

Efficacy of Three Different Plant Species for Arsenic Phytoextraction from Hydroponic System

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Abstract

Arsenic (As) is one of the heavy metals which causes acute bio-toxicity even at low concentration and has disastrous effect on environment. In some countries, As contamination has become alarming and increasing day by day as consequences of unsustainable management practices. Many existing physical, chemical and biological processes for As removal from water system are not feasible due to techno-economic limitations. The present study highlights the scope of biological strategy for As removal through phytoextraction. Arsenic uptake and accumulation in the biomass of three plant species and their As tolerance abilities have been investigated to develop an efficient phytoextraction system in combination of these plant species. Three non-crop plant species, *Pteris vittata*; *Mimosa pudica*, and *Eichhornia crassipes* were treated with 0–200 mg/L As in liquid nutrient solution for 14 days. *P. vittata* accumulated total 9,082.2 mg (8,223 mg in fronds) As/kg biomass and *Eichhornia* total 6,969 mg (4,517 mg in fronds)/kg biomass at 200 mg/L As concentration, respectively. Bioaccumulation factor (BF) and translocation factor (TF) were estimated to differentiate between excluders, accumulators and accumulation in above ground biomass. *Pteris* and *Eichhornia* have highest BF (67 and 17) and TF (64 and 3), respectively. In contrast, *Mimosa* accumulated up to 174 mg As/kg plant biomass which is low in comparison with other two plants, and both BF and TF were ≤ 1 . This study reveals that *Pteris* and *Eichhornia* are As hyperaccumulator, and potential candidates for As removal from water system.

Keywords: Arsenic, *Eichhornia crassipes*, Hyperaccumulators, *Mimosa pudica*, Phytoremediation, *Pteris vittata*

1. Introduction

Arsenic (As) contamination in water is one of the world's major environmental problems, posing significant risks to human life and ecosystem [1-3]. Arsenic is toxic, carcinogenic and a potent poison to different organisms. Arsenic toxicity is a serious threat to human life in many parts of the world due to contamination of potable water and food crops [4-6]. Arsenic concentration in water is increasing day by day due to various natural as well as intensified environmentally unsound activities, such as volcanic eruption, dissolution of minerals from sedimentary rocks, dilution of geothermal waters, smelting of ores, disposal of As containing industrial wastes, and others agricultural applications [7-9]. Arsenic is used in agriculture as herbicide and plaguicide in the form of synthetic compounds, such as sodium methyl arsenate disodium methyl arsenate, and dimethyl arsenic acid. Discharge of As containing effluent and irrigation of As contaminated ground or surface water are the main culprits for As contamination in land and water ecosystems [9-11] which gets accumulated in food crops, water and finds its way into the human system by way of direct ingestion or through consumer products [10, 12]. A wide range of technologies are available for remedia-

tion of metals-contaminated soil and groundwater. General approaches for remediation of metal contamination include: isolation, immobilization, toxicity reduction, physical separation & extraction, adsorption, coagulation and precipitation of metal ions from the contaminated matrix [13, 14]. Although, the above mentioned methods are successful in remediation at certain extents, they are not sustainable with respect to energy input and cost. Thus, development of eco-friendly and cost effective technology for mitigation of As pollution is a R&D priority. Phytoremediation is one of the emerging technologies that offer significant benefit in comparison with the conventional technologies, which employs plants for remediation purpose. It is aesthetically pleasing, ecologically sound and environmentally sustainable and safer for humans [15-17].

Phytoextraction phenomenon is based on the ability of plants to tolerate metal stress, metal accumulation in their above ground biomass, high growth rate and biomass accretion with prolific root system [12]. In the last few years, importance of metal accumulating hyperaccumulator plants have been realized, which are potential candidates to enhance the existing



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Received February 14, 2014 Accepted April 17, 2014

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phytoremediation process [18, 19]. In view of this, prospective plant species for metal tolerance and accumulation and other preferred characteristics are being actively tested to determine their metal phytoremediation potential. Different plant species primarily belonging to genus *Brassicaceae* and grasses have been identified which show tolerance for various metals [18, 20-22]. Some aquatic plants have also been reported having capability to accumulate heavy and toxic metals by different mechanisms, which are potential for purification of metal contaminated water [23-25]. *Pteris vittata* (brake fern) is the first As hyperaccumulator plant having remarkable ability to accumulate As in its shoots, followed by which 12 other As hyperaccumulator plants have been identified [12, 26]. However, a number of fern species and some plant species from the genus *Brassicaceae* have also been reported to be As non-tolerant [27, 28]. Due to this paradox, identification of candidate better plants species, for phytoremediation application is a R&D priority.

