Influence of EDTA and chemical species on arsenic accumulation in Spirodela polyrhiza L. (duckweed)

メタデータ	言語: English 出版者: 公開日: 2017-10-03 キーワード (Ja): キーワード (En): 作成者: Rahman, M.A., Hasegawa, Hiroshi, Ueda, K., Maki, T., Rahman, M.M. メールアドレス: 所属:
URL	http://hdl.handle.net/2297/9838

Influence of EDTA and Chemical Species on Arsenic Accumulation in *Spirodela polyrhiza* L. (Duckweed)

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

Tel/Fax: 81-76-234-4792

Abstract:

The influence of ethylenediaminetetraacetic acid (EDTA) and chemical species on arsenic accumulation in aquatic floating macrophyte *Spirodela polyrhiza* L. (Duckweed) was investigated. The uptake of inorganic arsenic species (arsenate; As(V) and arsenite; As(III)) into the plant tissue and their adsorption on iron plaque of plant surfaces were significantly ($p < 0.05$) higher then those of organic species (monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA)). The addition of EDTA to the culture media increased the uptake of As(V) and As(III) into the plant tissue though the MMAA and DMAA uptake were not affected. About 4-6% of the inorganic arsenic species were desorbed or mobilized from iron plaque by EDTA. Desorption of organic arsenic species was not affected by EDTA addition because the co-precipitation occurs only with inorganic species. Phosphate uptake was not affected by EDTA though its concentration in citrate-bicarbonate-EDTA (CBE)-extract was much higher than that of plant tissue. Iron uptake into the plant increased significantly ($p > 0.05$) by EDTA addition to the culture media while its concentration in CBE-extract decreased significantly ($p < 0.05$). The As(inorganic)/Fe ratios in plant were higher than those of CBE-extract which indicate the increased uptake of these arsenic species into the plant relative to the iron. The lower As(organic)/Fe ratios in plant and on CBE-extract suggest the reduction of accumulation of these arsenic species relative to the iron.

Keywords: Arsenic, EDTA, *Spirodela polyrhiza* L. (Duckweed), Iron plaque, CBE-extract.

Source of Funding

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

Introduction:

Arsenic is a ubiquitous and potentially toxic element in the environment. The decontamination of arsenic polluted environment becomes an important issue to reduce health risks (De La Rosa et al., 2006). Phytoremediation is a plant based green technology for removing or reducing the toxicity of contaminants which has been considered to be the environment friendly promising remediation measure for soil and water. Several terrestrial plant species such as *Agrostis castellana*; *Agrostis delicatula* (De Koe, 1994), *Bidens cynapiifolia* (Bech et al., 1997), Chinese brake fern (*Pteris vittata* L.) (Ma et al., 2001) and silver fern (*Pityrogramma calomelanos* L.) (Gulz et al., 2005) have been reported to accumulate arsenic from soil. In particular, Chinese brake ferns remove a formidable quantity of arsenic from soil (Gulz et al., 2005; Komar et al., 1998), and store it in the fronds (Gulz et al., 2005; Tu et al., 2002). However, phytoremediation of arsenic contaminated water is more difficult because of limited information about the mechanism of arsenic hyperaccumulation in aquatic plants. In this context, the identification of suitable aquatic plant species is indispensable. In addition, understanding the mechanisms of arsenic hyperaccumulation into aquatic plant is of paramount importance because it could lead to the improvement of the technology.

In general, plants store high concentrations of metal(loid)s in their roots compared to the above ground parts (Gulz et al., 2005; Meharg and Whitaker, 2002; Rahman et al., 2007). However, the hyperaccumulators are able to translocate a huge amount of metal(loid)s from

roots to the above ground parts (Ma et al., 2001; Gulz et al., 2005; Komar et al., 1998; Tu et al., 2002). The mechanisms involved in metal attenuation and cycling within plants are complex and involve both external (i.e., deposition on the root surfaces) and internal (i.e., deposition within the root cell walls and vacuoles) root exclusion (Hansel et al., 2002). Oxygenation of the rhizosphere by wetland plants leads to precipitation of iron hydroxides in the rhizosphere and on the roots of the plants (Otte et al., 1995). Iron plaque formation in the rhizosphere, however, may result iron deficiency to the plants. The synthetic chelating agents or phytochelatins solubilize the precipitated iron and make it bioavailable. For that reason, synthetic chelating agents have been very popular to the farmers in many countries.

The precipitation of iron hydroxide is also known as iron plaque to which arsenic has a high binding affinity, may be due to the co-precipitation of iron (FeIII) with arsenic (AsIII and AsV) (Gerth et al., 1993; Belzile and Tessier, 1990). In nature, plant roots or rhizospheric microbes exude phytosiderophores or siderophores to the root-plaque interface, respectively (Liu et al., 2005). These siderophores or phytosiderophores may form complex with iron and solubilize iron-bound arsenic (arsenic that co-precipitated with iron) and lender the arsenic bioavailable.

