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# **The arsenic tolerance of plant species: Arsenic accumulation and transport**

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Abstract—There is a naturally occurring source of arsenic in the environment due to anthropogenic and geological processes (Zhao et al., 2010). It is estimated that millions of people around the world, particularly in South East Asia, have been poisoned by As polluted ground water and food (Kile et al., 2007, Zhu et al., 2008; Pal et al., 2009). There is increasing interest in Phytoremediation, a plant-based green method that can be used to clean up As-contaminated soil and water (Kertulis –Tartar and Yong, 2010). It is possible to harvest and remove the toxins from a contaminated location by harvesting plant tissues that have accumulated the contaminants. In order to ingest arsenic from the soil, plants use phosphate uptake pathways, i.e., apoplastic or symplastic mechanisms (shoots and leaves). The effectiveness of a plant's phytoremediation process can be gauged by measuring the quantity of arsenic that moves from the roots to the shoots.

*Keywords*---arsenic tolerance, phytoremediation, yperaccumulators.

# **Introduction**

There is a naturally occurring source of arsenic in the environment due to anthropogenic and geological processes (Zhao et al., 2010). It is estimated that millions of people around the world, particularly in South East Asia, have been poisoned by As polluted ground water and food (Kile et al., 2007, Zhu et al., 2008; Pal et al., 2009). There is increasing interest in Phytoremediation, a plant-based green method that can be used to clean up As-contaminated soil and water (Kertulis –Tartar and Yong, 2010). It is possible to harvest and remove the toxins from a contaminated location by harvesting plant tissues that have accumulated the contaminants. In order to ingest arsenic from the soil, plants use phosphate uptake pathways, i.e., apoplastic or symplastic mechanisms (shoots and leaves). The effectiveness of a plant's phytoremediation process can be gauged by

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measuring the quantity of arsenic that moves from the roots to the shoots. As accumulation and translocation from roots to shoots in the Chinese brake fern (Pteris vittata L.) has been found to be the most efficient (Ma et al., 2001). As hyperaccumulators have recently been discovered in numerous plants. In Xie et al., (2009), the majority of the ferns described as naturally developed As accumulators are Pteris genus members. In addition to these efforts, aquatic and terrestrial plants have been identified as potential Asaccumulators (Tiwari et al., 2008; Tripathi et al., 2012; Ozturk et al., 2010; Xie and Yan, 2009). More plants must be screened, however, in order to locate the most suitable option for a specific region that may be used globally. As-phytoremediation could benefit greatly from the use of indigenous plants that are tolerant and capable of accumulating As. Research into arsenic tolerance and detoxification is just as vital as finding novel phytoremediators. The goal of this research is to identify indigenous plants with high As tolerance and accumulation capacity that might be used in phytoextraction or phytostabilization of polluted soil.

## **Methods and Materials**

## **Composition of plants**

To screen for As-tolerant plants, two types of plants were used: I ferns, which have been known to be As-accumulators, and (ii) some angiosperms, which have been shown to be metal accumulators. Around four-month ferns include Pteris vittata Linn, Christella dentata Forssk, Adiantum capillus veneris Linn, Nephrolepis tuberosa (Bory ex. Wild.) C. Presl, Nephrolepis cordifolia (L.) C. Presl, Nephrolepis bissrata (Sw.) Schott, Ampelopteris prolifera (Retz) copel, Co (C. Chr.) Microsorium punctatum Linn, C. Chr., and Microsorium altenifolium (Willd.) Copel. were collected from the NBRI fernery. Vetiveria zizinoides (L.) Nash ex. Small, Phragmitis karka (Cav.) Trin. Ex. Steud, Helianthus annus Linn., and Withania sommnifera (L.) Dunal were used as angiosperms. H. annus and W. somnifera seeds were obtained from NBRI germplasm and originally cultivated for four weeks in a commercial potting medium in a greenhouse. Following the growth phase, similar-sized seedlings were selected for the experiment. V. zizinoides and P. karka slips were collected at the NBRI farm.

