

Phytoremediation of the Arsenic Contaminated Soils by Different Fern Species in Northern of Iran

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Abstract: Phytoremediation is the use of plants to remove, metabolize or degrade toxic environmental materials. The prerequisite for successful phytoremediation is the existence of hyperaccumulator plants. Designed to search for new arsenic (As) hyperaccumulators, an experiment was conducted under greenhouse conditions in a completely randomized design with three replications. Speciation and distribution of arsenic in the plant can provide important information helpful to understanding the mechanisms for arsenic accumulation, translocation and transformation. We considered four species of native ferns in Iran as follow: *Asplenium nidus* (AN), *Pteris umbrosia* (PU), *Polypodium vulgare* (PV) and *Pteris cretica* (PC). The average As concentration ranged from 164 to 4820 mg.kg⁻¹ DW in the fronds and from 23 to 510 mg.kg⁻¹ in the roots of (AN), (PU), (PV) and (PC) after growing in 120 mg As kg⁻¹ soil for 50 days. The results showed that percentage of As (III) was greater than As (V) in the fronds and roots of fern species. Based on our study, *Pteris umbrosia* is suitable for environmental conditions in northern of Iran. The nutrient requirements or distributions within the fern species were altered distinctly when the plants were exposed to As.

Key words: *Arsenic · ferns · Hyperaccumulators · Phytoremediation · Pteris umbrosia*

INTRODUCTION

Arsenic (As) is a toxic metalloid widely distributed in the environment. It is commonly associated with metal ores of Cu, Pb and Au. It has been employed in a number of industrial and agricultural applications and this includes pesticides and a component of pressure treatments for wood [1]. The evidence of health risks from As contamination is so compelling that in 2002 the Environmental Protection Agency (EPA) lowered the maximum contaminant level of As in drinking water from 50 to 10 µg.l⁻¹, making remediation of As contaminated water an increasingly important and potentially expensive issue [2]. Arsenate As (V) and arsenite As (III) are the most common inorganic forms of arsenic in the environment [3]. Both As (III) and As (V) are toxic and as such inorganic arsenic is regarded as a major environmental pollutant based on United State Environmental Protection Agency's evaluation [4, 5]. Phytoremediation, a technology using plants to remove contaminants from soils or waters, has been

intensively studied during the past decade due to its cost-effectiveness and environmental harmonies [6]. According to Reeves and Baker [7], hyperaccumulators are plants that can accumulate more than 100 times concentration of metals or metalloids in their aerial parts than normal plants. In addition, hyperaccumulators have more metals or metalloids concentrated in shoots than roots, demonstrating efficient translocation [8]. Arsenic hyperaccumulators usually have the ability to uptake large concentration of As, even in soils with low levels of As, illustrating efficient bioaccumulation, which is an important factor in phytoremediation [9]. Uptake and accumulation of As from soils by plants are influenced by; plant species [10], soil As concentration [10, 11], the presence of other ions [12], exposure time and plant age. Although the definition of arsenic hyperaccumulators is not clearly defined and can be considered arbitrary, the working definition is a plant that accumulates a minimum As concentration of 1000 mg kg⁻¹ in the aboveground biomass and has a higher concentration in the aboveground biomass than in both the roots and the soil

[13]. The first known As hyperaccumulating plant is called *Pteris vittata* L., (or Chinese brake fern) and was discovered by Komar *et al.* [14] from an arsenic-contaminated site that was contaminated from pressure-treating lumber using chromated-copper-arsenate (CCA). In this study, we hypothesized that screening ferns could identify more As hyperaccumulators. But two of them were hyperaccumulator (*Pteris umbrosia* and *Pteris cretica*) in Iran. We investigated these hypothesis by growing different ferns species under controlled greenhouse conditions, the biomass and the accumulation of either As or phosphorus (P) in these fern species and their relations were also studied.