Mechanisms of arsenic exclusion and accumulation are well characterized for some terrestrial and wetland plants (Liu et al., 2005; Chen et al., 2005; Hansel et al., 2002; Otte et al., 1995). The effective accumulation of metal(loid)s by aquatic plants has been reported in literature (Rahman et al., 2007; Robinson et al., 2003; Ingole and Bhole, 2002; Alam et al., 1995; Low et al., 1994; Sen and Bhattacharyya, 1993; Selvapathy and Sreedhar, 1991). The encouraging results of previous studies draw the attention of researchers and scientists to investigation the effectiveness of those aquatic plants in phytoremediation technology (Rahman et al., 2007; Robinson et al., 2006; Mkandawire et al., 2004a,b; Mkandawire et al., 2003; Lee et al., 1991).

However, the formation of iron plaque on roots and surfaces of aquatic floating macrophytes and its influences on the accumulation of arsenic have not been widely investigated. Consequently, we studied the effect of the formation of iron plaque on roots and lower surfaces of fronds of an aquatic macrophyte namely, *Spirodela polyrhiza* L. (Duckweed) on uptake of arsenic species.

Materials and Methods:

Stock Culture

The *Spirodela polyrhiza* L. were collected from flower shop and cultured on soil dishes as stock in green house for 2 weeks. The temperature in green house fluctuated between 20 and 30 ºC and the humidity between 65 and 75%. The light schedule was roughly 14 h day /10 h night, 500 – 1100 µmol m^{-2} s^{-1}. The water for stock culture was collected from nearby river.

Conditions in Growth Chamber

The experiment was conducted in an incubator for a total of 3 weeks with the conditions 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.

Iron Plaque Induction

Iron plaque was induced on roots and lower surfaces of fronds of *Spirodela polyrhiza* L. as follows. Healthy strains of *Spirodela polyrhiza* L. were collected from the stock-culture and rinsed three times with deionized water (EPW using an E-pure system (Barnstead)). All plants were then grown on 1.5 litters of deionized water for 12 hours to minimize the interferences of other elements with iron (Liu et al., 2005). They were then transferred into 1 litter MS solution with 0.36 mM of Fe as $FeSO_4 \cdot 7H_2O$. The nutrients of MS culture solution are

presented in Table 1. The pH of the culture solution was adjusted to 6.0 using 0.1 M KOH or HCl. Iron was not added to the MS culture solution of control plants (pH 6.0). Plants were grown for 2 days in order to iron plaque induction.

Arsenic Treatments

After the induction of iron plaque, all plants were rinsed with DI water for 3 times and grown in 1/3 strength MS culture solution for 3 days before exposure to arsenic treatments. The control plants were also rinsed and grown in 1/3 strength MS culture solution separately.

About 100 ml 1/3 strength MS culture solution was taken into 200-ml polystylene test vessels (118 x 86 x 60 mm) and 6.0 μM of As(V), As(III), MMAA and DMAA were added to the solutions. The As(V), As(III), MMAA and DMAA solutions were prepared from $Na_2HAsO_4 \cdot 7H_2O$, $NaAsO_2$, $CH_3AsO_3Na_2$ and $(CH_3)_2AsO_2Na \cdot 3H_2O$, respectively. Each vessel contained only one species of arsenic. After the addition of arsenic to the culture solutions, the iron plaque induced plants were transferred into the solutions and grown for 2 weeks. The solutions were changed at every 72 hours. Importantly, arsenic was not added to the control treatments. The pH of the solutions was maintained at 6.0.

EDTA Application

After 2 weeks growth in arsenic treated solutions, arsenic was adsorbed on iron plaque or co-precipitated with iron on roots and fronds of *Spirodela polyrhiza* L. Then the plants of each vessel were divided into two equal groups. One group was transferred into 1/3 strength MS solutions containing 50 μM of EDTA and the other group was transferred into solutions having no EDTA. Plants were allowed to grow in this condition for 2 days. As the plants were grown on 1/3 strength MS solution, the standard concentration of EDTA could interfere with

the EDTA treatment. The experiment was arranged following randomized design (RD) with 3 replicates of each treatment.

EDTA is a strong and commonly used chelating agent. We supposed that the EDTA would form complex with iron and desorbs or mobilize arsenic from iron plaque and lender the arsenic bioavailable. To justify this hypothesis, we used EDTA in this experiment.

CBE-Extraction of Fe-plaques

Iron plaque from plant surfaces was extracted by CBE-extraction method, a modification of DCB-extraction procedure of Taylor and Crowder (1983) and Otte et al. (1991). CBE-solution was prepared with 0.03, 0.125 and 0.050 M of sodium citrate, sodium bicarbonate and EDTA, respectively. Plants were treated with 30 ml of CBE-solution for an hour. at room temperature. The plants were then rinsed with DI water for 3 times and the rinsed water was added to the CBE-extract to make a total volume of 50 ml.

Sample Preparation and Chemical Analysis

After CBE-extraction, plants were kept on clean absorbent paper to remove the water from plant surfaces. Then the samples were placed into an oven at 65 ºC for 24 hours until they reached a constant weight. About 0.10-0.20g of dried sample was taken into 50-ml polyethylene tubes (*Digi*Tubes, SCP Science, Canada). Five ml of 65% HNO_3 was added to the samples and kept standing overnight under a fume hood. The samples were heated on a heating block (*Digi*PREP, SCP Science, Canada) at 95 ºC for 2 hours. After cooling to room temperature, 3 ml of 30% H_2O_2 was added to the digests and the samples were heated again at 105 ºC for 20 min. Again the digests were cooled to room temperature and diluted to 10 ml using DI water and stored in 15-ml polyethylene bottles (HDPE, NALGENE®, Nalge Nunc International, Rochester, NY).

