EFFECTS OF SOIL AND PLANT ON ARSENIC ACCUMULATION BY ARSENIC HYPERACCUMULATOR *Pteris vittata* L

By

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By

Maria Isidoria Silva Gonzaga
To the memory of my father Henrique, whose love and inspiration have accompanied me through the years; to my mother Ines who, together with my father, gave me all the values that guide my life; and to my kids Jamile, Thomas and Genna, the best fruits of my existence.
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EFFECTS OF SOIL AND PLANT ON ARSENIC ACCUMULATION BY ARSENIC HYPERACCUMULATOR *Pteris vittata* L

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Phytoextraction using *Pteris vittata* L., an arsenic hyperaccumulator plant, could be potentially applied to remediate arsenic-contaminated sites worldwide. *Pteris vittata* grew well and took up large amounts of arsenic in six arsenic-contaminated soils with different properties and different sources of arsenic contamination. However, efficiency of the ferns in continually taking up arsenic from the soils decreased with time, and varied with growing season. The arsenic concentrations in different chemical fractions before and after plant uptake showed that *P. vittata* took up arsenic from the most available and also from the less available pools in all soils. The change in arsenic availability in the rhizosphere was evaluated by comparing *P. vittata* with a non-arsenic-hyperaccumulator *Nephrolepis exaltata*. As expected, *P. vittata* removed more arsenic from the soil than the non-arsenic-hyperaccumulator fern. Besides the efficient detoxification mechanisms of *P. vittata*, its more extensive and finer root systems, and its greater capacity to change the soil pH and produce root exudates in the rhizosphere likely
contributed to the difference. Furthermore, the experiments with *P. vittata* of different physiological ages revealed that younger plants (because of their higher metabolic and growth rate) were more efficient than older plants in taking up arsenic. Therefore, younger plants are recommended for phytoremediation. Comparing two arsenic hyperaccumulators in the genus *Pteris* showed that arsenic hyperaccumulator ferns differed in their ability to take up arsenic when growing under the same soils and environmental conditions. The concentrations of arsenic in both plants increased with the soil arsenic concentration, however, *P. vittata* performed better, regardless of the arsenic level in soils. After 4 weeks of growth, *P. biaurita* showed signs of stress and probably would not have survived longer under those conditions, while *P. vittata* showed no toxicity symptom. Our results showed that, in implementing an arsenic phytoextraction project, many aspects related to the plant must be considered.
Arsenic contamination in the environment from both anthropogenic and natural sources occurs in many parts of the world and is a global problem. Arsenic-contaminated soils, sediments, and sludge are the major sources of arsenic contamination in food chain, surface water, groundwater, and drinking water (Frankenberger and Arshad, 2002). Other potential sources of arsenic contamination are the chemicals used extensively in agriculture such as pesticides, insecticides, defoliants, wood preservatives, and soil sterilants (Azcue and Nriagu, 1994).

In many areas, arsenic levels in the environment have exceeded the safe threshold for human health. Epidemiological studies have documented various adverse effects of arsenic on humans and animals. Conventional remediation technologies have been used to clean up metal-contaminated sites because they are relatively insensitive to the heterogeneity in contaminated matrix, and can function over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials (Cunningham et al., 1997). However, they are expensive and time-consuming, often hazardous to workers, and produce secondary wastes (Lombi et al., 2000). Of the disadvantages of conventional remediation methods, cost is the primary driving force behind the search for alternative remediation technologies, such as phytoremediation.

Phytoremediation is the use of plants and their associated microbes to reduce, remove, degrade, or immobilize environmental contaminants in soil and water systems and can be applied for both organic and inorganic pollutants (Salt et al., 1998).
Phytoextraction, one of the strategies of phytoremediation, has attracted much attention as an environmentally-friendly low-input remediation technique that uses plants that extract heavy metals from the soil and accumulate them in the harvestable aboveground biomass (McGrath et al., 2002). However, the effectiveness of a phytoremediation plan is plant-dependent. Phytoextraction can be accomplished by using either tolerant high biomass plant species or hyperaccumulator plant species. Plants native to the target area should be considered, since they are adapted to the local climate, insects, and diseases.

Hyperaccumulators are plants that can take up and concentrate more than 0.1% of a given element in their tissue (Brooks, 1998). Metal hyperaccumulation is a rare phenomenon in terrestrial higher plants. The first arsenic hyperaccumulator, *Pteris vittata* L., was identified by Ma et al. (2001), followed by *Pityrogramma calomelanos* L. (Francesconi et al., 2002) and many other species of the *Pteris* genus such as *P. cretica* L., *P. longifolia* L., *P. umbrosa* L., and *P. argyraea* L. (Zhao et al., 2002), and *P. quadriaurita* L., *P. ryiunkensis* L. and *P. biaurita* (Srivastava et al., 2006).

*Pteris vittata* is a fast-growing fern and has the potential to produce relatively high biomass while accumulating a large amount of arsenic in its aboveground tissue. These characteristics and its ability to survive in different environments make this plant an exceptional candidate for use in phytoremediation of arsenic-contaminated soils.

To increase the efficiency of phytoextraction of arsenic contaminated soils using *P. vittata*, besides understanding its arsenic tolerance and detoxification mechanisms, it is important to learn more about the biological processes involved, such as plant arsenic uptake, plant nutritional requirements, rhizosphere processes and mobilization and
bioavailability of arsenic in the soil. Experiments included in our study had the following objectives:

1. Evaluate the efficiency of *P. vittata* in extracting arsenic from different arsenic-contaminated soils
2. Evaluate the importance of the physiological age of *P. vittata* on its arsenic uptake and plant growth
3. Determine the characteristics of the root systems and changes in the rhizosphere of *P. vittata* in comparison with a non-hyperaccumulator
4. Compare the efficiency of *P. vittata* in arsenic phytoextraction in comparison with *Pteris biaurita*, another arsenic hyperaccumulator.
Occurrence, Availability and Toxicity

Arsenic concentrations range from below 10 mg kg$^{-1}$ in non-contaminated soils (Adriano, 1986) to as high as 30,000 mg kg$^{-1}$ in contaminated soils (Vaughan, 1993). In rocks, arsenic is concentrated in magmatic sulphide minerals and iron ore. The most important arsenic ores include arsenical pyrite or arsenopyrite (FeAsS), realgar (AsS), and orpiment (As$_2$S$_3$).

Arsenic has been identified as a major toxic contaminant in many countries. Various human activities have elevated the arsenic levels in soils such as the production and the use of arsenical pesticides (fungicides, herbicides, and insecticides). In addition, manufacture of arsenic-based compounds, smelting of arsenic-containing ores, and combustion of fossil fuels have also contributed to arsenic contamination in soils, water, and atmosphere (Azcue and Nriagu, 1994). An increase in industrialization has also lead to an increase in the amount of arsenic present in biosolids. Arsenic deposits from the atmosphere, runoff, and effluents of industries often increase the concentration of arsenic in biosolids. Woolson (1983) reported a range of 0 to 188 mg kg$^{-1}$ As dry weight of biosolids. Biosolids are often disposed on land and may then increase arsenic concentrations in the top 20 cm of soil by up to 0.15% (O’Neill, 1990).

Sorption onto soil particles is an important process for arsenic immobilization in soils. Many studies have been devoted to arsenic sorption on well-characterized minerals.
or soil particles (clay, oxides of Al, Fe, and Mn, calcium carbonates, and/or organic matter) (Sadiq, 1997; Dobran and Zagury, 2005). The high affinity of arsenic for oxide surfaces is well known and is affected by several biogeochemical factors such as pH, redox potential, and competing ions (Adriano, 2001). The activity of arsenic in soil solution is mostly controlled by surface complexation reactions on oxides/hydroxides of Al, Mn, and especially Fe (Inskeep et al., 2002). The mobility of arsenic in soil is pH dependent as arsenate is preferentially sorbed onto hydrous oxides in the pH range of 4 to 7; whereas, arsenite is preferentially sorbed for in the pH range of 7 to 10 (Pierce and Moore, 1982). Frost and Griffin (1977) have shown that arsenic sorption onto kaolinite and montmorillonite is pH dependent, while Lin and Puls (2000) confirmed that arsenate is more strongly sorbed onto these minerals than arsenite. Furthermore, arsenate adsorption onto humic substances reaches a maximum at pH 5.5, while arsenite adsorption peaks at much higher pH of 8.5 (Thanabalasingam and Pickering, 1986).

The bioavailability, toxicity, and chemical behavior of arsenic compounds are largely influenced by the form and speciation of arsenic. In natural systems, arsenic can exist in four oxidation states: (-3), (0), (+3), and (+5). Arsenate [As(+5)] and arsenite [As(+3)] are the main forms present in soils (Harper and Haswell, 1988). Arsenate prevails under aerobic conditions and is somewhat less toxic and also less mobile than arsenite (dominant form under anaerobic conditions) because arsenate sorbs more strongly than arsenite onto minerals (Pierce and Moore, 1982). Generally, inorganic arsenicals are more toxic than organic arsenicals while the trivalent oxidation state is more toxic than the pentavalent oxidation state (Adriano, 2001).
Remediation of Arsenic Contaminated Soils

Many remediation techniques are available to address the problems in contaminated sites. However, the relatively high capital expenditure, unsuitability for large areas, and environmental disruption are some of the shortcomings of those techniques. No single soil-remediation technique is suitable for all situations. Careful investigation of the contaminated site characteristics, contaminant problem, treatment options and treatment timeframe must be considered.

Current remediation methods for arsenic-contaminated soils include soil removal and washing, physical stabilization, and/or the use of chemical amendments, all of which are expensive and disruptive, with an average cost of $404,700 per ha (Raskin et al., 1997). Following are some selected current remediation technologies for arsenic-contaminated soils (USEPA, 2002a).

a. **Capping:** a hard cover is placed on the surface of a contaminated soil. It is a simple method to reduce contaminant exposure. However, it does not remove contaminants from the soil.

b. **Solidification and stabilization:** the contaminated soil is mixed with stabilizers to reduce the arsenic mobility in a soil. It can be relatively costly.

c. **Vitrification:** arsenic is chemically bonded inside a glass matrix forming, silicoarsenates.

d. **Soil flushing:** uses water, chemicals, or organics to mobilize arsenic in a soil and then flush it from the soil.
e. **Phytoremediation/phytoextraction:** uses plants to take up arsenic from soil.

