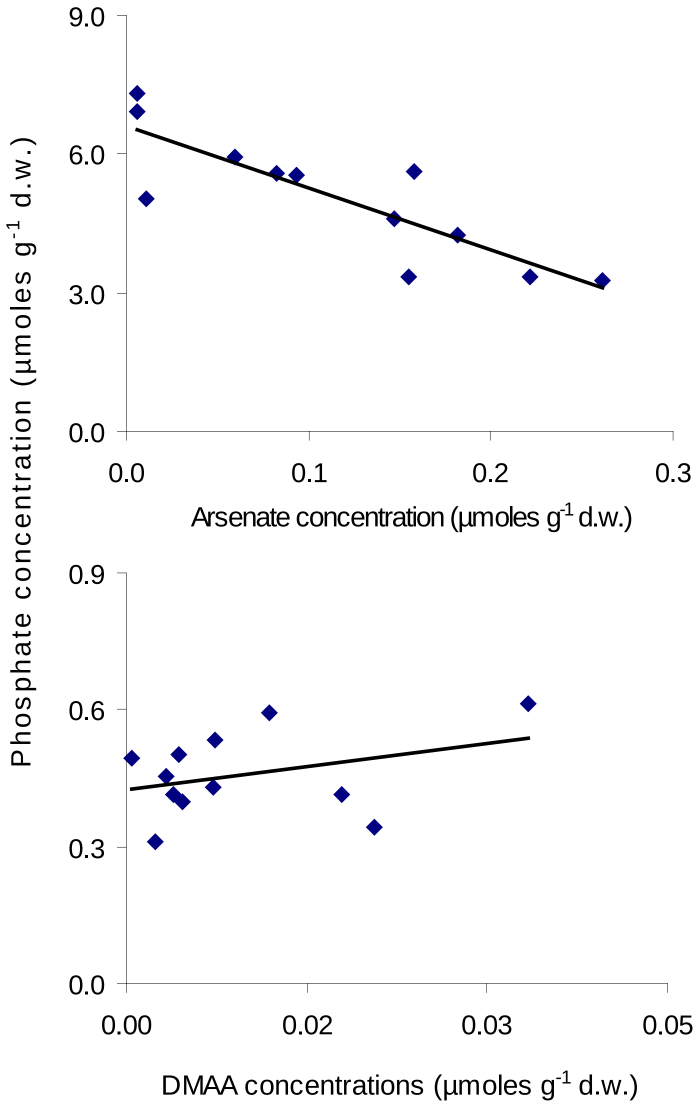

Figure 2: Correlation between arsenic and phosphate in of *Salvinia natans* L.