This investigation was carried out to identify fast-growing, high biomass non-crop plant species for metal phytoextraction from polluted water. Three different plant species, i.e., *Pteris vittata*, *Mimosa pudica*, and *Eichhornia crassipes* were selected for treatment with different As concentration and assess their response with reference to morphological changes of the plant and metal accumulation in their biomass. Hydroponic screening method reported by Kumar et al. [29] was used to identify the potential plant species out of the three varieties.

2. Materials and Methods

2.1. Experimental Setup

Three plant species namely *P. vittata*, *M. pudica* and *E. crassipes* of similar age were selected for the present study. All the three plant species were having 4–6 whirls of leaves, 6–8" height in case of *Pteris* and *Mimosa* and 2–6 leaves and 2–2.5" height in the case of *Eichhornia*. These plants were already maintained in the glass house at the Council of Scientific & Industrial Research–National Environmental Engineering Research Institute, Nagpur, India. The plants used for the treatment study were acclimatized in the hydroponic condition in 20% Hoagland nutrient solution with pH 5.4–6 [30] for 2 weeks under controlled temperature and humidity. Subsequently, these plants were transferred into culture vessels containing 500 mL nutrient solution supplemented with 0, 25, 50, 100, 150, and 200 mg/L of arsenate in the form of sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$), control plants were grown in the nutrient solution without As amendment. During the treatment duration, volume of the treatment medium was maintained with Milli-Q water. The experimental setups were conducted in a closed manner to prevent loss of As from the liquid medium. The experiments were conducted in glass house under 25°C–30°C temperature and 70%–90% humidity, in natural light intensity and 14:10 (light:dark) photoperiod. After 14 days, plants were harvested, thoroughly washed with tap water followed by distilled water, and separated into fronds and roots. The plant samples were oven dried at 60°C for 72 hr, and dry weight of each sample was recorded to determine the biomass accumulation. Known dry weight of the root and shoot biomass were taken for As estimation.

2.2. Effect of As on Plant Growth and As Removal Efficiency

To determine the effect of As on plant growth, the fresh weight and height of plant at initial and final day of treatment were measured. Plant height was measured from the crown to the apex of the highest shoot. Physiological and morphological changes of the plants with respect to As exposure were also recorded by visual observations. Relative growth rates (RGR) of the plants were calculated as per the formula given in the Eq. (1).

$$\text{RGR} = (\ln W_1 - \ln W_0) / (t_1 - t_0) \quad (1)$$

where, W_0 and W_1 are the initial and final weights and t_1 and t_0 are the beginning and end of treatment durations, respectively. Two indices, i.e., bioaccumulation factor (BF) and translocation factor (TF) were calculated to estimate the phytoextraction ability of selected plants. The BF for As accumulation in plant biomass was determined as the ratio of As accumulated in the biomass to that of the treated nutrient solution. The TF was calculated as the ratio of As accumulated in shoot to that of root to evaluate the plant's ability to translocate metal from roots to the aerial part to determine its phytoextraction potentiality.

2.3. Arsenic Determination in Plant Samples

Known weight of dried plant biomass (0.1–0.5 g) was grinded in mortar and pestle, mixed with 10 mL concentrated nitric acid and digested in Microwave Digestion (Ethos 900; Milestone Inc., Shelton, CT, USA) under 300 W for 15 min. The digested samples were cooled and volume adjusted to 50 mL with Milli-Q water, filtered through Whatman Grade 42 filter paper and stored in capped polypropylene bottles until As estimation. Arsenic content of the digested samples were analyzed using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES; PerkinElmer, Waltham, MA, USA) against blank and 2, 5, and 10 ppm As standards (Merck Cat. No. OC486486).