Ex situ remediation methods

f. **Excavation:** physical removal and disposal of a contaminated soil in a designated landfill. Even though it produces rapid results, excavation is often expensive because of the operation, transport, and special landfill requirements.

g. **Soil washing/Acid extraction:** based on suspension or dissolution of arsenic in a water-based solution to concentrate the contaminant.

**Phytoremediation**

Phytoremediation includes several methods that use plants to either remove contaminants or render them harmless in soil and water systems. It can be applied to both organic and inorganic contaminants in soil and water (Salt et al., 1998). This practice has been growing in popularity, because of its overall cost-effectiveness (Watanabe, 1997; Salt et al., 1998; Kabata-Pendias and Pendias, 2001). The term phytoremediation includes the following strategies.

**Phytoextraction**

Phytoextraction is the use of contaminant-accumulating plants, which are able to extract and translocate contaminants to the harvestable parts. Phytoextraction is the most effective strategy of phytoremediation, although technically the most difficult one. It uses tolerant plants that concentrate soil contaminants in their aboveground biomass and the contaminant-enriched biomass can then be properly disposed (Kramer, 2005).
Phytostabilization

Phytostabilization refers to the use of contaminant-tolerant plants for mechanical stabilization of contaminated soils to prevent soil erosion and to reduce air-borne transport and leaching of contaminants. It is used to provide a vegetation cover for a contaminated site, thus preventing wind and water erosion (Kramer, 2005). Plants that are suitable for phytostabilization have an extensive root system, provide a good soil cover, are tolerant to the contaminants, and ideally immobilize the contaminants in the rhizosphere. Arsenic-tolerant plants that may be potentially used for phytostabilization purposes have been known for a long time (Rocovich and West, 1975; Porter and Peterson, 1977; Benson et al., 1981).

Phytomobilization

Phytomobilization is the use of plants to decrease the mobility and bioavailability of contaminants by altering soil factors that lower contaminant mobility by formation of precipitates and insoluble compounds and by sorption onto the roots. Based on the chemical similarities between arsenic and phosphorus, there may be formation of arsenic/lead compounds as shown for phosphorus/lead precipitates in the rhizosphere of Agrostis capillaris (Cotter-Howells et al., 1999). Other plant-mediated processes of arsenic immobilization at the soil-root interface involve pH reduction and oxidation of the root environment by O₂ release from roots. Doyle and Otte (1997) found accumulation of arsenic onto the iron plaque in the oxidized rhizosphere of salt marsh plants, which may provide an effective immobilization and detoxification mechanism for the plants.
**Phytovolatilization**

Phytovolatilization is the use of plants to volatilize contaminants and has been demonstrated for Hg and Se. In the case of Hg, this was achieved by genetic manipulation of plants (Rugh et al., 1996); whereas phytovolatilization of Se occurs naturally in plants (Terry and Zayed, 1994). De Souza et al. (1999) demonstrated that rhizosphere bacteria can enhance the Se volatilization and accumulation in plants. Volatilization of arsenic is also known to occur in natural environments (Frankenberger and Arshad, 2002), but rhizosphere studies have not been reported for the formation of gaseous arsenicals enhancement at the soil-root interface. Available information on arsenic volatilization for soil suggests that in the absence of plant roots, volatile compounds account only for small proportions of total arsenic (Turpeinen et al., 1999).

**Phytoextraction of Arsenic**

Arsenic is a nonessential element for plants, and inorganic arsenic species are generally phytotoxic. Under normal conditions, arsenic concentrations in terrestrial plants are usually less than 10 mg kg$^{-1}$ (Matschullat, 2000). Different plants contain different levels of arsenic. The following plants contain arsenic in an increasing order: cabbage (0.020 to 0.050 mg kg$^{-1}$) < carrots (0.040 to 0.080) < grass (0.020 to 0.160) < potatoes (0.020 to 0.200) < lettuce (0.020 to 0.250) < mosses and lichens (0.26) < ferns (1.3) (Matschullat, 2000).

An average toxicity threshold of 40 mg kg$^{-1}$ was established for crop plants (Sheppard, 1992). The chemical behavior of arsenic is largely similar to that of phosphorus in soils and plants. In all plant species tested so far, arsenate is taken up via the phosphate transport systems (Asher and Reay, 1979; Lee, 1992; Meharg and Macnair, 1992). As a phosphate analog, arsenate can replace phosphate in many biochemical
processes, thus disrupt phosphate metabolism in plants. For example, arsenate can disrupt mitochondrial oxidative phosphorylation, thus the production of nucleotide adenosine triphosphate, the main energy source for cells. This process is known as arsenolysis, or the hydrolytic process whose first step is the replacement of arsenate for phosphate (Oremland and Stolz, 2003). Unlike arsenate, arsenite reacts with sulfhydryl groups of enzymes and tissue proteins, leading to inhibition of cellular function and death (Meharg and Hartley-Whitaker, 2002).

The transfer of arsenic from soils to plants is low for most plant species. This is due to several factors including: i) the restricted uptake by plant roots, ii) the limited translocation of arsenic from roots to shoots, iii) arsenic phytotoxicity even at low concentrations in plant tissues, and iv) the low bioavailability of arsenic in soil (Wang et al., 2002).

Among phytoremediation methods, phytoextraction is the most suitable for arsenic contaminated soils. It represents one of the largest economic opportunities for phytoremediation. This is due to the size and scope of environmental problems associated with arsenic contaminated soils, minimal environmental disturbance, public acceptance and the competitive advantage offered by the plant-based remediation technology (Raskin et al., 1997; Susarla, 2002).

After harvesting arsenic-enriched plants, the weight and volume of contaminated material can be further reduced, transported, and disposed off site as hazardous material (Salt et al., 1998; Ma et al., 2001). Successful application of phytoextraction to arsenic contaminated soils depends on many factors, among which the concentration and
bioavailability of arsenic, the nutrient and water status of the soils and the capacity of the plant to access the arsenic (Figure 2-1).

The arsenic bioavailability in soils is regulated by various physical, chemical and biological processes and their interactions (Ernst, 1996). The physical characteristics of soils have a great influence on the bioavailability of arsenic. For instance, soils with very fine texture offer a physical resistance that may demand a lot of energy from the plant in order to penetrate deeper layers. In this situation, arsenic may be inaccessible for plant uptake.

![Figure 2-1. Factors that influence the efficiency of phytoextraction](image)

Arsenic speciation and chemical conditions such as soil acidity and redox potential can largely determine arsenic bioavailability (Wenzel et al., 2002). The soil biota can strongly modify the chemical and physical conditions and processes, which determine arsenic bioavailability. Rhizosphere interactions plays a key role in controlling nutrient bioavailability to crop plants (Hinsinger, 2001) as well as in the understanding of the processes occurring during phytoremediation (Lombi et al., 2001). The availability of
arsenic in a soil around the roots is strongly affected by root exudates and root
depositions (mucilage and border cells) but also by microbial activities (Lombi et al.,
2000). The processes occurring in the rhizosphere such as plant uptake, changes of pH
and redox potential, root exudation and etc. alter the chemical compositions of the soil-
root interface. It may also influence the bioavailability of arsenic in the soil and
consequently the efficiency of phytoextraction. For instance, under oxic soil conditions,
an increase in the rhizosphere pH could favor mobilization of labile and exchangeable
As(V), enhancing plant uptake (Wenzel et al., 2002). Also, nitrogen fertilization as
nitrate (NO₃⁻) can potentially increase the rhizosphere pH, and thus possibly enhance
arsenic accumulation in plant tissues.

The symbiotic association of plant roots with mycorrhizal fungi in arsenic
contaminated soils can influence its bioavailability by exploiting a greater soil volume
and by solubilizing the arsenic. Therefore, the interactions of arsenic with soil matrix and
the ability of plants to continually accumulate and detoxify arsenic in their shoot system
are essential to the phytoremediation concept.

Phytoextraction can be accomplished by using either tolerant high biomass plant
species or hyperaccumulator plant species. The growth and remediation potential has
been assessed for various plants including cottonwood (Populus deltoides Bartr.), cypress
(Taxodium distichum L.), eucalyptus (Eucalyptus amplifolia Naudin, E. camaldulensis
Dehn., and E. grandis Hill), and leucaena (Leucaena leucocephala L.), which are all
potential high biomass species. However, the use of hyperaccumulator plants has the
advantage of producing a more concentrated residue, reducing the final disposal of the
contaminant-rich biomass.
Unfortunately, most metal hyperaccumulator plants grow slowly with low biomass, while plants that produce a high biomass are usually sensitive to high metal concentrations. The energy costs of metal tolerance mechanisms are partially responsible for this phenomenon (trade-off hypothesis). There are, however, exceptions (e.g. the nickel hyperaccumulator *Berkheya coddii* and arsenic hyperaccumulator ferns), indicating the capacity of a plant to accumulate and tolerate high metal concentrations in shoots and to produce high amounts of dry matter is not always mutually exclusive (Robinson et al., 1997; Ma et al., 2001).

Plant species used in the phytoextraction of arsenic should be able to thrive in a contaminated site and at the same time accumulating a substantial amount of arsenic in the aboveground tissues. They should also be responsive to agricultural practices designed to enhance arsenic accumulation and to allow repeated planting and harvesting of arsenic-rich biomass (Tu and Ma, 2002). However, continuous phytoextraction depends on the natural ability of a plant to accumulate, translocate and tolerate high concentrations of arsenic over the entire growth cycle (Garbisu and Alkorta, 2001) as well as the arsenic bioavailability in the soil.