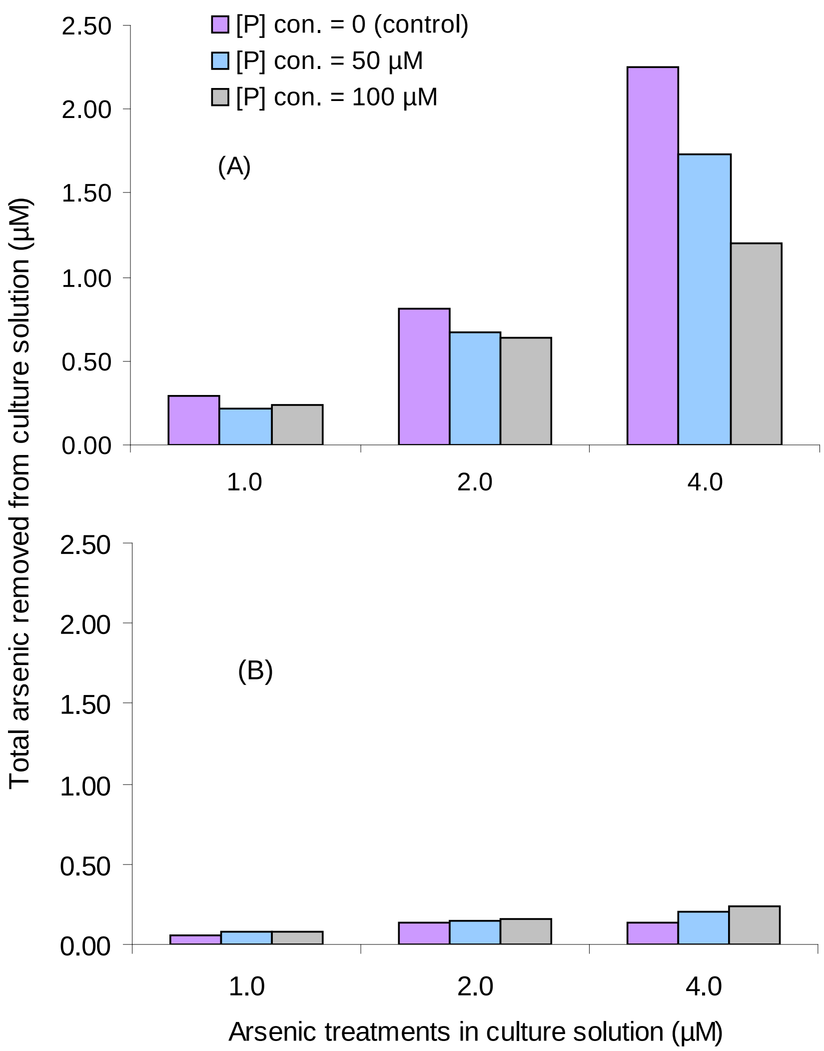

Figure 3: Arsenic removal efficiency of *Salvinia natans* L. from culture solutions containing different phosphate concentrations. The duration of exposure was 5 days. Arsenate (A); DMAA (B).

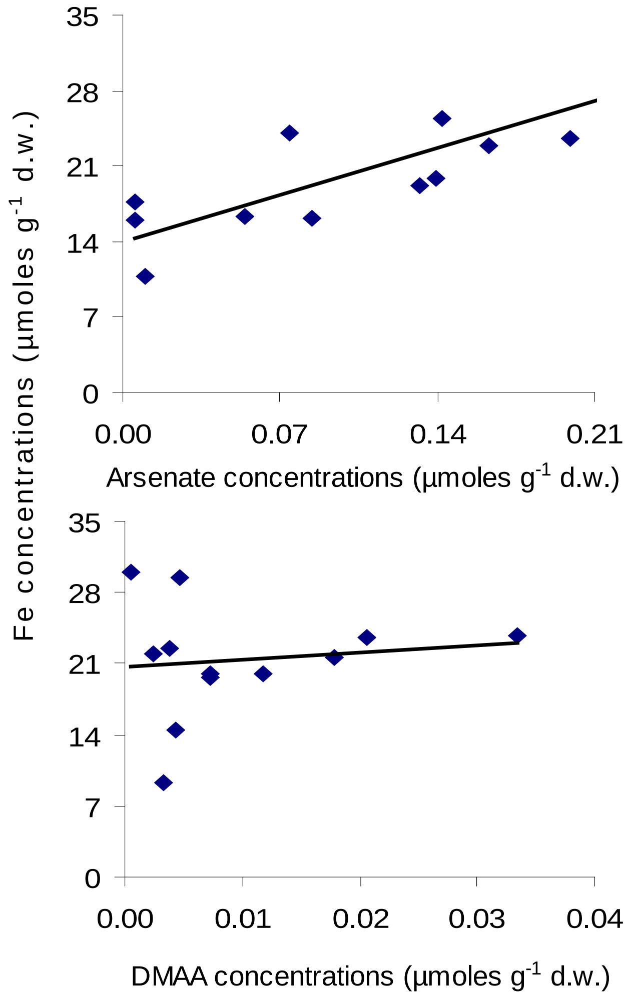

Figure 4: Correlation between arsenic and iron in *Salvinia natans* L.