2.4. Statistical Analysis

All experiments were carried out in three replicates. Data presented here are expressed as the mean \pm SD of three independent experiments. Statistical analysis was carried out using MINITAB 16 software and treatment effects were determined by analysis of variance using Tukey test given in the software.

3. Results and Discussion

3.1. Comparative Growth Performance of Selected Plants

The hydroponic screening experiment was carried out to test the effect of As on growth and metal accumulation in three non-crop plants belonging to different habitats, i.e., *Mimosa* (terrestrial) and *Pteris* and *Eichhornia* (lithophytic and macrophyte), respectively. In addition to the ability for efficient extraction of As from liquid medium, growth performance which is indicative of biomass accumulation is one of the essential features for identification of prospective plants for successful As phytoremediation application. Comparative growth performance of control and treated experimental plants were evaluated during the study period of two weeks and presented in this paper. Out of the three plants, As treated *Eichhornia* and *Mimosa* plants showed toxic-

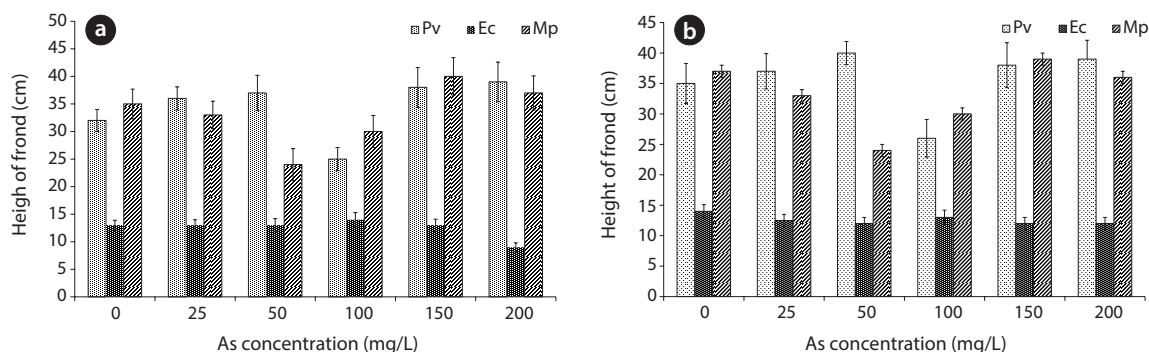


Fig. 1. Graph representing height of frond of three different plant species in 0–200 mg As/L medium at (a) 0 day and (b) 14th day. Pv: *Pteris vittata*, Ec: *Eichhornia crassipes*, Mp: *Mimosa pudica*.

ity symptoms in all concentration of As. Leaves started turning yellow and wilted after one week, whereas the control plants remain green and normal. At higher As concentration, onset of toxicity was early. In case of these two plants, leaves dried within two weeks, whereas in case of *Pteris* the plant remains green even after one month. This finding shows that *Pteris* is more tolerant to As stress in comparison to other two plants. Moreover, *Pteris* was able to survive even in 200 mg/L As concentration, although toxicity symptoms were visible lately.

One of the factors that plays important role in phytoextraction efficiency is plant biomass accretion under metal stress condition. This parameter was also considered in this study to evaluate stress response of the investigated plants to determine their As phytoextraction potentiality in addition to tolerance of the stress. Growth performances of these three plants with respect to height of the plant were compared at initial and final day of As treatment (Fig. 1). Out of these three plants, in *Pteris* root and shoot elongation was clearly evident and it flourished in all studied As concentration. Nevertheless, in lower concentrations of As up to 50 mg/L, *Pteris* biomass increased as compared to control plant. On the contrary, *Mimosa* and *Eichhornia* plants didn't show significant growth with respect to plant height in most concentration of As. Although *Mimosa* plant survived in 25 and 50 mg/L As, but at higher As concentrations toxicity was prominent. The RGR of the studied plants treated with all concentrations of As was determined with reference to controls (Table 1). The results clearly indicate that in all As treatment concentrations *Pteris* showed highest RGR in comparison to the other two plant species.