**Hyperaccumulator Plants**

Plants show several response patterns when growing in the presence of toxic metals, such as tolerance, indicator and hyperaccumulation (Figure 2-2). Most plants are sensitive even to low concentrations, others have developed resistance and a reduced number behave as hyperaccumulators of a metal (Baker and Brooks, 1989; Schat et al., 1999). Tolerant species are those that can grow in a soil with metal concentrations toxic to most other plants. However, they are not necessarily indicators or hyperaccumulators,
as tolerant non-accumulators can exclude metals from entering the root tissue. Both indicator species and hyperaccumulators are also tolerant (Assunção et al. 2001; Bert et al. 2003). On the other hand, hyperaccumulators take up particularly high amounts of a toxic element in their shoots during normal growth and reproduction cycle (Baker and Whiting 2002). Metal resistance in species with exclusion strategy is frequently based on reduced metal uptake into the roots, preferential storage of metals in the root vacuoles and restricted translocation into the shoots. Hyperaccumulators, in contrast, take up more metals, store a lower proportion of them in the root vacuoles, and export higher amounts to the shoots.

Hyperaccumulators are plants that can take up and concentrate greater than 0.1% of a given element in their tissue (Brooks, 1998). Metal hyperaccumulation is a rare phenomenon in terrestrial higher plants. To date, over 400 plant species have been identified as metal hyperaccumulators, representing <0.2% of all angiosperms (Brooks, 1998; Baker et al., 2000; Ma et al.’ 2001). Approximately two-thirds of the known hyperaccumulators are Ni accumulators. This is because of the widespread occurrence of Ni-rich ultramafic (serpentine) soils and the long history of geobotanical studies of ultramafic floras. Plant species that are able to hyperaccumulate Cd, Co, Cu, Pb, Zn, and As are much less numerous.

Hyperaccumulator plants have evolved internal mechanisms that allow them to take up and tolerate high concentrations of metals that would be toxic to other organisms (Lasat, 2002). They are adapted to the particular environmental conditions of their habitat and high metal accumulation may have contributed to their defense against herbivores and fungal infections (Martens and Boyd, 2002).
The definition of a hyperaccumulator has to take into consideration not only the metal concentration in the aboveground biomass, but also the metal concentration in the soil. Both bioaccumulation factor (BF) and translocation factor (TF) have to be considered while evaluating whether a particular plant is a hyperaccumulator (Ma et al., 2001). The term BF, defined as the ratio of metal concentration in plant biomass to that in the soil, has been used to determine the effectiveness of plants in removing metals from soil. The term TF, defined as the ratio of metal concentrations in the shoots to those in the roots of a plant, has been used to determine the effectiveness of plants in translocating metals from the roots to the shoots (Tu and Ma, 2002). Therefore, an arsenic hyperaccumulator plant should have BF > 1 and TF > 1 as well as total accumulation > 1,000 mg kg\(^{-1}\) arsenic in plant biomass.

![Figure 2-2](image)

**Figure 2-2.** Conceptual response of hyperaccumulator, indicator and excluder plants for metal concentrations in the aboveground tissues in relation to increasing metal concentrations in a soil. (Adapted from Ghosh and Singh (2005).

While some plants can survive in an environment containing high concentrations of metals, they may not show an ability of hyperaccumulating the metals. For example, *Agrostis tenuis* concentrated 3,470 mg kg\(^{-1}\) of arsenic when growing in a soil containing
as high as 26,500 mg kg$^{-1}$ of arsenic. Even though the plant arsenic concentration was very high, it can not be characterized as an arsenic hyperaccumulator. This is because the BF and TF were both lower than one.

**Arsenic Hyperaccumulation by *Pteris vittata***

Arsenic hyperaccumulators were discovered only recently and the majority of them are fern species in the *Pteris* genus. Ma et al. (2001) reported the first known arsenic hyperaccumulator plant, *Pteris vittata* L, commonly known as Chinese Brake fern. The plant was found on a site contaminated with the wood preservative chromated copper arsenate in Central Florida. Afterwards, other arsenic hyperaccumulator ferns have been identified in Florida and elsewhere (Francesconi et al., 2002; Zhao et al., 2002; Srivastava et al., 2005).

Other non fern plants have also been reported as “arsenic hyperaccumulators” (>1,000 $\mu$g g$^{-1}$ As) growing on mine wastes from various sites in the United Kingdom (Porter and Peterson, 1977) and on smelter wastes in northeast Portugal (De Koe, 1994). However, though accumulating large amounts of arsenic, these plants do not concentrate arsenic, i.e. arsenic concentrations in the plant are lower than those in the soil. Hence, they should not be classified as arsenic hyperaccumulators.

Although there are a number of ferns known to accumulate arsenic, *P. vittata* is by far the most studied arsenic hyperaccumulator plant. It is native from China (Nelson, 2000) but is widespread in the old world, occurring from Europe to Asia. In the U.S., this fern grows in the southeast and southern California (Ma et al., 2001). In Florida, *P. vittata* is one of the only three naturalized exotic ferns (Nelson, 2000). *Pteris vittata* ferns are very diverse and have survived in great numbers and adapted to both a vastly
changing environment and competition from seed plants. They are widespread thriving in both temperate and tropical climates (Matschullat, 2000). Also, the distribution of *P. vittata* is controlled by its requirement of a well-drained alkaline substrate exposed to abundant sunshine (Ma et al., 2001).

*Pteris vittata* has an extraordinary capacity not only to tolerate and take up arsenic but also translocate large amounts of arsenic to the fronds, with frond concentrations reaching levels up to 100-fold greater than the soil concentrations. In addition, this plant, with its high biomass, is an easy-to-grow, vigorous perennial that is resistant to disease and pests, and exhibits a high arsenic accumulation rate. While natural arsenic concentrations in most plants seldom exceed 1 mg kg\(^{-1}\) (Porter and Peterson, 1977), after 6 weeks of growth *P. vittata* accumulated 438-755 mg kg\(^{-1}\) As in a uncontaminated soil and 3,525 to 6,805 mg kg\(^{-1}\) As in a contaminated soil (Ma et al., 2001). It also removed 26% of the added arsenate after 18 weeks of growth (Tu and Ma, 2002). Liao et al. (2004) showed that, within seven months, *P. vittata* could extract up to 7.8% of the arsenic from a soil containing 64 mg kg\(^{-1}\) As.

The ability of *P. vittata* to take up high concentrations of arsenic and sequester it into aboveground portions when grown on either uncontaminated or arsenic-enriched soils implies that the fern has highly effective arsenic scavenging mechanisms. Another interesting characteristic of the ferns is their pereniality, which allows for successive cuttings of the aboveground biomass while growing in contaminated soils.

An intriguing observation was made by Tu et al. (2002). In a pot experiment, they observed that addition of 50 mg kg\(^{-1}\) arsenate to a sandy soil increased the fern biomass by 107%. After 12 weeks of growth, *P. vittata* produced more aboveground biomass in
soils containing 50 and 100 mg kg\(^{-1}\) arsenic compared to the control. The increase in plant biomass may be due to the arsenic-enhanced phosphate concentrations in the soils. However, when the soil arsenic concentration reached 200 mg kg\(^{-1}\), there was a slight decrease in fern biomass. Another interesting fact is that arsenic has been shown to leach from \textit{P. vittata} fronds as they senesce (Tu et al., 2003). This may pose a potential drawback to the use of \textit{P. vittata} in phytoremediation of arsenic contaminated soils, as the arsenic may be returned to the soil.

To date, the only non-\textit{Pteris} fern to exhibit the ability of arsenic hyperaccumulation is \textit{Pityrogramma calomelanos} (Francesconi et al., 2002). Its fronds accumulated 2,760 to 8,350 mg kg\(^{-1}\) arsenic when growing in soil containing 135 to 510 mg kg\(^{-1}\) arsenic. Interestingly, the fronds with the greatest arsenic concentration were collected from the ferns growing in the lowest arsenic concentration in the soil (135 mg kg\(^{-1}\) As) (Francesconi et al. (2002). In the case of \textit{P. vittata}, studies have shown that the arsenic concentration in the fronds increase with increasing arsenic concentration in the soil.

\textbf{Arsenic Species in \textit{Pteris vittata}}

The form of arsenic accumulated by plants is important in determining its suitability to remediate arsenic contaminated sites, because the arsenic-rich fronds will need to be disposed properly to avoid further contamination (Ma et al., 2001). \textit{Pteris vittata} has been shown to be capable of taking up both inorganic and organic arsenic species including arsenate, arsenite and monomethylarsonic acid (MMA), concentrating up to 93\% of the arsenic in the fronds (Ma et al., 2001; Kertulis et al., 2005). Research on arsenic hyperaccumulation by \textit{P. vittata} showed that arsenic exists in the plant mostly as inorganic species, and up to 94 \% of the arsenic in the fronds is present as arsenite. Similar results were observed when arsenic was supplied to the ferns in several different
forms (Zhang et al., 2002; Tu et al., 2003; Kertulis et al., 2005). Regardless of the arsenic species supplied to the fern, >90% of the total arsenic in the roots is present as arsenate, versus approximately 94% arsenite in the fronds. In both studies, low concentrations of organic arsenic were found in the fern. Similarly, in a study involving *P. calomelanos*, most of the arsenic in its fronds was present as arsenite. Only trace amounts of monomethylarsonate (MMA) and dimethylarsinate (DMA) were found in a few samples (Francesconi et al., 2002).

The uptake rates of arsenite and arsenate by *P. vittata* roots may not be equal, because the species are taken up through different mechanisms. For instance, Wang et al. (2002) found that arsenate was taken up more quickly by *P. vittata* than was arsenite, especially in the absence of phosphate. The authors suggest that this is due to arsenate being assimilated via phosphorus-suppressible uptake system in the roots.