### **Configuration of the experiment**

Eight-inch pots were taken and filled with four kilogrammes of dirt (fertile garden soil). Table 3 contains soil zero-day data. 1 Arsenic in the form of sodium arsenate was administered in quantities of 25, 50, 75, and 100 mg kg-1, with garden soil serving as a control. Three duplicates were taken for each treatment. The pots were left for one week to allow the arsenic to acclimate. After one week of therapy, the plants were transferred to the container. After six months, harvesting was completed. To determine the effects of arsenic exposure, chlorophyll, protein, antioxidant enzymes, and glutathione levels were determined in the plants. Following harvest, a portion of plant samples were frozen in liquid nitrogen and stored at -80oC for enzyme analysis and biochemical parameters, while the remainder of the plant samples were dried in an oven at 65oC for 48 hours for digestion and analysis. For analysis, soil samples were collected, air dried, and sieved (1mm mesh).

<b>Parameters</b>		<b>Values</b>			
pH		$9 \pm 0.17$			
$EC (\mu S)$		$158.83 \pm 5.26$			
$TOC$ $(\%)$		$1.18 \pm 0.04$			
A.P (mg/g)		$0.07 \pm 0.006$			
A.N(%)		$0.08 \pm 0.01$			
A.K (mg/g)		$0.11 \pm 0.01$			
	Dehydrogenase (µg/tpf/g24hr)	$0.95 \pm 0.03$			
	Alkaline Phosphatse (µg/pnp/g/hr)	$1.5 \pm 0.09$			
As (ppm)		<b>ND</b>			
<b>Texture</b>	Clay (%)	$11.9 \pm 1.15$			
	Silt $(\%)$	$21 \pm 3.46$			
	Sand (%)	66±3.05			
	Bulk Density (g/c.c)	$1.18 \pm 0.004$			
	Particle Density (g/c.c)	$1.69 \pm 0.01$			
Porocity (%)		30.16±0.87			
	Water Holding Capacity (%)	$30 \pm 0.5$			

Table 1 Soil analysis on the same day

# **Capacity for phytoextraction**

The phytoextraction capacity of plants was determined as follows:

$$
TF=[As]_{\text{shoot}}/\,[As]_{\text{root}}
$$

#### **Results**

# **Plants resistant to as are screened**

The resistance to arsenic of fifteen indigenous tropical Indian plants was evaluated. Fifteen of the species were ferns, with the remaining four being angiosperms. A number of previous research (Ma et al., 2001; Wei et al., 2010; Francesconi et al., 2002; Du et al., 2005; Srivastava et al., 2006; Wang et al., 2006; Koller et al., 2007; Luango and Ma, 2005; Singh et al., 2010) have shown that ferns are naturally developed as accumulators of carbon dioxide (CO2). This collection also included P. karka, V.zizinoides, W. somnifera, and H. annus, all of which have been shown to be tolerant to heavy metal pollution. This plant's resistance to As is depicted in Table 3.2 at various As concentrations. All of these plants were sensitive to As and could not thrive even in soil containing 25 mg kg-1 As of As. As at 25 mg kg-1 was tolerated by N. bissrata. In addition to P. vittata, hyperaccumulator A. capillus veneris, C. dentata, N. tuberosa, N. cordifolia, A. prolifera, and V. zizinoides P. karka were all capable of tolerating As concentrations of up to 100 mg kg-1 in soil, as was P. vittata. But after a month of growth, the Nephro l I species' leaves began to display necrosis and eventually dried out. After surviving for six months, A. prolifera's development slowed dramatically. Weather circumstances caused the leaves of V. zizinoides to dry out. Arsenic resistance was found in A. capillus veneris, C. dentata, and P. karka, in addition to the previously described As hyperaccumulator, P. vittata, when the samples were harvested (six months after exposure).

# **The impact of arsenic on plant species' biomass and survival**

Toxicology signs were not present in the plants after six months of growth, as depicted by the figures in Figure 1 and 2. As the soil's arsenic concentration rose, so did biomass. Table 3 shows the increase in shoot and root length in the studied plant species. As arsenic's time in the soil rose, so did the length of the plants' shoots and roots. These findings show that these plant species were wellversed in arsenic tolerance.