The concentrations of arsenic and iron were analyzed using graphite-furnace atomic absorption spectrometer (GF-AAS, Z-8100, Hitachi, Japan). During arsenic determination, 5 µl of 0.05 M nickel nitrate was added to a 10-µl sample in the cuvette as matrix solution. Two reagent blanks and certified standard reference materials (1573a, tomato leaf from NIST, USA) were included to verify the accuracy of the analysis. The concentration of arsenic in certified standard reference materials was reported to be 0.112±0.004 µg g^{-1} while the measured arsenic concentration was 0.114±0.002 µg g^{-1}. The concentrations detected in all samples were above the instrumental limits of detection (≥ 0.01 µM in water sample). Total phosphate was determined spectrophotometrically (Lenore et al., 1998).

All chemicals were of analytical grade. Glassware was washed with detergent solution, 3 M HCl and finally with DI water for eight times before use. At least three replicates were included in each analytical batch.

Data Analysis

Elemental concentrations in CBE-extracts and plant tissues were calculated on dry weight basis. The data were subjected to analysis of variance (ANOVA) according to the Duncan Multiple Range Test (DMRT) using SPSS statistical package (version 10.0 for windows). In addition, Pearson correlation coefficient (r) was determined using the same statistical package at 5% level.

Results and Discussions:

Effect of Arsenic Species on its Uptake by *Spirodela polyrhiza* L. (**Duckweed)**

The accumulation of inorganic arsenic species (arsenate and arsenite) in *Spirodela polyrhiza* L. (Duckweed) from solutions treated with EDTA was higher than those of without EDTA. However, the uptake of organic species (MMAA and DMAA) was not affected by EDTA (Fig.

1). The results indicate that EDTA influences only the accumulation of As(V) and As(III) in *Spirodela polyrhiza* L. (Duckweed). The accumulation of As(V), As(III), MMAA and DMAA in *Spirodela polyrhiza* L. is presented in Table 2. It is clear that the trend of arsenic accumulation was- As(V) > As(III) > MMAA > DMAA.

Arsenic uptake into the *Spirodela polyrhiza* L. did not differ significantly ($p > 0.05$) when the plants were grown in solutions treated with As(V) and As(III) (Fig. 1A). The result is not in agreement with the previous report of De La Rosa et al. (2006) and Aldrich (2004). In a study with a wetland plant tumbleweed (*Salsola kali*), De La Rosa, et al. (2006) observed higher arsenic concentration in tissues of the plant exposed to As(III) than that of As(V). Aldrich (2004) 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). The completely different results of these two studies might be because of the difference in plant species and plant habitat. The tumbleweed (*Salsola kali*) is a wetland plant and grows in wetland (anaerobic condition). On the other hand, *Prosopis spp.* is a terrestrial plant and grows in aerobic condition. Thus, they might show different uptake behavior of arsenic species in different growth conditions. In another study with rice (*Oryza sativa* L.), Liu et al. (2005) reported higher accumulation of arsenic both in roots and shoots when the plants were grown in culture solution containing As(V) compared to those plants grown in solution having As(III). However, the concentrations of arsenic in shoots of rice (*Oryza sativa* L.) did not differ significantly ($p > 0.05$) when the culture solutions contained either As(V) or As(III).

After a comprehensive greenhouse study with an aquatic floating macrophyte *Lemna gibba* L., Mkandawire et al. (2004) observed that the arsenic concentration in tissues of the plant was higher when they were exposed to As(V) than those plants exposed to As(III) though the differences were not significant ($p > 0.05$). The data of the present study on arsenic accumulation into the tissues of *Spirodela polyrhiza* L. are different of tumbleweed (*Salsola*

kali) and *Prosopis spp*. but similar to rice (*Oryza sativa* L.) and *Lemna gibba* L. Though rice (*Oryza sativa* L.) is a wetland plant the arsenic accumulation behavior in it differed from that of tumbleweed (*Salsola kali*). This might be because rice plant was grown hydroponically in the experiment. On the basis of these observations, it can be suggested that the effect of arsenic species on its uptake into the plant tissues is definitely related to the plant species and the growth conditions (habitat). It is revealed through review of literatures (Mkandawire et al., 2004; Liu et al., 2005) that the aquatic plants uptake inorganic arsenic species with no significant variations which is also supported by the results of present study. A few studies (De La Rosa, et al., 2006) though have reported that As(III) uptake is preferred to As(V) by a number of terrestrial plants.

The present study also reports that EDTA increases arsenic uptake into the tissues of aquatic plant when exposed to inorganic arsenic species [As(V) and As(III)] than those of methyl species (MMAA and DMAA). This phenomenon might be because the EDTA desorbed or mobilized the inorganic arsenic from the iron plaque of plant surfaces and lender the arsenic bioavailable. The uptake of organic arsenic species (MMAA and DMAA) into aquatic plant species has not been adequately investigated and discussed in literature. The present study demonstrates that the accumulation of MMAA and DMAA into *Spirodela polyrhiza* L. was significantly less ($p < 0.05$) than those of organic species (Fig. 1A). The EDTA had no significant influence on the accumulation of inorganic arsenic species into the plant. Concentration of inorganic arsenic species in *Spirodela polyrhiza* L. was about 3 folds compared to MMAA and 10 folds compared to DMAA. Thus it is evident from the present resutls that the aquatic macrophyte *Spirodela polyrhiza* L. uptakes inorganic arsenic species more potentially than those of organic species.