3.2. As Accumulation in Plant Biomass

After 2 weeks, the older and green leaves of the plants were

taken for As estimation as described in the materials and methods. The results of As accumulation inside the shoot and root of both control and As treated plants of *Pteris*, *Eichhornia*, and *Mimosa* species are presented in Fig. 2. *P. vittata* plant shows the highest As accumulation in shoot, i.e., 8,223 mg/kg dry biomass. Arsenic accumulation in *Eichhornia* shoot, i.e., 4,517 mg/kg biomass was nearly half that of *Pteris*; whereas, *Mimosa* plant show very low accumulation in its shoots. On the other hand, in the case of *Pteris*, extent of Arsenic accumulation in shoots increased with increase in concentration. As accumulation in roots also followed the same trend as that of shoot. Previous studies have reported Pb, Cu and Cd phytoextraction ability of *Mimosa* [31] with the adsorption efficiency 71%, 81% and 33%, respectively. Ashraf et al. [32] have also reported Zn accumulation in *M. pudica*, i.e., 38.94 mg/kg biomass. Although *Mimosa* is reported to be accumulator for the other heavy metals our study shows that it is not suitable for extraction of As. Previously, *Eichhornia* has been utilized for As removal from water matrix which showed 1.8 ± 0.5 mg As/kg removal under the assay conditions at 0.15 mg/L concentration of As [33]. They suggested that high As removal efficiency of *Eichhornia* was due to high biomass. Our results also show high As accumulation by *Eichhornia* and this findings is similar to the other studies reported earlier.

It is reported that As hyperaccumulator plants can accumulate As up to 2% dry weight of their biomass in the above ground parts with As TF usually exceeding 1 [12, 34]. Arsenic tolerance by a plant system depends on its inherent ability for avoidance of metal entry to the plant body as well as biochemical adaptation to tolerate intracellular As stress. The plant with high BF and TF >1 has been categorized as hyperaccumulator [35]. *Pteris* and *Eichhornia* plant have high As BF in the biomass, i.e. 67 and 64, respectively, at 25 mg/kg in Fig. 3(a). Further, the BF increased up to 50 mg/L As concentration and high TF indicated more As ac-

Table 1. Relative growth rate (RGR) of experimental plants at 14th day

Arsenic concentration (mg/L)	<i>Eichhornia crassipes</i>	<i>Mimosa pudica</i>	<i>Pteris vittata</i>
0	0.003	0.002	0.013
25	0.000	0.000	0.017
50	-0.004	-0.000	0.020
100	-0.005	-0.000	0.022
150	-0.006	-0.001	0.001
200	-0.008	-0.000	0.002

Negative value shows RGR reduction over control and positive value shows RGR increase over control.

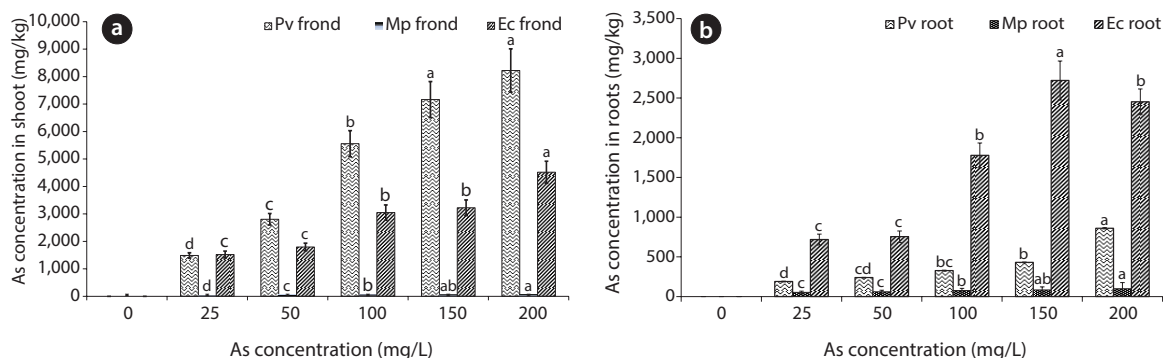


Fig. 2. Graph representing arsenic accumulation in (a) fronds and (b) roots of different plants in 0–200 mg As/L medium. Pv: *Pteris vittata*, Ec: *Eichhornia crassipes*, Mp: *Mimosa pudica*.