MATERIALS AND METHODS

Germination: Spores of *Asplenium nidus* (AN), *Pteris umbrosia* (PU), *Polypodium vulgare* (PV) and *Pteris cretica* (PC) were collected from the natural habitat (80-90 percent relative humidity and 25°C temperature) of each species. Spores of these species were sprinkled onto a moist soil (40% sand, 30% peat and 30% garden soil) contained in a seed tray. The trays were covered with a plastic film to maintain moisture (field capacity moisture). After spore germination and the prothalli development, fertilizer was applied. Once sporelings grew to having 2 or 3 fronds, they were transplanted individually into plastic pots with a volume of 3500 cm³ containing just potting soil. After one month, these plants were transferred to pots containing 2.5 kg of soil (clay loam texture). The experiment was conducted in controlled environmental conditions 25°C /20°C day/night temperature and 75-85% relative humidity. The study was set up as a completely randomized design in a 4×2 factorial treatment combinations. Four fern species AN, PU, PV and PC were grown in a clean soil (control) and a soil spiked with 120 mg As kg⁻¹, added as Na₂HAsO₄•7H₂O. Each treatment was replicated three times. Fifty days after transplanting, the plants were harvested and washed with de-ionized water.

Chemical analysis: Plant roots and fronds were harvested, washed with tap water, rinsed with de-ionized distilled water and oven dried at 50-55°C for three days. After that, frond and root biomass were determined and the dried plant material was then ground (mortar/pestle). Samples were prepared for the As concentration analysis by using the dry ashing method. From 20 to 50 mg accurate weight (±0.01 mg) of the dried powder was mixed into a crucible with 1.5 ml freshly prepared slurry

(30 g Mg(NO₃)₂, 50 g MgO and 500 ml of distilled water). The mixture was dried overnight at 80°C and digested in a muffle furnace (200°C for one hr, 300°C for one hr and 500°C for eight hrs). Subsequently, the residue was dissolved in 6 M HCl (2.5 ml) and the solution mixed with distilled water (2.5 ml). Total As concentrations in these solutions were determined by graphite furnace atomic absorption spectrophotometer (AAS) (Plus AB). Phosphorus was determined by a modified molybdenum blue method [15]. Briefly, the pH of the digestion solution was adjusted to around seven with NaOH and H₂SO₄. Ten mls of the solution was then pipetted into a 20 ml glass test tube, to which 0.5 ml of L-cysteine (5% w/v in 0.6 M HCl) was added. The test tube was capped tightly and incubated for five mins at 80°C to allow complete reduction of arsenate into arsenite. The solution was then cooled to room temperature and P was determined by the molybdenum blue method [16]. Arsenic speciation was performed by extracting fresh plant samples ultrasonically in 10 ml of a methanol / water mixture (1/1 (v/v)) three times for a total of six hrs at 25°C [17]. The four extracts were decanted into a 50 ml volumetric flask and diluted to 50 ml with water. Arsenate and arsenite were separated using an As speciation cartridge (Metal Soft Center, Highland Park, NJ), which retains arsenate [18]. The sum of As (III) and As (V) concentrations determined by HPLC-ICP-MS and the total As concentration determined by As cartridge-GFAAS agreed by 96±13.

Statistical analysis: Treatment effects were determined by analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) (SAS Inst., 1996). Means were separated using the Duncan Multiple Range Test (DMRT) at 5%. Single correlation analyses were performed to investigate the relationships between arsenic in the plant parts of each fern species and macronutrient contents using the correlation procedures (PROC CORR) [19].

RESULTS AND DISCUSSION

Plant growth and arsenic accumulation in frond and root tissues: The data in Table 1 indicated that the plant growth as indicated by biomass of either frond or root parts for fern species could be arranged in the following order; PU>AN>PC>PV. In addition, variation in the As accumulation was greatly influenced by the ability of the plant species to translocate As. The highest values of As accumulation in both fronds and roots organs was observed in *Pteris umbrosia* specie.

Table 1: Biomass and As-uptake by different fern species

Fern species	Plant biomass (g/plant)		As uptake (mg/plant)	
	Fronds	Roots	Fronds	Roots
PV	1.12B	0.78B	0.18D	0.01D
PC	1.25B	0.83B	2.72B	0.15B
AN	1.78A	1.12A	0.64C	0.05C
PU	1.82A	1.18A	8.70A	0.59A

Values with the same letter in each column are not significant at $p = 0.05$ according to Duncan test