**Arsenic Tolerance and Detoxification Mechanisms**

Plants often contain trace concentrations of contaminants of concern. At low levels, plants can usually metabolize or dispose of these compounds without significant injury. Generally, at high concentrations in soil or water, plants are not able to metabolize contaminants. However, some plants can survive and even grow well when they accumulate high concentrations of toxic elements as is the case of the hyperaccumulator plants. In ferns like *P. vittata*, arsenic is taken up at high rates and at concentrations proportional to the arsenic concentrations in the growth media at least up to a certain point where arsenic availability becomes a limiting factor and its arsenic concentration exceeds its detoxification ability (Ma et al., 2001; Zhang et al., 2002; Wang et al., 2002; Kertulis et al., 2005).
The fact that *P. vittata* could survive in a soil spiked with 1,500 mg kg\(^{-1}\) arsenic and concentrate 2.3 % of arsenic in its biomass indicates that it is equipped with efficient mechanisms for detoxifying accumulated arsenic. Mechanisms employed by plants to detoxify metals include chelation, compartmentalization, biotransformation and cellular repair (Salt et al., 1998). For example, heavy metals are generally transported and deposited in a vacuole as metal chelates. Baker et al. (2000) reported that the solution concentration of free metal ions taken up by plants into their tissues is reduced greatly when they are chelated by specific high-affinity ligands (like oxygen-donor, sulfur-donor, or nitrogen-donor ligands). Sulfur-donor ligands (like metallothioneins and phytochelatins) form highly stable complexes with heavy metals, because sulfur is a better electron donor than oxygen. In fact, the reduction of arsenate to arsenite is catalyzed by glutathione (GSH) in microbes (Rosen, 2002). However, this has not been demonstrated in *P. vittata*.

The formation of arsenite-tris-thiolate complexes has been demonstrated both *in vivo* and *in vitro* by electrospray ionization mass spectroscopy (Schmoger et al., 2000) and x-ray absorption spectroscopy (Pickering et al., 2000) with the thiolate derived from either GSH or phytochelatins (PCs). The majority of the reports on arsenic toxicity in plants show a clear role for PCs in the detoxification of arsenic (Schmoger et al., 2000; Hartley-Whitaker et al., 2001, 2002; Schat et al., 2002). Reina et al. (2005) demonstrated that PCs were the most abundant thiols in white lupin under higher arsenic exposure levels than other plants could tolerate. Together, GSH and PCs were able to complex the majority of arsenic in the shoots. However, the role of PCs seems to be minor in arsenic hyperaccumulator ferns (Zhao et al., 2003; Raab et al., 2004; Zhang et al., 2004).
As for arsenic hyperaccumulators *P. vittata* and *P. calomelanos* the reduction of arsenate to arsenide inside plant cells occurs as well (Ma et al., 2001; Francesconi et al., 2002). This reduction of arsenate inside the plant cells is intriguing because arsenide is more toxic than arsenate. Additionally, *P. vittata* was shown to have only 4.5% of its arsenic complexed with phytochelatins, as a glutathione-arsenide-phytochelatin complex (Zhao et al., 2003). In a study by Raab et al. (2004), the arsenic hyperaccumulator, *P. cretica*, had only 1% of its arsenic complexed with phytochelatins. The conclusion reached in both studies was that the phytochelatins may act as shuttles for transporting arsenic in a non-toxic form through the cytoplasm and into the vacuoles. However, arsenic complexation with phytochelatins by itself does not account for the high efficient detoxification mechanism in arsenic hyperaccumulator ferns, suggesting a novel mechanism of arsenic tolerance in *P. vittata*.

Arsenic detoxification in microorganisms includes methylation and biotransformation. Some bacteria enzymatically reduce arsenate to arsenide by Ars C, and the arsenide is then pumped out by the membrane protein Ars B (Cai and Ma, 2003). None of these mechanisms were identified in the ferns. Therefore, an important gap in the arsenic hyperaccumulation mechanism is how the ferns rapidly translocate arsenic from the roots to the fronds, and still able to survive the exceedingly high concentrations of arsenide in the fronds, which may perturb the cellular function by disrupting the sulfhydryl groups of proteins.

**Effects of Heavy Metals on Arsenic Hyperaccumulation**

Many contaminated sites contain a variety of contaminants. So another important point to be considered in phytoremediation is the ability of arsenic hyperaccumulator plants to grow in soils contaminated with other heavy metals. The ability of *P. vittata* to
survive in soil contaminated with Cu and Cr is confirmed by the fact that this fern was first discovered in a soil contaminated with CCA (chromated-copper-arsenate). The total arsenic in that soil was 131 mg kg\(^{-1}\), Cr was 40.6 mg kg\(^{-1}\), and Cu was 8.30 mg kg\(^{-1}\).

Fayiga et al. (2004) studied the effects of Cd, Ni, Pb, and Zn on the arsenic accumulation by *P. vittata*. They observed the concentrations of heavy metals (50 and 200 mg kg\(^{-1}\)) negatively affect the growth of the fern. Still total plant biomass increased by 12-fold after 8 weeks of growth.

However, the efficiency of arsenic accumulation by the fern depended on the concentration of the metals in the soil (Fayiga et al., 2004; An et al., 2006). In the study of Fayiga et al. (2004), arsenic uptake decreased with an increase in the metal concentration except in Pb-treated soils. Though effective in taking up arsenic, *P. vittata* had a limited capability to take up other metals. Arsenic transfer factors ranging from 15.8 to 46.1, indicating that *P. vittata* was able to effectively translocate arsenic in the presence of other metals. Caille et al. (2004) reported that *P. vittata* grew poorly in a soil heavily contaminated with As, Cu, Pb and Zn, probably as a result of Zn and Cu toxicity. The concentrations of Zn and Cu in the plants were well above the toxicity thresholds of 100-500 mg kg\(^{-1}\) Zn and 20 mg kg\(^{-1}\) Cu, respectively (Kabata-Pendias and Pendias, 2001). Phytotoxicity of Zn and Cu not only decreased plant growth, but also arsenic uptake by *P. vittata*, resulting in a negligible phytoextraction of arsenic from the soil.

Contamination of multiple metals or metalloids, particularly at high concentrations, thus presents a challenge for phytoremediation.

**Effect of Phosphate on Arsenic Hyperaccumulation**

The competitive effect of arsenate and phosphate in soils (Livesey and Huang, 1981; Manning and Goldberg, 1997; Smith et al., 2002; Tu and Ma, 2003) and their
interactions in plants (Asher and Reay, 1979; Meharg and Macnair, 1992; Fourquerean and Cai, 2001) have been demonstrated. Basically, the uptake of arsenate and phosphate by plants has been reported to be competitive (Tu and Ma, 2003). Moreover, arsenate interferes with phosphate metabolism causing toxicity in plants. On the other hand, phosphate may be able to alleviate arsenate toxicity by improving phosphate nutrition (Sneller et al., 1999). Thus, the presence of phosphorus in contaminated soils plays a role in the phytoextraction process.

Tu and Ma (2003) studied the influence of phosphate on the arsenic phytoextraction by *P. vittata*. They observed that, at low and medium arsenate levels (50 to 200 mg kg$^{-1}$, respectively), phosphate had slight effect on arsenate accumulation by and growth of *P. vittata*. However, phosphate substantially increased plant biomass and arsenate accumulation by alleviating arsenate phytotoxicity at high arsenate levels (400 mg kg$^{-1}$). The authors suggested a minimum P/As molar ratio of 1:2 in soil solution for effective removal of arsenic by the plant.

Fayiga et al. (2006) reported that addition of phosphate rock in a soil spiked with arsenic and other metals increased plant arsenic uptake, from 608 to 1,046 mg kg$^{-1}$ in the fronds. This shows the ability of phosphate rock to aid in arsenic accumulation by *P. vittata* in a multi-metal system by reducing the toxic effects of the metals on the fern; thereby, enhancing plant arsenic uptake. Boisson et al. (1999) also reported an increase in arsenic uptake by plants (*Zea mays* cv. Volga and *Phaseolus vulgaris* cv. Limburgse vroege) after applying hydroxyapatite to a soil contaminated with Zn, Pb, Cu, Cd and As. They suggested that the increased phosphate concentration in the soil solution might be
responsible for increased plant uptake, since phosphate can displace adsorbed arsenate from soils and increase the availability of arsenate (Smith and Naidu, 1998).

In a chromated-copper-arsenate contaminated sandy soil, addition of a large amount of rock phosphate (15 g kg\(^{-1}\)) also increased arsenate uptake by *P. vittata* (Cao et al., 2003). Therefore, phosphorus additions, in the first place, enhanced plant growth, and secondly, mobilized exchangeable arsenic resulting in an increased total arsenic uptake.

Despite the appeal of increased arsenic availability from application of soil amendments such as phosphorus fertilizers for the purpose of phytoremediation, it is environmentally paradoxical in that the benefit of enhanced arsenic removal from soils may be offset by the risk of increased arsenic leaching into ground water. Therefore, the rate of arsenic uptake should be higher than the rate of arsenic release in the soil. In this regard, a very extensive root system is a valuable characteristic in a hyperaccumulator plant.

**Mycorrhizae Association and its Role in Arsenic Hyperaccumulation**

Mycorrhizae have a well-documented role in increasing the plant uptake of phosphorus (Smith and Read, 1997) and other poorly mobile elements, and are recognized as an important component of bioremediation strategies for soils contaminated with heavy metals (Khan et al., 2000). In fact, mycorrhizae are integral, functional components of plant root systems and the fungi involved can play an important role either in alleviating metal toxicity in plants or enhancing metal uptake. For instance, Weissenhorn and Leyval (1995) reported higher concentrations of heavy metals in plants, even resulting in toxic levels, due to arbuscular mycorrhizae (AM) colonization, while Heggo et al. (1990) observed reduced metal concentrations in the shoots due to
mycorrhizal colonization. On the other hand, Galli et al. (1995) found no effects exerted by AM fungi on metal concentrations in plant shoots and roots.

Since arsenic and phosphorus are chemical analogues, it is expected that mycorrhizal symbiosis may be involved in arsenic uptake as well as P uptake by arsenic hyperaccumulator plants. However, few studies have been done in this regard. Ying et al. (2004) investigated the colonization and diversity of AM fungi associated with common pteridophytes and found no defined relationship between mycorrhizae and plants under field condition. Out of all arsenic hyperaccumulator ferns, only association of *P. vittata* with mycorrhizae has been investigated (Alagely et al., 2005; Leung et al., 2006).

In a study on the effect of increasing levels of arsenic and phosphorus on *P. vittata* infected with mycorrhizae, Al Agely et al. (2005) observed that the AM fungi not only tolerated arsenic amendment, but its presence increased the frond biomass at the highest arsenic application rate (100 mg kg\(^{-1}\)). The AM fungi also increased arsenic uptake across a range of phosphorus levels, while phosphorus uptake was generally increased only when there was no arsenic amendment, suggesting an important role of AM fungi in arsenic accumulation by *P. vittata*.