Adsorption of arsenic on Fe plaque and the Effect of EDTA on its Uptake

The concentrations of As(V) and As(III) in CBE-extract were significantly higher ($p < 0.05$) than those of MMAA and DMAA (Table 2). The concentration of As(V) and As(III) in CBE-extract decreased by the addition of EDTA to the culture solutions though MMAA and DMAA did not (Fig. 1B). It was calculated that about 4-6% of the inorganic arsenic species was desorbed or mobilized from iron plaque by EDTA. Desorption or mobilization of inorganic arsenic species from iron plaque might be due to the complexion of iron with the chelating agent which leads to the increase of arsenic availability to the plant. However, desorption of organic arsenic species by EDTA was negligible (0.5-1%). This might be because the co-precipitation of arsenic occurs only with inorganic species and for this reason, organic arsenic species can not be influenced by EDTA addition.

Though the arsenic adsorption on iron plaque of plant roots has been reported and discussed in a good number of literatures (Hansel et al., 2002; Liu et al., 2005; Blute et al., 2004; Chen et al., 2005; Otte et al., 1995), little has been known about the influence of chelating agent on arsenic desorption and uptake into plant tissue. EDTA is a strong chelating agent which has been found to enhance metal uptake from soil to plants (Chiu et al., 2005; Madrid et al., 2003; Lim et al., 2004). The chelating agents solubilize metal cations from soil particles and increase the availability of the metals to plants (Huang et al., 1997; Blaylock et al., 1997; Wu, et al., 1999). Chiu et al. (2005) reported that the chelating agents solubilize the arsenic from soil particles and increase its concentrations significantly in tissues of terrestrial plants like *Vetivera zizanioides* and *Zea mays*. The results of the present study suggest that the uptake of inorganic arsenic species into the aquatic plant *Spirodela polyrhiza* L. increased by EDTA.

Effect of Arsenic Species on Phosphate Uptake

All four arsenic species significantly ($p < 0.05$) reduced the phosphorus contents in both plant tissue and CBE-extract as compared to the control (Table 3). Phosphorus content in CBE-

extract was higher than that of plant tissue. The addition of EDTA to the culture solution did not affect significantly ($p > 0.05$) the phosphate uptake into the plants grown in As(III), MMAA and DMAA treated solutions. However, the concentration of phosphate in tissue was significantly ($p < 0.05$) higher when the plants were exposed to As(V). On the other hand, phosphate content in CBE-extract decreased significantly ($p < 0.05$) by EDTA when the plants were exposed to MMAA and DMAA.

The As/P ratios in plant tissue and CBE-extract (Fig. 2) indicate enrichment or depletion of arsenic species in relation to the phosphate. The As/P ration in plant tissue was significantly ($p < 0.05$) higher when the plants were grown in solutions treated with As(V) and As(III) than those plants treated with MMAA and DMAA (Fig. 2A). Moreover, the EDTA decreased As/P ratio in plant tissue significantly when the plants were exposed to As(V) though the ratio increased significantly when exposed to As(III). The significant decrease in As/P ration in tissues of plant exposed to As(V) solution indicates depletion of arsenic uptake relative to phosphate than that plant exposed to As(III) solution. Studies have reported on the reduction of arsenic uptake into plants exposed to As(V) because the phosphate is stronger than arsenate in surface chemistry competition (Mkandawire et al., 2003; Aracil et al., 2001; Meharg et al., 2002). Thus arsenic uptake into the tissues of *Spirodela polyrhiza* L. occurs via phosphate uptake pathway as the same mechanism has been reported for other plant species (Wang et al., 2002).

The As/P rations in CBE-extract was significantly ($p < 0.05$) higher when the plants were grown in solutions treated with As(V) and As(III) than those plants treated with MMAA and DMAA (Fig. 2A). It implies that more arsenic was adsorbed on iron plaque compared to phosphate when exposed to As(V) and As(III) than exposed to MMAA and DMAA. The As/P ratios in CBE-extract decreased by EDTA when the plants were grown in either As(V) or

As(III) treated solutions (Fig. 2B). The results also indicate the desorption of arsenic from iron plaque formed on root surfaces of plats treated with As(V) and As(III).

Effect of Arsenic Species on Iron Uptake

Iron uptake into the plant tissues increased while its concentrations in CBE-extracts decreased significantly ($p < 0.05$) by the application of EDTA to the culture solution (Table 4). Hansel et al. (2002) reported that the precipitation of iron hydroxide or formation of plaque on the plant surfaces was likely the result of radial oxygen diffusion and subsequent oxidation of ferrous iron. Iron precipitation or plaque formation in the medium may result iron deficiency to the plant. Moreover, the high sorptive affinity of arsenic to iron plaque results attenuation of arsenic into the plant (Belzile and Tessier, 1990; Gerth et al., 1993). In nature, plant roots or rhizospheric microbes exude phytosiderophores or siderophores, respectively to the root-plaque interface (Liu et al., 2005). These siderophores or phytosiderophores complex with iron and make iron and arsenic available to the plants. The present study showed that the EDTA application increased the arsenic and iron uptake by *Spirodela polyrhiza* L. It might be because the EDTA complex with iron of plant surfaces increased iron availability to the plant as siderophores or phytosiderophores do.