## Table 2 Testing for arsenic resistance in indigenous ferns and other plants



Figure 3.1 Effect of arsenic on biomass of different plant species. Vertical bars indicate means  $\pm$  S.D. of three replicates. Bars with same letter were not significantly different at p < 0.05 within a species according to DMRT (Duncan's multiple range test)

### **Intake and storage of arsenic by plants**

Phytoremediation relies on plant uptake of contaminants. Plant components with the highest arsenic concentrations were found in areas where arsenic levels rose in the soil (Figure 3.2 A and B). For arsenic accumulation, P. vittata was the most effective plant investigated; this species has proven to be an effective arsenic accumulater in the field (Wang et al., 2007). A. capillus veneris, C. dentata, P. karka, V. zizinoides, and A. prolifera were the next species to be discovered. When exposed to 100 mg kg-1 arsenic daily for six months, P. vittata's roots and shoots (fronds) accumulated 23436.67 and 11115.33 mg kg-1 dry weight of arsenic, respectively. Whereas, in the root and shoot of A. capillus veneris, 9532.33 and 14800 mg kg-1 As were found to be present. This plant accumulated arsenic at 5848 and 6072.33 mg kg-1 As in the root and shoot parts, respectively, while P.karka had 4774.33 mg kg-1 As in shoot and root parts. As concentrations increased in the roots and shoots of PV, AC, CD and PK, with the shoot concentration rising faster than that in the roots. However, AP, NT, NC, NB, and VZ were all As accumulators, although the root section of the plant had the most uptake. All other treatments saw an increase in arsenic ac mulation when soil arsenic content rose (25, 50 and 75 mg kg-1).

It is possible to calculate a plant's efficiency in moving As from roots to shoots using the translocation factor (TF), which may be seen in Figure 3.3. When the TF was greater than 1, it indicated a hyperaccumulator property in PV, AC, CD, and PK. However, in NT the TF was greater than 1 up to 50 mg/kg-1 treatment, but subsequently it did accumulate As, but only in the root system. After being exposed to 25 mg kg-1 of arsenic, rats in the NB experiment demonstrated TF>1. The leaf and root sections of the selected accumulator plant species against arsenic were subjected to several cluster analyses. Figure 3 clearly shows the dendogram analysis for the treated leaf using Euclidean distance. Four different clusters, designated A, B, C, and D, were developed. Cluster 1 gathered plants 1 and 2, and Cluster 2 grouped plants 3 and 4 based on their D2 value, which revealed 99 percent of similarity index for these plants. To make up Cluster 3, the D2 values of plants 6, 7, 8, and 9 were all at 100%, while the D2 values of plants 5 in Cluster 4 were all at 0%. In the root cluster analysis, there were found to be

five unique clusters. There was an 84% D 2 value in the first cluster, which included the numbers 1, 2, 8 and 9. In clusters 2, 3, 4, and 5, the plants are arranged differently, but cluster 5 contains 3 and 6 plants that have a D 2 value of 83%. For example, P. vittata (plant 1 and 2 cluster 1) exhibited similar arsenic accumulation behaviour to A. capillus-veneris while C. dentata (plant 3 and 4 cluster 2) showed similar arsenic accumulation behaviour.



Figure 3.2 Arsenic accumulation A) leaf B) root of different plant species. Vertical bars indicate means  $\pm$  S.D. of three replicates. Bars with same letter were not significantly different at p < 0.05 within a species according to DMRT (Duncan's multiple range test)





Note: Means followed by the same letter were not significantly different at  $p < 0.05$  between different arsenic concentration for a given plant species at a given arsenic treatment