The percentage of mycorrhizal infection in *P. vittata* increased as soil arsenic concentrations increased (Leung et al., 2006). The authors also reported an increase in arsenic accumulation by *P. vittata* infected with mycorrhizae and improvement of the nutrient status of the plants. The presence of mycorrhizae likely increased the amount of P transporters at hyphae level and, consequently, the amount of arsenic taken up by the plant. The presence of mycorrhizae also seems to be related to the formation of thiol like glutathione (Schutzendubel and Andrea, 2002), a phenolic defense system. It is
hypothesized that the production of thiols by *P. vittata*, induced by the increased arsenic concentration, alleviated the toxicity of arsenic (Cai et al., 2004) and the plant can readily retain arsenic with the aid of mycorrhizae (Diaz et al., 1996). Therefore, it is possible that *P. vittata* may derive some benefit from the symbiosis by forming low molecular weight thiols.
CHAPTER 3
ARSENIC ACCUMULATION BY *Pteris vittata* L FROM ARSENIC CONTAMINATED SOILS

**Introduction**

Arsenic contamination in soils, groundwater, and drinking water is a serious concern as it can affect both human and animal health. The extensive use of arsenic compounds such as pesticides, insecticides, defoliants, wood preservatives, and soil sterilants in the past (Azcue and Nriagu, 1994) has left a legacy in the history of arsenic contamination in the environment and a burden for the future generations.

For instance, pesticides containing arsenic trioxide were largely used in cattle dip vats in the southeastern USA to eradicate cattle fever tick resulting in many soils contaminated with arsenic (Thomas *et al.*, 2000). The production of the wood preservative containing chromated-copper-arsenate (CCA) represented 67 % of the arsenic used in 1992. Besides the contamination of soils in the sites of operation, CCA-treated wood can potentially contaminate soils through the leaching of arsenic from the wood. Smelting and mining activities represent significant sources of arsenic contamination because pyrometallurgical production processes lead to large emissions of Pb, Zn, Cu, Cd and As (Boisson *et al.*, 1999). Moreover, herbicides containing monosodium methane arsonate (MSMA) are still used for weed control in Florida (Cai *et al.*, 2002).

The use of plants to remove arsenic from soils is a fairly new technology. It is an efficient and less costly way to treat contaminated soils. Hyperaccumulator plants possess
highly efficient mechanisms to acquire and concentrate arsenic in the plants. However, not all contaminated sites are suitable to be treated by means of phytoextraction. This is because the arsenic concentration in a soil, its availability and the depth of arsenic contamination can be limiting factors. Moreover, the plant to be used for this purpose must be tolerant to arsenic, adapted to the local soil and climate characteristics and take up a large amount of arsenic.

During phytoextraction process, several crops of hyperaccumulator plants may be needed to sequentially reduce soil arsenic concentration (Raskin et al., 1994). However, continuous phytoextraction depends on the natural ability of a plant to accumulate, translocate and tolerate high concentrations of metals over the whole growth cycle (Garbisu and Alkorta, 2001).

*Pteris vittata* is a fern species identified by Ma et al. (2001) as an efficient arsenic-hyperaccumulator plant. Because it hyperaccumulates an extremely high level of arsenic (up to 2.3%) in its aboveground biomass and it is easy to grow in a variety of soil environments, this plant has the potential to clean up arsenic contaminated sites nationwide, and potentially worldwide. Furthermore, the perennial nature of *P. vittata* make the phytoextraction process even more cost-effective since no replanting after harvest is needed. However, practical issues such as the time required to achieve a given target level, the long term efficiency of the process, and the arsenic pools depleted by the plant still need to be addressed.

The aim of this study was thus to 1) assess the efficiency of repeated harvests of the fronds of *P. vittata* growing in soils with different sources of arsenic contamination, and 2) investigate the effects of plant arsenic uptake on arsenic redistribution in soils.
Materials and Methods

Soil Collection and Characterization

Five arsenic contaminated soils plus a soil with naturally high arsenic (Marl soil) were used for this study. The soils were: contaminated with arsenical insecticide (Avon soil), arsenical wood preservative (CCA soil), arsenical pesticide (CDV soil), arsenical herbicide (EDS soil), and mining activities (Mining soil). Selected properties of the soils are shown in Table 3-1. The soils were collected, air-dried, and passed through 2 mm sieve. They were analyzed for: CEC by the ammonium acetate method (Thomas, 1982), organic matter content by the Walkley Black method (Nelson and Sommers (1982), soil texture by the pipette method (Day, 1965) and soil pH in 1:2 soil: water. Concentrations of P, Ca, Mg, and trace elements were determined using the EPA Method 3050.

Experimental Design and Statistical Analyses

Six soils with different sources of arsenic contamination were used to grow *P. vittata*. Each treatment was replicated four times and pots without plants were included as controls. The pots were arranged in a completely randomized design.

SAS software was used for all statistical analyses (SAS Institute, 1987). Means and standard deviations of arsenic concentrations were calculated for different treatments. Analysis of variance was used to assess significant differences among treatments.

Experimental Set up

*Pteris vittata* used for the experiments were of similar age and size. One-year-old ferns were transferred (1 per pot) to 2- gallon-size plastic pots filled with 4 kg of arsenic contaminated soil. The soils were thoroughly mixed with Osmocote, extended time-release base fertilizer (N-P-K = 18-6-12) at a rate of 2 g kg\(^{-1}\) soil. The fertilization was
applied annually. After transplanting, the ferns were watered to 60 % of the field capacity.

Table 3-1. Selected chemical and physical characteristics of six arsenic contaminated soils

<table>
<thead>
<tr>
<th>Soil characteristic</th>
<th>Marl</th>
<th>Avon</th>
<th>CCA</th>
<th>CDV</th>
<th>EDS</th>
<th>Mining</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.85</td>
<td>6.70</td>
<td>7.00</td>
<td>6.76</td>
<td>6.70</td>
<td>6.75</td>
</tr>
<tr>
<td>CEC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.6</td>
<td>4.50</td>
<td>4.40</td>
<td>16.8</td>
<td>22.8</td>
<td>12.0</td>
</tr>
<tr>
<td>OM (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.80</td>
<td>19.6</td>
<td>11.0</td>
<td>26.5</td>
<td>28.0</td>
<td>4.20</td>
</tr>
<tr>
<td>Total As (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22.2</td>
<td>26.5</td>
<td>110</td>
<td>211</td>
<td>640</td>
<td>214</td>
</tr>
<tr>
<td>Extractable As&lt;sup&gt;c&lt;/sup&gt; (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22.7</td>
<td>14.8</td>
<td>58.8</td>
<td>45.9</td>
<td>499</td>
<td>22.5</td>
</tr>
<tr>
<td>Extractable P (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.36</td>
<td>31.9</td>
<td>24.8</td>
<td>19.2</td>
<td>96.0</td>
<td>8.07</td>
</tr>
<tr>
<td>Extractable Ca (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>21000</td>
<td>1892</td>
<td>2960</td>
<td>2040</td>
<td>1602</td>
<td>4920</td>
</tr>
<tr>
<td>Extractable Mg (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>120</td>
<td>28.8</td>
<td>130</td>
<td>64.8</td>
<td>336</td>
<td>63.6</td>
</tr>
<tr>
<td>Fe oxides (mg kg&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.96</td>
<td>509</td>
<td>1322</td>
<td>5577</td>
<td>1117</td>
<td>1626</td>
</tr>
<tr>
<td>Al oxides (mg kg&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.23</td>
<td>632</td>
<td>884</td>
<td>1163</td>
<td>1056</td>
<td>179</td>
</tr>
<tr>
<td>Sand (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>401</td>
<td>866</td>
<td>882</td>
<td>840</td>
<td>851</td>
<td>807</td>
</tr>
<tr>
<td>Silt (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>410</td>
<td>89</td>
<td>91</td>
<td>140</td>
<td>117</td>
<td>152</td>
</tr>
<tr>
<td>Clay (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>189</td>
<td>45</td>
<td>27</td>
<td>20</td>
<td>32</td>
<td>41</td>
</tr>
</tbody>
</table>

a: CEC: cation exchange capacity (Cmolc kg<sup>-1</sup>)

b: Amorphous Fe and Al extracted by 0.2 M oxalic acid + ammonium oxalate solution

c: Extractable elements were obtained using Mehlich III solution.

The first harvest was performed in the month of October, 2003, four months after the transplant. The second (April, 2004) and third (October, 2004) harvests were performed in a six months interval from the first harvest and from each other. The plants aboveground biomass was analyzed for dry biomass weight and total arsenic concentration.
**Sampling, Digestion and Analysis**

The plant samples were dried in a 65°C oven for approximately 48 hours, weighed and then ground into powder through a 1-mm mesh screen using a Wiley Mill for digestion. Also, soil samples collected together with the plant samples were air-dried and analyzed.

The soil samples were digested according to the EPA Method 3051a and the plant samples a modified EPA Method 3051 (Chen and Ma, 1998; Komar et al., 1999). One blank, one Standard Reference Material from National Institute of Standards and Technology (NIST), one duplicate and one spiked sample were included for every 20 samples. Soil and plant samples were digested with nitric acid using a Hot Block digestion system (Environmental Express, Mt. Pleasant, SC; EPA Method 3051).

Approximately 0.5-1.0 g of air-dried soil or 0.1–0.5 g of dry plant sample were mixed with 1:1 HNO₃ and allowed to set for approximately 24 hours. They were heated at 105°C for 2 hours and then cooled for 3 minutes. The samples were mixed with 1 ml of 30% H₂O₂ and placed on the block digester for 15 additional minutes. After the second heating, the samples were cooled completely and diluted to a 50 mL volume with distilled water. A filter cartridge was placed in the bottom of the digestion tube. The digested samples were analyzed for As concentration with a SIMMA 6000 graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin-Elmer, Norwalk, CT) using the EPA SW 846 method 7060 A.