The As/Fe ratios in plant tissue (Fig. 3A) and CBE-extract (Fig. 3B) give the indications of enhancement or reduction of arsenic concentrations relative to iron. The As/Fe ratios both in plant tissue and CBE-extract were significantly ($p < 0.05$) higher when the plants were grown in solutions treated with As(V) and As(III) than those treated with MMAA and DMAA. The results imply that the adsorption of As(V) and As(III) on iron plaque of plant surfaces was much higher than those of MMAA and DMAA. The significantly higher As/Fe ratio in plant tissue due to the application of EDTA in the culture solutions also suggests that the chelating agent enhanced arsenic uptake into the plant compared to iron. The poor As/Fe rations in

plant tissue and CBE-extract imply poor uptake of arsenic into plant tissue and adsorption on iron plaque when the *Spirodela polyrhiza* L. were grown in solutions treated with MMAA and DMAA.

Acknowledgements:

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

References:

Alam, B., Chatterjee, A.K., Duttagupta, S., 1995. Bioaccumulation of Cd (II) by water lettuce. Pollut. Res. 14, 59-64.

Aldrich, M.V., 2004. Toxicity and accumulation of chromium, lead, copper, and arsenic in mesquite (*Prosopis* spp.). Ph. D. dissertation, University of Texas at El Paso, Texas.

Aracil, P., Burlo, F., Lario, Y., Martinez-Romerio, D., Valero, D., Carbonell-Barrachina, A.A., 2001. Total arsenic accumulation in edible pods and seeds of *Phaseolus valgaris*. J. Environ. Sci. Health. 36, 849-861.

Bech, J., Poschenrieder, C., Llugany, M., Barcelo, J., Tume, P., Toloias, F.J., 1997. As and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. Sci. Tot. Environ. 203, 83-91.

Belzile, N., Tessier, A., 1990. Interactions between arsenic and iron oxyhyroxides in lacustrine sediments. Geochim. Cosmochim. Acta. 54, 103-109.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced Accumulation of Pb in Indian Mustard by Soil-Applied Chelating Agents. Environ. Sci. Technol. 31(3), 860-865.

Blute, N.K., Brabander, D.J., Hemond, H.F., Sutton, S.R., Newville, M.G., Rivers, M.L., 2004. Arsenic Sequestration by Ferric Iron Plaque on Cattail Roots. Environ. Sci. Technol. 38 (22), 6074 -6077.

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

Chiu, K.K., Ye, Z.H., Wong, M.H., 2005. Enhanced uptake of As, Zn and Cu by *Vetiveria zizanioides* and *Zea mays* using chelating agents. Chemosphere. 60, 1365-1375.

De La Rosa, G., Parsons, J.G., Martinez-Martinez, A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2006. Spectroscopic study of the impact of arsenic speciation on arsenic/phosphate uptake and plant growth in tumbleweed (*Salsola kali*). Environ. Sci. Technol. 40, 1991-1996.

De Koe, T., 1994. *Agrostic castellana* and *Agrostis delicatula* on heavy metal and arsenic enriched sites in NE Portugal. Sci. Total. Environ.145, 103-109.

Gerth, J., Brümmer, G.W., Tiller, K.G., 1993. Retention of Ni, Zn and Cd by Si-associated goethite. Z. Pflanzenernähr Bodenkd. 156, 123-129.

Gulz, P.A., Gupta, S.K., Schulin, R., 2005. Arsenic accumulation of common plants from contaminated soils. Plant soil. 272, 337-347.

Hansel, C.M., La Force, M.J., Fendorf, S., Sutton, S., 2002. Spatial and temporal association of As and Fe species on aquatic plat roots. Environ. Sci. Technol. 36, 1988-1994.

Huang, J.W., Chen, J., Berti, W.R., Cunningham, S.D., 1997. Phytoremediation of Lead-Contaminated Soils: Role of Synthetic Chelates in Lead Phytoextraction. Environ. Sci. Technol. 31, 800-805.

Ingole, N.W., Ting, J.P., 2002. Study on nutrient removal potential of selected aquatic macrophytes. J. Inst. Eng. (India) Environ. Eng. Div. 83, 1-6.

Komar, K., Ma, L.Q., Rockwood, D., Syed, A.A., 1998. Identification of arsenic tolerant and hyperaccumulating plants from arsenic contaminated soils in Florida. Agron. Abstr. p 343.

Lee, C.K., Low, K.S., Hew, N.S., 1991. Accumulation of arsenic by aquatic plants. Sci. Total Environ., 103, 215-227.

Lenore, S.C., Arnold, E.G., Andrew, D.E., (Eds.). Standard methods for the examination of water and wastewater, 20th ed. 1998; APHA, AWWA and WEF.

Lim, J.-M., Salido, A.L., Butcher, D.J., 2004. phytoremediation of lead using Indian mustard (*Brassica juncea*) with EDTA and electrodics. Microchem. J. 76, 3-9.

Liu, W.-J., Zhu, Y.-G., Smith, F.A., 2005. Effects of iron and manganese plaques on arsenic uptake by rice seedlings (*Oryza sativa* L.) grown in solution culture supplied with arsenate and arsenite. Plant Soil. 277, 127-138.