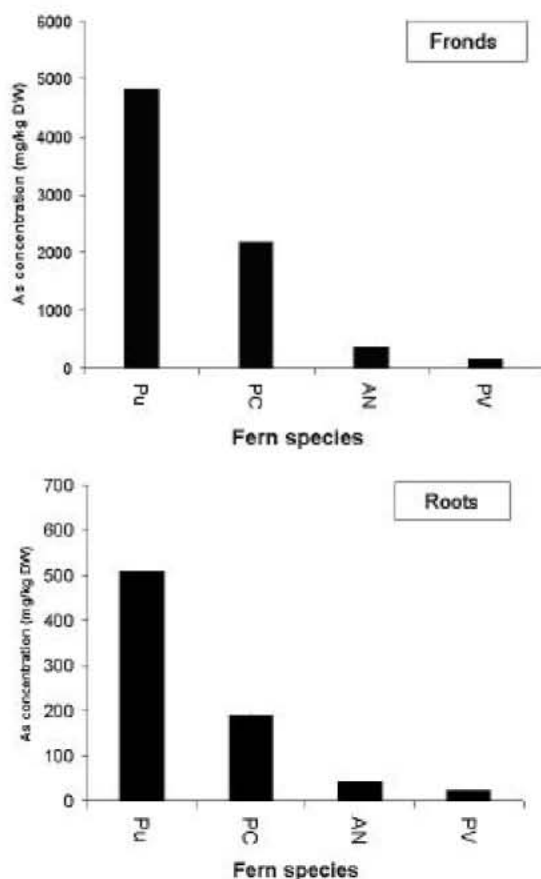


Fig. 1: Concentrations of arsenic in frond and root tissues of different fern species

Arsenic accumulation was determined in the frond and root tissues of the four ferns species. Results in Fig. 1 showed that after 50 days of exposure to $120 \text{ mg As kg}^{-1}$, all the plants were healthy and did not show any phytotoxicity symptom. This may suggest that these fern species were might be relatively tolerant to As. Fronds and roots As concentrations varied from 164 to $4820 \text{ mg.kg}^{-1} \text{ DW}$ and from 23 to $510 \text{ mg kg}^{-1} \text{ DW}$ respectively with highest values in *Pteris umbrosia* specie. This study was in agreement with Zhao *et al.*[20].

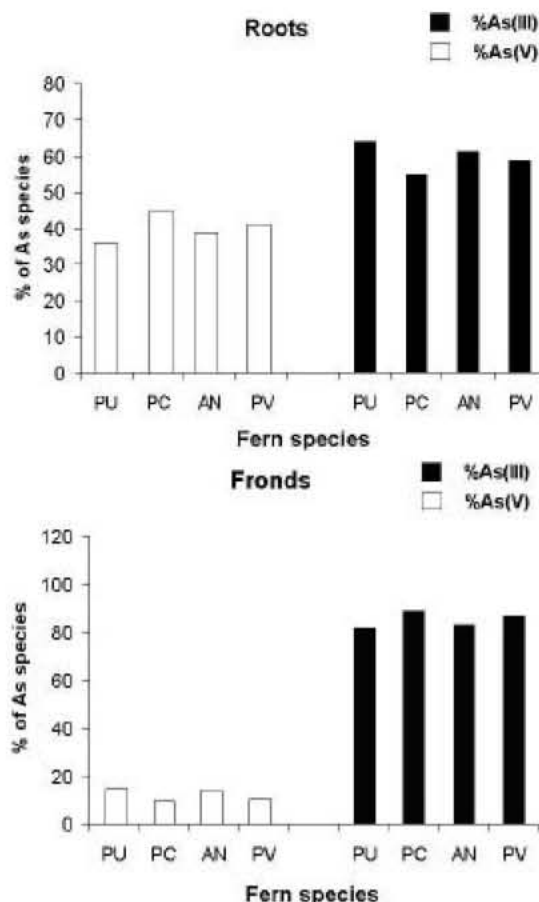


Fig. 2: Distribution of arsenic forms in frond and root tissues of different fern species

Arsenic speciation in plant parts: The As (III) and As (V) were the principal arsenic species found in the present study. This finding is in agreement with Tu *et al.* [21] and Srivastava *et al.*[22] who also recorded the same As forms in their study using *Pteris* species. The distribution of As forms in these fern species is shown in Fig. 2. In general, fronds of plants contained a greater percentage of As (III) (83-89%) than As (V).