Figure 3.3 TF of Plant species at different arsenic concentrations



Figure 3.4 Cluster Analysis A) leaf B) root of different plant species

## **Arsenic's effect on soil's physical and chemical properties**

Different soil parameters and arsenic concentrations are shown in Table 4 A, B, C, and D. The texture of the soil was sandy loam, which had a high alkaline content. All the arsenic accumulator plant species showed an increase in arsenic concentration with an increase in soil arsenic concentration. Arsenic removal from the soil was accelerated thanks to these plants' expertise in arsenic uptake from treated soil (Figure 3.5). One of the plant species classified as an arsenic hyperaccumulator (P. vittata) eliminated arsenic at a far faster rate than the other three. At 25, 50, 75 and 100 mg kg of arsenic per acre, P. vittata eliminated 63.68, 46.89, 41.48 and 34.8 percent of the arsenic in the soil. For example, A. capillus veneris eliminated arsenic at levels as low as 25 mg kg-1 and as high as 100 mg kg-1 when exposed to the same concentrations as A. capillus veneris. When exposed to 25, 50, 75, and 100 mg kg -1 arsenic, C. dentata eliminated 77.01, 49.33, 48.76, and 48.78 percent of the arsenic. Ultimately, P. karka was able to remove 73.53% of arsenic from soil in 25 mg kg-1, 50 mg kg-1, 75 mg kg-1, and 100 mg kg-1 arsenic treatments. In contrast to the control, EC declined as arsenic levels in the soil rose, except in the case of P. vittata, when EC actually rose. All plant species discovered N.P.K in the soil to be the same as the control. After increasing the arsenic concentration further, TOC dropped in both 25 and 50 mg kg-1 arsenic treatments, compared to the control. Since enzymes are involved in the movement of the most critical nutrients, they are an useful indicator of soil fertility. All plant species tested exhibited no significant changes in DHA or APA activity when compared to the control at various arsenic doses.



Figure 3.5 Arsenic Removal of Plant species at different arsenic concentrations

Table 3.4 Physicochemical analysis of soil A) P.vittata B) A. capillus veneris C) C. dentata D) P. karka after 180 days of growth.

A (P.vittata)										
<b>Treatment</b>								<b>APA</b>	<b>DHA</b>	
	As $(mg kg-1)$	рH	EC (µS)	$A.P$ (mg g <sup>-1</sup> )	$A.K(mg g-1)$	A.N (%)	TOC $(\%)$	$(\mu$ gpnpg <sup>-1</sup> hr <sup>-1</sup> )	$(\mu g t p f g^{-1} 24 h r)$	
<b>PVControl</b>	ND	$9.97 \pm 0.06$ <sup>c</sup>	$148.63 \pm 20.43^a$	$0.03 \pm 0^a$	$0.09 \pm 0.01^3$	$0.03 \pm 0^a$	$1.45 \pm 0.14^{ab}$	$1.25 \pm 0.02^a$	$0.31 \pm 0.12^a$	
<b>PV25</b>	$14.94 \pm 0.45^b$	$9.70 \pm 0^3$	$149.50 \pm 17.33$ <sup>a</sup>	$0.03 \pm 0^4$	$0.12 \pm 0.01^b$	$0.03 \pm 0^a$	$1.49 \pm 0.06^{ab}$	$1.26 \pm 0.04$ <sup>ab</sup>	$0.36 \pm 0.15^a$	
<b>PV50</b>	$22.30\pm0.86$ °	$9.77 \pm 0.06^{ab}$	$159.63 \pm 18.65^a$	$0.03 \pm 0^2$	$0.13 \pm 0.01^b$	$0.02 \pm 0^a$	$.66{\pm}0.20^b$	$1.27 \pm 0.02$ <sup>ab</sup>	$0.29 \pm 0.05^a$	
<b>PV75</b>	$30.66 \pm 0.15^{\circ}$	$9.83 \pm 0.06$ <sup>abc</sup>	$166.07 \pm 15.14^{\circ}$	$0.03 \pm 0^4$	$0.12 \pm 0.01^{\circ}$	$0.03 \pm 0^a$	$1.46 \pm 0.06^{ab}$	$1.35 \pm 0.06^{\circ}$	$0.39 \pm 1.14$ <sup>a</sup>	
<b>PV100</b>	$33.60 \pm 0.17$ <sup>c</sup>	$9.87 \pm 0.06^{\rm bc}$	$186.20 \pm 5.40^a$	$0.03 \pm 0^4$	$0.11 \pm 0^b$	$0.04 \pm 0^{b}$	$1.23 \pm 0.04^a$	$1.29 \pm 0.01$ <sup>ab</sup>	$0.38 \pm 0.08$ <sup>a</sup>	











Note: Means followed by the same letter were not significantly different at p < 0.05 between different arsenic concentration for a given plant species at a given arsenic treatment