Low, K.S., Lee, C.K., Tai, C.H., 1994. Biosorption of copper by water hyacinth roots. J Environ. Sci. Health. A. 29(1), 171-188.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D., 2001. A fern that hyperaccumulates arsenic. Nature. 409, 579.

Madrid, F., Liphadzi, M.S., Kirkham, M.B., 2003. Heavy metal displacement in chelate-irrigated soil during phytoremediation. J. Hydrol. 272, 107-119.

Meharg, A.M., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Physiol, 154, 29-43.

Mkandawire, M., Taubert, B., Dudel, E.G., 2003. Effects of phosphate and ammonium amendments on arsenic and uranium immobilization by *Lemna* sp. from tailing waters, presented at the 2nd European Bioremediation Conference, June-July, Chania, Greece.

Mkandawire, M., Lyubun, Y.V., Kosterin, P.V., Dudel, E.G., 2004a. Toxicity of arsenic species to *Lemna gibba* L. and the influence of phosphate on arsenic bioavailability. Environ. Toxicol. 19, 26-35.

Mkandawire, M., Taubert, B., Dudel, E.G., 2004b. Capacity of Lemna gibba L. (duckweed) for uranium and arsenic phytoremediation in mine tailing waters. Int. J. Phytoremediation. 6(4), 347-362.

Otte, M.L., Dekkers, M.J., Rozema, J., Broekman, R.A., 1991. Uptake of arsenic by *Aster tripolium* in relation to rhizosphere oxidation. Canadian J. Bot**.** 69, 2670-2677.

Otte, M.L., Kearns, C.C., Doyle, M.O., 1995. Accumulation of arsenic and zinc in the rhizosphere of wetland plants. Bull. Environ. Contam. Toxicol. 55, 154-161.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.)- A good option for phytoremediation. Chemosphere. In press, doi:10.1016/j.chemosphere.2007.04.019.

Rahman, M.A., Hasegawa, H., Rahman, M.M., Rahman, M.A., Miah, M.A.M., 2007. Accumulation of arsenic in tissues of rice plant (*Oryza sativa* L.) and its distribution in fractions of rice grain. Chemosphere. In press, doi:10.1016/j.chemosphere.2007.05.044.

Robinson, B., Duwig, C., Bolan, N., Kannathasan M., Saravanan A., 2003. Uptake of arsenic by New Zealand watercress (*Lepidium sativum*). Sci. Total Environ. 301, 67-73.

Robinson, B., Kim, N., Marchetti, M., Moni, C., Schroeter, L., Dijssel, C., Milne, G., Clothier, B., 2006. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo volcanic zone, New Zeeland. Environ. Exp. Bot., 58, 206-215.

Selvapathy, P., Sreedhar, P., 1991. Heavy metals removal by water hyacinth. J. Indian. Pub. Health Eng. 3, 11-17.

Sen, A.K., Bhattacharyya, M., 1993. Studies on uptake and toxic effects of lead on *Salvinia natans*. Indian J. Environ. Health. 35, 308-320.

Taylor, G.J., Crowder, A.A., 1983. Use of DCB technique for extraction of hydrous iron oxides from roots of wetland plants. American J. Bot**.** 70, 1254-1257.

Tu, C., Ma, L.Q., Bondada, B., 2002. Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediation. Environ. Qual. 31, 1671-1675.

Wang, J., Zhao, F.J., Meharg, A.A., Raab, A., Feldman, J., McGrath, S.P., 2002. Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiol. 130, 1552-1561.

Wu, J., Hsu, F.C., Cunningham, S.D., 1999. Chelate-Assisted Pb Phytoextraction: Pb Availability, Uptake, and Translocation Constraints. Environ. Sci. Technol. 33(11), 1898-1904.

Table 1: Nutrients of Murashige & Skoog (MS) culture medium used for the hydroponic growth of *Spirodela polyrhiza* L.

Nutrients	Concentration (mg l^{-1})
KNO_3	1900.00
NH_4NO_3	1650.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
K_2HPO_4	170.00
$FeSO_4.7H_2O$	27.80
$MnSO_4.5H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
H_3BO_3	6.20
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Na_2-EDTA	37.30

Table-2: Arsenic concentrations in citrate-bicarbonate-EDTA (CBE) extracts and tissues of duckweed (*Spirodela polyrhiza* L.) exposed to nutrient solutions containing different arsenic species at 6.0 µM for 2 weeks

As treatments in culture solutions	µM As (g dry weight)$^{-1}$					
	Plant tissue		CBE-extract		Total	
	+ [a] EDTA	- [b] EDTA	+ EDTA	- EDTA	+ EDTA	- EDTA
Control [c]	0.04±0.01d		0.02±0.00c		0.06±0.02d	
As (V)	1.39±0.13a	1.31±0.12a	0.40±0.07a	0.49±0.062a	1.79±0.12a	1.80±0.26a
As (III)	1.34±0.12a	1.24 ±0.11a	0.44±0.05a	0.55±0.08a	1.78±0.24a	1.79±0.18a
MMAA	0.40±0.11b	0.43±0.02b	0.10±0.01b	0.11±0.01b	0.50±0.12b	0.54±0.03b
DMAA	0.12±0.08c	0.14±0.02c	0.04±0.02c	0.10±0.03b	0.16±0.06c	0.24±0.04c

In a column, values having different letters indicate significant differences ($p < 0.05$) between them. Data are mean ± SD ($n = 3$).