**Arsenic Fractionation**

Since it is assumed that plant arsenic uptake will substantially reduce soil arsenic availability, it is important to determine how plant arsenic uptake affects arsenic
redistribution in a soil. A sequential extraction procedure was used to account for the changes in different soil arsenic-pools as a function of plant uptake. According to Wenzel et al. (2001), soil samples were fractionated into five arsenic fractions with decreasing availability: (N) non-specifically bound, (S) specifically bound, (A) amorphous hydrous oxide-bound, (C) crystalline hydrous oxide-bound and (R) residual (Table 3-2). Samples from each fraction, with the exception of the residual fraction, were centrifuged at 3,500 rpm for 15 min and 20°C after each extraction and/or wash. The supernatants were collected and filtered through Whatman 42 filter paper and analyzed for arsenic concentration using GFAAS.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Extracting solution</th>
<th>Extraction condition</th>
<th>SSR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N- Non-specifically-bound</td>
<td>(NH₄)₂SO₄ 0.05M</td>
<td>4 h shaking, 20°C</td>
<td>1:25</td>
</tr>
<tr>
<td>S- Specifically-bound</td>
<td>(NH₄)H₂PO₄ 0.05M</td>
<td>16 h shaking, 20°C</td>
<td>1:25</td>
</tr>
<tr>
<td>A-Amorphous hydrous oxide-bound</td>
<td>NH₄-oxalate buffer (0.2 M) pH 3.25</td>
<td>4 h shaking, 20°C</td>
<td>1:25</td>
</tr>
<tr>
<td>C-Crystalline-hydrous oxide-bound</td>
<td>NH₄-oxalate buffer (0.2 M) + ascorbic acid (0.1M) pH 3.25</td>
<td>30 min in a water basin at 96 ±3 °C in the light</td>
<td>1:25</td>
</tr>
<tr>
<td>R-Residual</td>
<td>HNO₃/H₂O₂</td>
<td>Hot block digestion</td>
<td>1:50</td>
</tr>
</tbody>
</table>

*SSR=Soil solution ratio

**Single Extractions**

Water-extractable arsenic was obtained using a 1:10 soil to deionized water ratio, shaking for two hours and centrifuging at 3500 rpm for 15 min (Olsen and Sommers, 1982). Mehlich III method (Mehlich, 1984) was commonly used as a standard procedure in soil testing to assess the amount of P available in the soil for growing plants. As a phosphorus analogue, this method was chosen to correlate plant arsenic availability in
soil as well. Mehlich III extractable arsenic was obtained using Mehlich III extracting solution (0.2 \( M \) \( \text{CH}_3\text{COOH} \), 0.25 \( M \) \( \text{NH}_4\text{NO}_3 \), 0.015 \( M \) \( \text{NH}_4\text{F} \), 0.013 \( M \) \( \text{HNO}_3 \) and 0.001 \( M \) \( \text{EDTA} \)) after shaking for 5 min. Arsenic was also extracted with 1.6 mM organic acids (phytic acid -1.4mM + oxalic acid - 0.2 mM), in a 1:20 soil to solution ratio, shaking for 24 hours and centrifuging at 3500 rpm for 15 min. The concentrations of organic acids used in this study were similar to those reported by Tu et al. (2004) in the root exudates of \( P. \text{vittata} \). All the extracts were filtered through Whatman 42 filter paper and analyzed for arsenic concentration using GFAAS.

**Results and Discussion**

**Plant Growth**

The aboveground biomass production and the regrowth capacity are important factors in the phytoextraction of arsenic using perennial plants since multiple harvests will be needed in order to remove the arsenic from the soil (Fayiga and Ma, 2005).

Despite the different soil properties and arsenic concentration among the six soils, the ferns grew well in all six soils during the first four months of the experiment (June-October 2003), without showing any toxicity symptom (Figure 3-1). The plants biomass was uniform in all soils ranging from 24.8 to 33.5 g plant\(^{-1}\), greater than the biomass data reported for \( P. \text{vittata} \) growing in pot experiments (Tu and Ma, 2002; Fayiga et al., 2004). It should be pointed out that the 12-month-old plants used in this study were small, with 5-6 fronds.

There was a significant reduction in the frond biomass production in the subsequent harvests, except for the ferns growing in the Marl and Mining soils in the third harvest. For all soils, there was a significant decrease in the frond biomass in the
second harvest (40, 81, 82, 84, 79, and 71 % for the Marl, Avon, CCA, CDV, EDS and Mining soils, respectively), likely due to the seasonal effect (cooler climate from October to April). For all soils, October harvests yielded larger plant biomass than the April harvest, except for the Marl and EDS soils.

Figure 3-1. Frond biomass of *Pteris vittata* after first, second and third harvests in six soils. Means followed by the same letter within the same soil are not different by the Duncan test at p < 0.05.

Due to the alkaline nature of the Marl soil, condition that favors the fern growth, dry matter yield was comparable in all three harvests. On the other hand, plants drastically failed to regrow in the EDS soil after the first harvest, probably because of the extremely high solubility of arsenic in this soil (Table 3-1).

Even though *P. vittata* has shown to be very tolerant to arsenic exposition, Tu and Ma (2002) reported that addition of 500 mg kg\(^{-1}\) As to a sandy soil reduced the fern
biomass by 64 %, a common symptom of arsenic toxicity (Kabata Pendias and Pendias, 2001). However, in their study, addition of 200 mg kg⁻¹ As did not affect biomass production, suggesting a much higher tolerance of *P. vittata* as compared to normal plants. The difference in arsenic concentrations in the Avon, CCA and CDV soils (Table 3-1) did not influence the dry matter yield since all had the same trends among different harvests (Figure 3-1). However, the better performance of the CCA soil was likely due to the higher pH and Ca concentration, and also due to the fact that this was the soil where *P. vittata* was found naturally growing (Ma et al., 2001).

Overall, the regrowth capacity of the plants was related to the temperature effect. That is, the difference in plant performance between harvests is due to colder temperature prevailing during December to April. According to Jones (1998), *P. vittata* prefers warmer climate. In a field experiment, Kertulius (2005) also reported greater biomass production when *P. vittata* plants were harvested in the months of October and December. Another factor that, associated with the temperature, could also explain the poor regrowth capacity of the plants after the first harvest was the clipping procedure. All the fronds, including the coiled young fronds (fiddleheads), were removed in the first harvest. This did not happen in the second harvest.

The effect of successive fern cultivation on plant biomass and arsenic removal by *P. vittata* is relatively scarce and inconsistent. For instance, pot experiments using *P. vittata* have shown that the amount of biomass harvested decreased after two or three successive cuttings as well as the amount of arsenic extracted from a soil (McGrath et al., 2002). However, Kertulius (2005) found positive results when growing *P. vittata* under field conditions. Likely, the exploitation of a greater soil volume and the amount of
residue produced at field condition may account for some of the differences between the studies in pot (McGrath et al., 2002) and field condition (Ketulius, 2005).

**Arsenic Concentrations in the Frond Biomass**

The total arsenic concentration in the soils used in this study varied from 22 mg kg\(^{-1}\) in the Marl soil to 640 mg kg\(^{-1}\) in the EDS soil (Table 3-1). The arsenic concentration in the EDS soil was greater than the arsenic level of 500 mg kg\(^{-1}\) used by Tu and Ma (2002), which was spiked as arsenate. Despite the high arsenic level, the plants grew well and took up arsenic from all soils.

The frond arsenic concentrations ranged from 166 to 6,151 mg kg\(^{-1}\) in the first harvest, from 110 to 3,056 mg kg\(^{-1}\) in the second harvest, and from 162 to 2,139 mg kg\(^{-1}\) in the third harvest (Table 3-3). The highest arsenic concentrations in plants were observed in the EDS (from 2139 to 6151 mg kg\(^{-1}\)), CDV (477 to 1872 mg kg\(^{-1}\)) and Mining (423 to 1079 mg kg\(^{-1}\)) soils (higher soil arsenic concentrations) and the lowest in the Marl (110 to 166 mg kg\(^{-1}\)) soil (the lowest soil arsenic concentration).

Even though the Marl and Avon soils had similar arsenic concentrations, the plants took up more arsenic from the Avon soil.

There was no significant difference in the frond arsenic concentrations of *P. vittata* in the first and third harvest for all soils, except for the CDV and EDS soils. However, cooler climate conditions not only affected plant biomass production but also plant arsenic accumulation. Fern frond arsenic concentrations were 34, 46, 51, 75, 61 and 50% lower in the second harvest compared to those in the first harvest for the Marl, Avon, CCA, CDV, Mining and EDS soils, respectively (Table 3-3).
Table 3-3. The frond arsenic concentrations of *Pteris vittata* after the first, second and, third harvest in six soils.

<table>
<thead>
<tr>
<th>Soil</th>
<th>As levels mg kg⁻¹</th>
<th>Frond arsenic (mg kg⁻¹)</th>
<th>Harvest 1</th>
<th>Harvest 2</th>
<th>Harvest 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marl</td>
<td>22</td>
<td>166 a</td>
<td>110 b</td>
<td>162 a</td>
<td></td>
</tr>
<tr>
<td>Avon</td>
<td>27</td>
<td>336 a</td>
<td>181 b</td>
<td>280 a</td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td>110</td>
<td>659 a</td>
<td>325 b</td>
<td>747 a</td>
<td></td>
</tr>
<tr>
<td>CDV</td>
<td>211</td>
<td>1872 a</td>
<td>477 b</td>
<td>715 b</td>
<td></td>
</tr>
<tr>
<td>Mining</td>
<td>215</td>
<td>1079 a</td>
<td>423.4 b</td>
<td>962 a</td>
<td></td>
</tr>
<tr>
<td>EDS</td>
<td>638</td>
<td>6151 a</td>
<td>3056 b</td>
<td>2139 b</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter in a row and within the same soil are not different by the Duncan test at $P < 0.05$ within the same soil.

The frond arsenic concentrations increased with soil arsenic concentrations, in all three harvests with a correlation coefficient of 0.97-0.98. However, the ability of the plants to remove arsenic from the soils reduced with time. The highest arsenic concentration (6,151 mg kg⁻¹) was observed in the plants growing in the EDS soil, which also had the highest soil arsenic concentration, both total and extractable (638 and 498 mg Kg⁻¹ As, respectively; Table 3-1).