### **Discussion**

In the wake of the crises in Southeast Asia, the fate and behaviour of As in the environment has come to the fore. Inorganic As species, particularly AsV and AsIII, are more hazardous to plants than organic As species (Tamaki and Frankenberger, 1992). Recent years have seen a lot of interest in the use of plants to clean up contaminated soil (Baker et al., 2000). Soil contamination can be remedied through the use of plants. Since its discovery in 2001 by Ma et al., P. vittata, the first known As hyperaccumulator, has garnered much attention. One of phytoremediation's limitations is the necessity for plant species to thrive at contaminated environments. As a result, the process of choosing plant species for phytoremediation is never-ending. There have been several studies that have

uncovered plant species with the ability to accumulate aboveground biomass of more than 1000 mg kg As. These include Visoottiviseth et al., (2001), Sridokchan et al., (2005); Srivastava et al., (2006); Wang and Singh (2007; 2010), and Visoottiviseth and Singh (2002).

The three parameters for successful As phytoremediation are as follows: Soil As must be taken up and remedied by the roots of the plants, the plants must be able to transport and accumulate As in the shoots that can be harvested later, and the plants must be equipped with an effective detoxification system to protect themselves from the toxic effects of high levels of As (Gumaelius et al., 2004). The fern family, specifically the Pteris genus, is home to various naturally developed As accumulators that have recently undergone screening. The arsenic hyperaccumulation of some Pteris species is not universal (Meharg, 2003). Fern species from different families and several higher plants belonging to different genera were screened at arsenic concentrations of 25, 50 and 75 mg kg. Four of the 15 plant species studied (P. vittata, A. capillus veneris, C. dentata, and P. karka) had high biomass. Successful phytoextraction relies heavily on biomass. Arsenic concentration in the soil had a positive effect on plant biomass, shoot length, and root length. Singh et al., 2010 found similar outcomes. A. capillus veneris, C. dentata, and P. karka, all closely related to P. vittata, met the criteria for being classified as hyperaccumulators in this study. High arsenic concentrations (100 mg kg-1) were tolerated by the plants, and they accumulated more arsenic in the shoot portion than in the root portion; iii) the translocation factor was determined to be more than 1. Singh et al., (2010), Srivastava et al., (2010), and Mirza et al., (2010) all found similar results in various plant species.

As concentration in the fertiliser solution increased, so did soil arsenic concentrations (Shaibur and Kawai, 2009). The nutritional solution was thought to have a high concentration of As. Our study found that the amount of As in the medium had a direct effect on the amount of As that could be absorbed by plants. As compared to the control, arsenic reduced soil Ec. Due to the high carbon concentration in soil, the soil was determined to be alkaline. An integrated labile pool for plant nutrients, TOC is found in soils with high levels, indicating that nutrients are readily available. NPK levels were low, indicating that the plant was utilising these nutrients for its own growth. For assessing the impact of metal contamination on the soil environment, the assay of potential enzyme activity is critical. As a sensitive indicator of how environmental factors affect microorganisms, soil enzyme activity is commonly used. In contrast to the garden soil, DHA and APA activity was shown to be reduced in the presence of arsenic. Ghosh et al., obtained similar results (2004). In order to use phytoremediation to clean up arsenic-contaminated soil, the soil must be rich in nutrients.

Figure 3.6 a Experimental setup of P. vittata and C. dentata with arsenic treatment



Figure 3.6 b Experimental setup of A. capillus veneris and P. karka with arsenic treatment







Figure 3.6 c Experimental setup of N. tuberosa, N. cordifolia and N. biserrata with arsenic treatment









#### Figure 3.6 d Experimental setup of V. zizanioides and A. prolifera with arsenic treatment

# **Conclusion**

Efforts to clean up arsenic-contaminated land are becoming more and more significant. Toxic metals like As can be removed from the environment through phytoremediation, a promising new method. Progress has been made in recent years in identifying arsenic hyperaccumulators and determining the mechanisms by which phytoremediation works. In the study, A. capillus veneris, C. dentata, and P. karka may be considered arsenic hyperaccumulator species. Phytoremediation mechanisms, including As uptake, As transformation, translocation, and detoxification, necessitate an integrative study approach. The ability of these plant species to accumulate arsenic and grow their biomass is a crucial factor in phytoremediation, and further research is needed.

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