[a] EDTA was applied to the culture solution.

[b] EDTA was not applied to the culture solution.

[c] Control treatments were not subjected to the application of EDTA and arsenic.

Table-3: Phosphate concentrations in citrate-bicarbonate-EDTA (CBE) extracts and tissues of duckweed (*Spirodela polyrhiza* L.) exposed to nutrient solutions containing different arsenic species at 6.0 μM for 2 weeks

As treatments in culture solutions	μM P (g dry weight)$^{-1}$			
	Plant tissues		CBE-extracts	
	+ [a] EDTA	- [b] EDTA	+ EDTA	- EDTA
Control [c]	0.096±0.024a		175.9±23.2a	
As (V)	0.066±0.014b	0.061±0.017b	74.3±12.3c	80.0±9.2e
As (III)	0.046±0.018c	0.047±0.012c	76.3±11.3c	84.6±14.3d
MMAA	0.049±0.006c	0.048±0.014c	94.1±18.5b	138.3±22.2b
DMAA	0.049±0.009c	0.060±0.007b	66.5±11.4c	93.9±16.1c

In a column, values having different letters indicate significant differences ($p < 0.05$) between them. Data are mean ± SD ($n = 3$).

[a] EDTA was applied to the culture solution.

[b] EDTA was not applied to the culture solution.

[c] Control treatments were not subjected to the application of EDTA and arsenic.

Table-4: Iron concentrations in citrate-bicarbonate-EDTA (CBE) extracts and tissues of duckweed (*Spirodela polyrhiza* L.) exposed to nutrient solutions containing different arsenic species at 6.0 µM for 2 weeks

As treatments in culture solutions	µM Fe (g dry weight)$^{-1}$			
	Plant tissues		CBE-extracts	
	+ [a] EDTA	- [b] EDTA	+ EDTA	- EDTA
Control [c]	65.2±0.2b		914±3a	
As (V)	82.6±1.0a	64.3±1.1b	472±4c	543±4b
As (III)	76.3±1.0a	63.7±0.6b	404±5d	547±2b
MMAA	58.8±0.7b	48.4±0.6c	429±3d	561±10b
DMAA	78.1±1.3a	50.2±0.5c	484±4c	345±5e

In a column, values having different letters indicate significant differences ($p < 0.05$) between them. Data are mean ± SD ($n = 3$).

[a] EDTA was applied to the culture solution.

[b] EDTA was not applied to the culture solution.

[c] Control treatments were not subjected to the application of EDTA and arsenic.

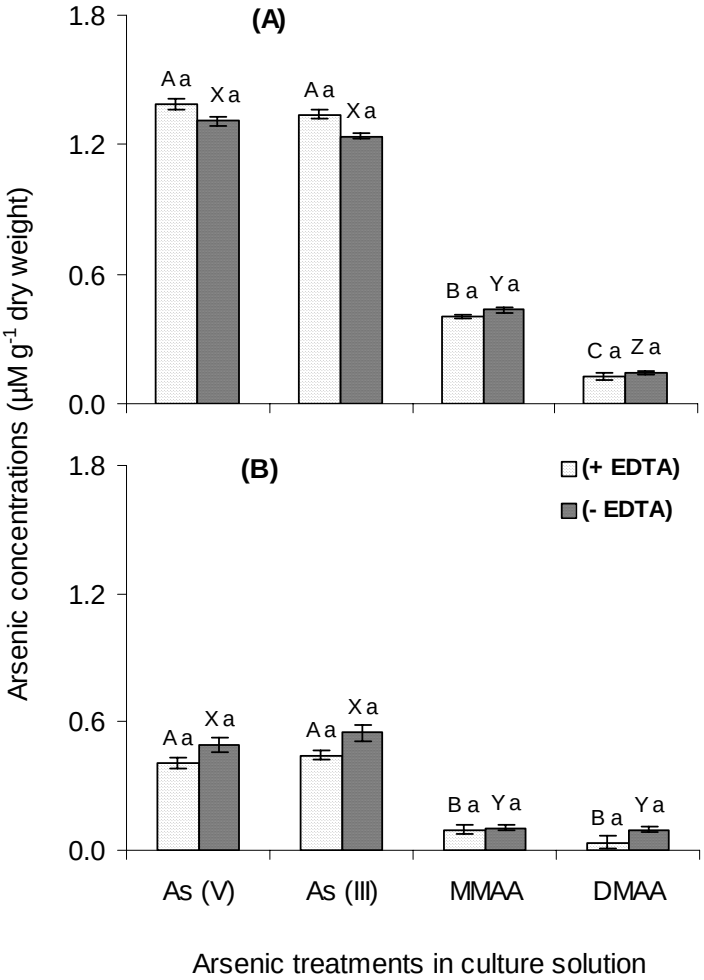

Figure 1: Arsenic uptake in tissue (A) and CBE-extract (B) of *Spirodela polyrhiza* L. Different capital letters (A, B, C are for EDTA treated samples (+EDTA) and X, Y, Z are for samples without EDTA treatment (-EDTA)) indicate significant differences among the arsenic species and small letters indicate significant differences between EDTA treatments (with or without EDTA application), at $p < 0.05$. Error bars express mean ± SD ($n = 3$).