Arsenic translocation and bio-concentration: One of the characteristics of hyperaccumulator plants is the ability to accumulate high concentrations of metal or metalloid in the aboveground biomass. The transfer factor has been used to describe a plant's ability to transfer an element from roots to aboveground biomass, which is defined as concentration ratio of an element in the aboveground biomass to that in the roots. Figure 3A show the arsenic translocation factor as indicated by their concentration ratios in either frond or root tissues of different fern species. The translocation factor of As in

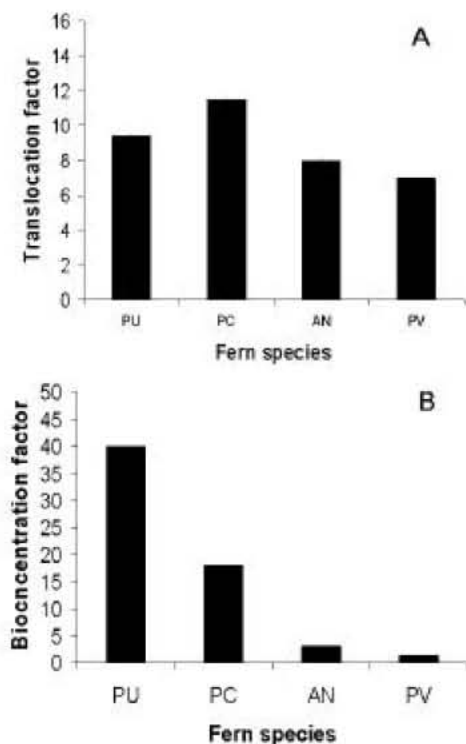


Fig. 3: Arsenic translocation factors and bio-concentration factors of different fern species

the plants varied from 7.13 to 11.5 as a results of growing plants in the soil spiked with 120 mg kg⁻¹ As. The PC plant specie was the most efficient in translocation As from roots to fronds. On the other hand, the bioaccumulation factor which measures the ability of a plant to concentrate the contaminant relative to the medium and is defined as the concentration ratio of an element in the plant to the soil medium. The bio-concentration factor ranged from 1.36 to 40.16 (Fig. 3B).

Relationship between arsenic and phosphors: The differences in the ability to concentrate nutrients by fern species could account for the differences between the species to remove As from the system. The results in Table 2 indicated that fern species have different phosphorus requirement and partitioning in the plant parts. The results also indicated that the phosphorus status and distribution varied with the amount of As present For example, in the absence of As, PC specie accumulate the highest concentration of P. However, when exposed to 120 mg kg⁻¹ of arsenic, PV was the species that accumulated more P than all of them. The relative content of phosphorus in the plant

Table 2: Phosphorus concentrations (Unites) in fronds and roots of different fern species

Fern species	Fronds		Roots	
	No AS	With AS	No AS	With AS
PV	5661Aa	6171Ab	2173Aa	2411Ab
PC	5956Ba	4291Bb	3423Ba	2285 Bb
AN	4247Ca	4953Cb	2294Ca	2550Cb
PU	4850Da	5232Db	2434Da	2722Db

Values with the same capital letter (in each column), and small letter (in each raw) are not significant at p= 0.05 according to Duncan test

parts, however, may change depending on arsenic concentrations in the soil. For example, regardless of the soil As concentration, P concentrations in the fronds were greater than that in the roots for all fern species evaluated. Exposure of the plants to As (120 mg kg⁻¹) increased the frond P concentration in all the species of fern during this study except PC. Increased P availability due to competitive adsorption [23, 24] and arsenic-induced physiological requirements of a plant [25] may be the reasons for the increased P uptake. However, PV was the species with the highest P concentration as a result of the mentioned processes.

Arsenic hyperaccumulators: It is reported that an arsenic hyperaccumulator should have concentrations in excess of 1000 mg As kg⁻¹ in its' aboveground biomass [26]. In addition, its transfer factor and bio-concentration factor should be greater than one. Using these definitions, we consider three species of fern that PU just acted as hyperaccumulator as similar to PC. These species are characterized by a high bio-concentration factor (>7.13) and high translocation factor (>1.36). That these species can withstand the high arsenic concentrations in the soil suggest that these plants may have a mechanism to detoxify accumulated As [27]. The present study has shown that most of the As in the frond tissues is readily extracted into methanol and water and this As is primarily present as inorganic As (III), i.e. arsenite. Because of its status as a hyperaccumulator and its relatively hardy nature, PU should be considered the most suitable species among the four fern species studied for phytoremediation purposes.

CONCLUSION

Greenhouse experiments were performed to investigate the phytoremediation of As. This research is conducted first time in Iran to introduce the suitable

species for northern areas of Iran. Based on our result, it is suggested that *Pteris umbrosia* is good specie for phytoremediation of arsenic-contaminated sites in humid and sub-humid climate conditions in northern part of Iran.

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