The high organic matter content of the EDS soil likely plays a role in the availability of arsenic. However, little information is known on arsenic reactions with organic matter. In fact, it is suggested that soil organic matter contributes very little to the arsenic sorption in soils due to their anionic nature (Livesey and Huang, 1981; Wenzel et al., 2001a). Therefore, considering the sandy nature and arsenic concentration of the EDS soil, phytoextraction might not be a good alternative for this soil, especially if there is any
risk of arsenic exposure. Besides the long time required to cleaning up, there is the risk of potential arsenic leaching.

**Plant Arsenic Removal from Soils**

*Pteris vittata* had significantly higher arsenic accumulation (arsenic concentration X plant biomass) in the first harvest as compared with the second and third harvest from the Avon, CDV and EDS soils (Figure 3-2). Therefore, the first harvest accounted for most of the arsenic removed from these soils. In the Marl, CCA and Mining soils, the accumulation of arsenic in the first harvest was similar to that of the third harvest.

![Figure 3-2. Arsenic accumulation by P. vittata after first, second and third harvests in six soils. Values represent mean ± standard deviation (n=4).](image)

If the Mining soil is not considered, arsenic accumulation followed the trend arsenic concentrations in the soils. The total arsenic removed by the plants varied from 1.71 to 4.54 mg pot\(^{-1}\) in the Marl soil and from 17.0 to 175 mg pot\(^{-1}\) in the EDS soil.

More specifically, the plants removed 10, 13.5, 42.7, 70.9, 53.3, and 210 mg arsenic per
pot from the Marl, Avon, CCA, CDV, Mining and EDS soils, respectively (Figure 3-2) after the three harvests. Even though arsenic hyperaccumulation did occur in the ferns growing in the EDS soil, the rate of arsenic removal was low relative to the amount of soluble and extractable arsenic in this soil.

The percentage of arsenic removed from each soil by the fern uptake was used as a parameter of relative comparison of the soils with a wide range of arsenic concentration. For instance, the plants removed 8.2 % of arsenic from the EDS soil (higher arsenic content) after the three harvests, while the plants growing in the Marl and Avon soils (soils with the lowest arsenic content) removed approximately 12 % of the soil arsenic. The lower percent of arsenic removal from the EDS soil as compared to the Marl and Avon soils was likely due to the failure of the plant in regrowing after the first harvest. Similarly, the percent of arsenic removed from the CCA soil (9.5 %) was greater than from the Mining soil (5.8 %). In this case, despite the similar growth of the plants in both soils, most of the arsenic in the Mining soil is unavailable to the plants.

In general, the accumulation of arsenic by \textit{P. vittata} translates to arsenic reduction of 6.4 to 13 % (Figure 3-3). In spite of the greater amounts of arsenic extracted from the first and third harvest as compared to the second harvest, it was still difficult to project the time required for soil arsenic reduction to meet the required limits because the plants failed to regrow after the third harvest.

\textbf{Soil Arsenic Distribution}

Despite all the criticisms to the fractionation procedures, it is important in environmental research because it may provide useful information on the reactivity, mobility and availability of the elements (Wenzel et al., 2001).
Figure 3-3. Arsenic removed from the soil by *Pteris vittata* after three harvests. Bars represent mean ± standard deviation (n=4).

Figure 3-4 shows arsenic distribution in the five fractions based on sequential extraction before and after plant growth. Among the five fractions, the N and S fractions are considered to be the most plant-available, whereas R the least plant-available. The sum of N and S fractions constituted 17.5, 22.0, 32.1, 21.7, 4.33 and 46.3 % of the arsenic in the Marl, Avon, CCA, CDV, Mining and EDS soils, respectively (Figure 3-4). As expected, arsenic in these soils was primarily associated with the A fraction, ranging from 40.0 to 58.7 %, except for the Marl soil which had a substantial amount of the arsenic (59.1%) associated with the C fraction and only 9.28 % associated with the N+S fraction. In the Mining soil, the A and C fractions constituted 84.3 % of the total arsenic. In the EDS soil, the majority of the arsenic was distributed among the first three fractions in the order A > S > N, further explaining the high arsenic availability in this soil (499 out of 640 mg kg⁻¹ total As was extractable As).
Figure 3-4. Arsenic concentrations (mg kg\(^{-1}\)) in different fractions in soils before and after plant growth. Values represent mean ± standard deviation (n=4). N= non-specifically bound, S= specifically bound, A= amorphous hydrous oxide-bound, C= crystalline hydrous oxide-bound and R= residual fraction.

Wenzel et al. (2001) fractionated arsenic in 20 arsenic contaminated soils and also showed that most of the arsenic was associated with the amorphous fraction. The different soils with different sources of arsenic contamination used in this study confirmed that, regardless of the source or form of arsenic that enter the soil, arsenic reacts with soil components and becomes less available. It seems that the predominant reactions occur with hydrous Fe and Al oxides which mostly coat soil particles (Pierce and Moore, 1980; Smith et al., 1998).
Plant growth influenced arsenic mobilization from all five arsenic fractions in the soils, except the residual fraction, which is the most stable one. Among the fractions, the $A$ fraction contributed the most for the arsenic reduction (45.3, 48.0, 72.0 and 59.0 %) for the Avon, CCA, CDV and mining soils, respectively (Table 3-4). This was not observed for the Marl and EDS soils (Figure 3-4; Table 3-4). For instance, in the Marl soil, the highest arsenic mobilization occurred in the $C$ fraction, which was also the fraction with the highest arsenic concentration. Even though the greatest arsenic mobilization in the EDS soil occurred in the $S$ fraction (46.4 %), both the $N$ and $A$ fractions also contributed to a great extent. The treatments without plants showed that the arsenic mobilization was primarily a result of plant growth, although some arsenic mobilization was also observed in the absence of plants. This was probably due to wetting and drying cycles during the experimental period.

Although arsenic has a high affinity to soil, when the environmental condition changes, the mobility and even speciation of arsenic can change. For instance, the presence of plants leads to the exudation of some organic anions into a soil, which directly impacts the mobilization of arsenic in the soil.

Total arsenic concentration is not a good predictor of its bioavailability because only arsenic dissolved in water can be transported to the roots and taken up by plants (Ritchie and Sposito, 1995). Plants tend to first take up the most available fraction of arsenic from the soils and as this pool becomes smaller, some of the arsenic from other fractions will be slowly transformed to water-soluble fraction to reestablish their equilibrium (McGrath et al., 2000).
Table 3-4. Arsenic reduction (%) in each fraction in six soils as a result of plant arsenic uptake by *P. vittata*.

<table>
<thead>
<tr>
<th>Soil</th>
<th>As removed (mg pot⁻¹)</th>
<th>N¹</th>
<th>S</th>
<th>A</th>
<th>C</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marl</td>
<td>9.93</td>
<td>13.9</td>
<td>9.65</td>
<td>3.47</td>
<td>62.3²</td>
<td>10.6</td>
</tr>
<tr>
<td>Avon</td>
<td>13.5</td>
<td>20.9</td>
<td>27.8</td>
<td>45.3</td>
<td>4.71</td>
<td>1.28</td>
</tr>
<tr>
<td>CCA</td>
<td>41.9</td>
<td>15.0</td>
<td>16.3</td>
<td>47.9</td>
<td>16.2</td>
<td>4.60</td>
</tr>
<tr>
<td>CDV</td>
<td>72.1</td>
<td>5.60</td>
<td>18.9</td>
<td>71.6</td>
<td>1.85</td>
<td>2.03</td>
</tr>
<tr>
<td>Mining</td>
<td>50.0</td>
<td>1.17</td>
<td>7.18</td>
<td>58.7</td>
<td>26.9</td>
<td>5.98</td>
</tr>
<tr>
<td>EDS</td>
<td>210</td>
<td>28.0</td>
<td>46.4</td>
<td>21.2</td>
<td>3.26</td>
<td>1.56</td>
</tr>
</tbody>
</table>

¹ N= non-specifically bound, S= specifically bound, A= amorphous hydrous oxide-bound, C= crystalline hydrous oxide-bound and R= residual fraction.
² the fraction with the highest arsenic reduction for a given soil was in bold font.

As plants take up more arsenic, there is less available arsenic present in the soils, resulting in reduced available arsenic in soils and reduced plant arsenic uptake. Therefore, it is reasonable to assume that, only arsenic in N fraction can be readily taken up by the plant and arsenic in other fractions has to convert to N fraction before being taken up by the plant. In other words, N fraction acts as both a source of arsenic for the plant and a sink for arsenic in the soil, thus linking arsenic in the soil with the arsenic taken up by the plant.

High proportion of the total arsenic in historically polluted soils is mostly present in the residual fraction (Voigt et al., 1996; Kavanagh et al., 1997). Thus, most of the schemes commonly used tend to underestimate arsenic availability (Gleyzes et al., 2001). However, the present results show that the presence of arsenic in the residual fraction was low, even in the soils where the presence of arsenic is of natural origin, such as Marl and Mining soils. This could indicate that the scheme we used seems to be more suitable for the study of arsenic fractionation in these polluted soils.
Comparison of Extractable Arsenic Using Single Extraction Methods

In this study, three single extraction methods were used in order to relate arsenic availability in soils and plant uptake. The arsenic concentrations extracted with these methods are shown in Table 3-5. Generally, the extractable arsenic followed the descending order of Mehlich III > organic acids > water-soluble in all soils. When calculated in percent of the total arsenic determined using the EPA 3050 method, the water soluble arsenic, Mehlich III and organic acid extractable arsenic represented 1.62, 102 and, 28.2 % (Marl), 5.81, 54.8 and, 49.3 % (Avon), 5.32, 53.4, 12.1% (CCA), 0.06, 21.7 and, 18.9 % (CDV), 1.46, 10.5 and, 3.96 % (Mining) and 21.3, 78 and 38 % (EDS), respectively. The Mehlich III was expected to extract more arsenic than the water and the organic acids, irrespective the soil because of its chemical composition, a dilute acid-fluoride-EDTA solution at pH 2.5. The result showed that the presence of low molecular weight organic acids in soils can be potentially dangerous as it increase the mobility and availability of arsenic in the soil. For instance, in the Avon and CDV soils, the organic acid extractable arsenic was comparable to the Mehlich III extractable arsenic. Therefore, the presence of typical plants that do not hyperaccumulate arsenic in arsenic contaminated soils has the potential to mobilize arsenic from the soil since the exudation of organic acids is a common phenomenon in the rhizosphere of the plants.