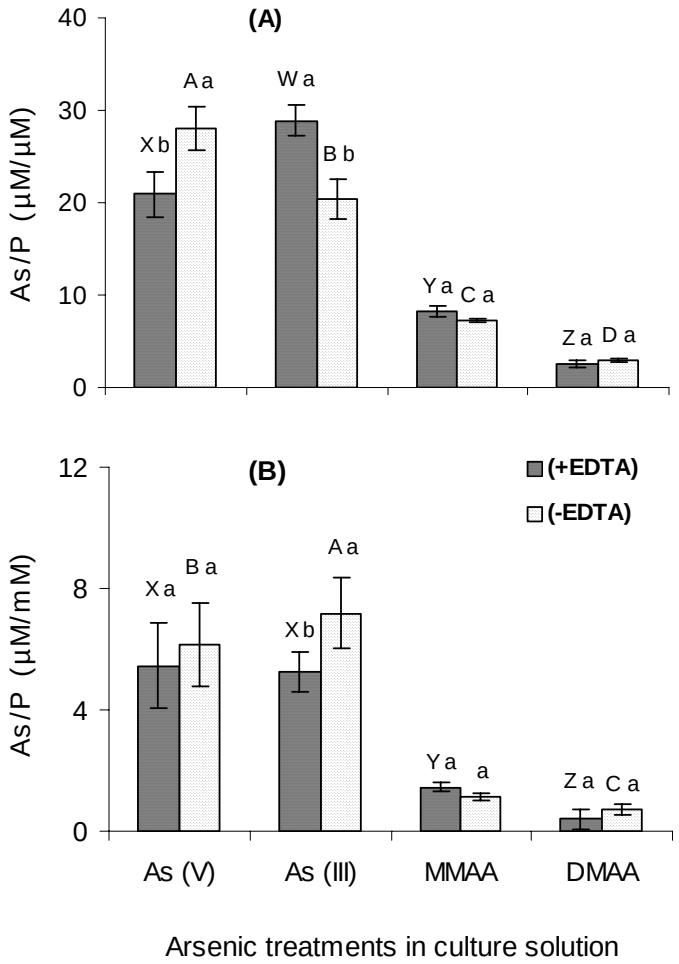

Figure 2: Mean As/P ratio in tissue (A) and CBE-extract (B) of *Spirodela polyrhiza* L. Different capital letters (A, B, C are for EDTA treated samples (+EDTA) and X, Y, Z are for samples without EDTA treatment (-EDTA)) indicate significant differences among the arsenic species and small letters indicate significant differences between EDTA treatments (with or without EDTA application), at $p < 0.05$. Error bars express mean ± SD ($n = 3$).

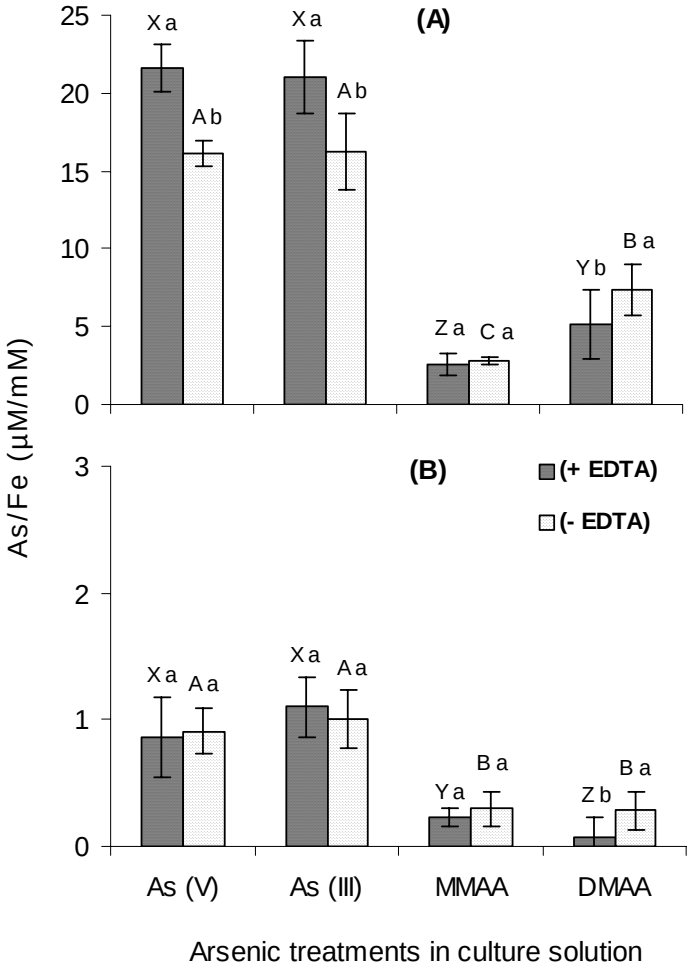

Figure 3: Mean As/Fe ratio (µM/mM) in *S. polyrhiza* L. Plant tissues (A); CBE-extracts (B). Different capital letters (X, Y, Z are for EDTA treated samples (+EDTA) and A, B, C are for samples without EDTA treatment (-EDTA)) indicate significant differences among the arsenic species and small letters indicate significant differences between EDTA treatments (with or without EDTA application), at $p < 0.05$. Error bars express mean ± SD ($n = 3$).