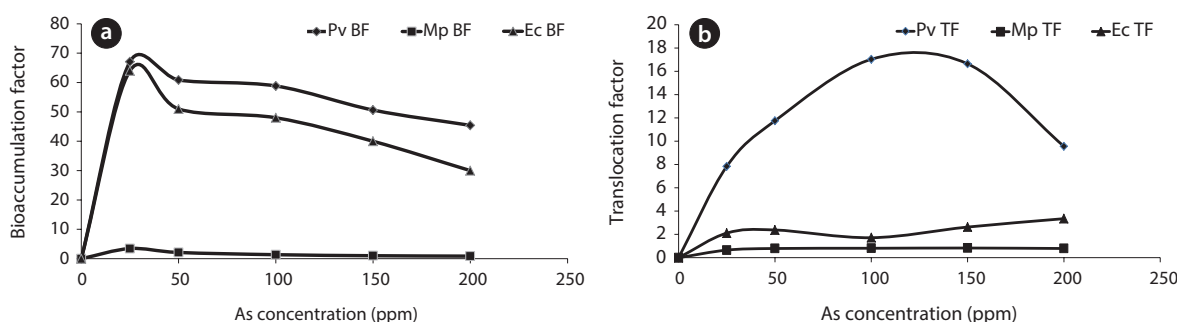


Fig. 3. Graph representing bioaccumulation factor (BF) a) and translocation factor (TF) b) of different plants in 0–200 mg As/L medium. Pv: *Pteris vittata*, Ec: *Eichhornia crassipes*, Mp: *Mimosa pudica*.

accumulation in shoot in comparison to roots. Fig. 3(a) also shows that *Pteris* and *Eichhornia* has As BF >1 which signifies one of the characteristics of hyperaccumulators; whereas, *Mimosa* has BF <1 indicating as As non-hyperaccumulator. BF for As in case of *Mimosa* was significantly different in comparison to other two plants. Arsenic translocation to the above ground biomass was highest in case of *P. vittata* showing TF up to 17, i.e. highest in comparison to other two plants (Fig. 3(b)). Arsenic treated *Eichhornia* plant died within 7 days unlike *Pteris*, which were green up to one month. Our finding shows that *Pteris* plant is tolerant up to 100 mg/L As. On the other hand, the plant continues to grow with increasing plant biomass under 50 mg/L As stress. Therefore, As extraction and accumulation in the plant biomass was possible simultaneously. Based on these findings it is certain that phytoextraction potentiality of this *P. vittata* genotype is more in comparison to the other two plants.

4. Conclusions

The results of this investigation show that *Pteris* and *Eichhornia* have significant capacity for As accumulation in the biomass, but *Pteris* has more tolerance to As toxicity. Arsenic accumulation was found to be more in shoot of *Pteris*; whereas, in case of *Eichhornia*, it was more in roots. The *Pteris* and *Eichhornia* plants accumulated $8,223 \pm 791$ and $4,517 \pm 402$ mg As/kg of dry shoot biomass and 859 ± 79 and $2,452 \pm 162$ mg in roots per kg of dry shoot biomass of the respective plants. They are more poten-

tial to accumulate As in plant biomass in comparison to *Mimosa* plant. The results indicate that *Pteris* plant has tolerance limit up to 100 mg/L As and has high As accumulation in the biomass with high BF up to 67. Under As stress, As accumulation in *Pteris* and *Eichhornia* plant biomass increased with increase in treatment concentration up to 200 mg/L. However, *Eichhornia* plant died after treatment with 50 mg As/L medium. High BF shows that both the plants can be used for As phytoextraction, but *P. vittata* is more suitable in comparison to *Eichhornia* due to higher TF for accumulation in above ground biomass and biomass accretion under As stress. The results of this study, along with the fact that this plant species are capable of thriving in As amended water support that *P. vittata* and *E. crassipes* could be the suitable species for sustainable As removal from contaminated water.

Acknowledgments

The authors acknowledge financial support to Sarita Tiwari by the CSIR under the 6th 5-Year Plan Project. The authors also wish to acknowledge encouragement of Director, NEERI and providing research facility to carry out the work.

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