Plant uptake and Arsenic Availability in Soils as Measured by Single Extractants

Arsenic phytoextraction from contaminated soils depends, among other factors, on arsenic bioavailability and the capacity of a plant to access arsenic. Arsenic bioavailability is regulated by physical, chemical and biological processes and their interactions, which makes the simulation of element availability to plants with chemical extractants a difficult task (Carter, 1993). On the other hand, the total concentration of an
element is only important for evaluating the toxicity potential of soils, but they say little about elemental bioavailability.

Table 3-5. Concentrations of arsenic extracted by different extraction methods in six soils before plant growth.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Arsenic extracting solutions</th>
<th>Water</th>
<th>Mehlich III</th>
<th>Organic acids</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>As (mg kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marl</td>
<td>0.36 ± 0.15 (1.6)$^a$</td>
<td>22.7 ± 1.70 (102)</td>
<td>6.26 ± 0.89 (28)</td>
<td>22.2 ± 4.11</td>
<td></td>
</tr>
<tr>
<td>Avon</td>
<td>1.57 ± 0.05 (5.9)</td>
<td>14.8 ± 0.88 (55)</td>
<td>13.3 ± 0.05 (49)</td>
<td>27.0 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td>5.85 ± 0.29 (5.3)</td>
<td>58.8 ± 5.02 (54)</td>
<td>13.3 ± 1.03 (12)</td>
<td>110 ± 1.73</td>
<td></td>
</tr>
<tr>
<td>CDV</td>
<td>0.12 ± 0.03 (0.1)</td>
<td>45.9 ± 20.5 (22)</td>
<td>39.9 ± 1.00 (19)</td>
<td>211 ± 2.65</td>
<td></td>
</tr>
<tr>
<td>Mining</td>
<td>3.13 ± 0.15 (1.5)</td>
<td>22.5 ± 1.00 (11)</td>
<td>8.49 ± 0.97 (4.0)</td>
<td>214 ± 16.5</td>
<td></td>
</tr>
<tr>
<td>EDS</td>
<td>136 ± 4.04 (21)</td>
<td>498 ± 57.5 (78)</td>
<td>242 ± 30.1 (38)</td>
<td>640 ± 5.52</td>
<td></td>
</tr>
</tbody>
</table>

Results are mean ± standard deviations (n=3). $^a$: Results in the brackets are percentage of the total.

In this section, the relationship between the soil arsenic extracted by three different methods and the arsenic taken up by the plants was evaluated.

Water-soluble arsenic grossly underestimated the plant arsenic uptake by 242% (based on the average of five soils, excluding the CDV soil), whereas the Mehlich III over-estimated by 72%. Therefore, neither water nor Mehlich III solution was good indicators of the plant available arsenic. On the other hand, arsenic taken up by *P. vittata* constituted 74% of the arsenic extracted using organic acids, only 26% underestimation.

Based on arsenic extracted with water, the arsenic taken up by *P. vittata* implies that a greater amount of arsenic was mobilized from other pools over time. This was particularly true for the CDV soil in which the amount of arsenic taken up by the plants was over 15,500 times greater than the amount of water-extractable arsenic (Table 3-6).
Because low molecular weight organic acids used in this study are products of root exudates, microbial secretions, and plant and animal residue decomposition in soils (Stevenson, 1986; Zhang et al., 1997), and they are found in the rhizosphere of *P. vittata* (Tu et al., 2004), we hypothesized that the organic acids extractable arsenic would be a good predictor of plant available arsenic in soils. Phytic acid and oxalic acid were the two main low molecular weight organic acids identified in the root exudates of *P. vittata* (Tu et al., 2004). Furthermore, the role of oxalic acid in mobilizing mineral elements has been widely reported (Jones, 1998).

In fact, in this study, the organic acid extractable arsenic was the best method to predict plant arsenic uptake by *P. vittata* (Table 3-6).

Arsenic bioavailability in soils has been found to be enhanced by plant root exudates (Dinkelaker et al., 1989; Ohwaki and Hirata, 1992), suggesting that root exudates could be important in the mobilization of soil arsenic and arsenic accumulation by a plant.

Table 3-6. Percent of the arsenic taken up by *P. vittata* in each extracting method.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Water-Soluble</th>
<th>Mehlich III</th>
<th>Organic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marl</td>
<td>836*</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>Avon</td>
<td>216</td>
<td>23</td>
<td>95</td>
</tr>
<tr>
<td>CCA</td>
<td>183</td>
<td>18</td>
<td>81</td>
</tr>
<tr>
<td>CDV</td>
<td>16475</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>Mining</td>
<td>435</td>
<td>60</td>
<td>159</td>
</tr>
<tr>
<td>EDS</td>
<td>39</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Average</td>
<td>342*</td>
<td>28</td>
<td>74</td>
</tr>
</tbody>
</table>

*Obtained by dividing the amount of arsenic taken up by *P. vittata* by the amount of water-soluble arsenic. A value of 836% indicates that *P. vittata* took up 736% more arsenic than the amount of water soluble arsenic.  

* excluding the CDV soil.
The percent of arsenic taken up by *P. vittata* according to the organic acids extraction method varied with soils. The best correlation was found for Avon (95 %), CCA (81 %) and Mining (159 %). In the Mining soil, *P. vittata* took up 59 % more arsenic than predicted by the organic acid method. However, the plants took up 60, 54 and 78 % less arsenic than predicted for the Marl, CDV and EDS soils, respectively.

Tu et al. (2004) reported that *P. vittata* exuded 40–106% more phytic acid and 300–500% times more oxalic acid under arsenic stress than a non arsenic hyperaccumulator, *N. exaltata* fern. It also mobilized significantly greater amounts of arsenic from both arsenic minerals and a CCA soil (data not shown). Under the same concentrations, phytic acid mobilized more arsenic from the soils than oxalic acid, likely due to its stronger complexation capability and greater acidity (Tu et al., 2004).

Oxalic acid has been widely used as an extractant for plant available nutrients from soil, including phosphate (Fransson, 2001; Sagoe et al., 1998) due to its typical presence in plant root exudates (Pinton et al., 2001) and its capability of both proton donation and ion complexation.

Arsenic extraction with water underestimated the availability of soil arsenic to *P. vittata* in all soils except the EDS soil. This was because 21% of the arsenic was water soluble in the EDS soil (Table 3-5) whereas the plant took up 39% water-soluble arsenic, i.e., the amount of arsenic taken up by the plant was less than the amount of water-soluble arsenic in the EDS soil. On the other hand, the concentrations of water-soluble arsenic were low in all other soils. The amount of arsenic taken up by *P. vittata* after three harvests was consistent with the fact that more arsenic was solubilized over time from other pools (Figure 3-3).
Because Mehlich III extracts a large proportion of total soil arsenic, it overestimated plant available arsenic. The amount of arsenic taken up by *P. vittata* was lower than the amount of arsenic extracted using Mehlich III extractant. Only Mehlich III extractable arsenic in the Mining soil was close to the amount of arsenic taken up by the plant (60%). Results from this study support the conclusion of McLaughlin (2002) that indices of metal phytoavailability in soils that are based on the determination of the most available metal pools (water-soluble and organic acids), while not perfect, appear to better predict risk from metal contamination than do total metal concentrations or metals removed from soils by strong extractants (Mehlich III). Therefore, organic acids might be more suitable than water and Mehlich III to assess arsenic availability to *P. vittata* in the soils used in this study.
CHAPTER 4
EFFECTS OF PLANT AGE ON THE ARSENIC ACCUMULATION BY THE HYPERACCUMULATION Pteris vittata L.

Introduction

Arsenic is a ubiquitous trace constituent in soil and plants. However, natural and anthropogenic activities have resulted in many areas polluted with arsenic. The extensive contamination of soil and water by arsenic worldwide has renewed the interest in more cost-effective remediation technologies such as phytoremediation/phytoextraction.

The potential use of hyperaccumulator plants such as Pteris vittata L. for the phytoremediation of arsenic-contaminated soils has been reported (Komar et al., 1998; Ma et al., 2001; Tu and Ma, 2002). Several desirable characteristics, such as the ability to concentrate arsenic in the fronds, large biomass, fast growth, easy reproduction, resistance to adverse soil characteristics and its perennial nature, make P. vittata suitable for phytoremediation (Ma et al., 2001).

It is known that during the sigmoidal phase of a plant growth cycle, dry matter and nutrient accumulation occur at a maximal rate, although the rate of nutrient uptake varies with developmental conditions of a plant and is different for different nutrients (Garcia et al., 2003). There exists a set of plant physiological characteristics that change with plant age, which either isolated or combined with other factors influence biomass production, and nutrient and contaminant accumulation. For instance, young roots are generally considered to have higher nutrient uptake activity than old roots (Eshel and Waisel, 1972; Mengel and Barber, 1974; Vegh, 1991; Yanai, 1994) and the root systems
at different growth stages may not have the same specific nutrient uptake activity (Chen and Barber, 1990; Smethurst and Comerford, 1993). This is important because the accumulation ability of a plant is highly influenced by the effectiveness of its root system.

Little information is available about arsenic hyperaccumulation in plants at different ages. Furthermore, no study has been reported for *P. vittata* of different ages growing in soil conditions. Therefore, identifying the plant age that allows maximal arsenic accumulation may play an important role in successful phytoextraction. The aim of this study was to assess the influence of the physiological ages of *P. vittata* on its arsenic removal from a contaminated soil.

**Materials and Methods**

**Plant Propagation**

*Pteris vittata* plants used in this study were propagated in our laboratory. An assembly of cool-white fluorescent lamps supplied an 8-h photoperiod to the plants with an average photon flux of 825 µmol m^{-2} s^{-1}.

**Soil Characterization**

The soil used in this study (sandy, siliceous, hyperthermic grossarenic paleudult) was collected from an abandoned chromated- copper-arsenate (CCA) wood preservation site in north central Florida. The soil had pH (1:2 soil:H_{2}O) 7.30; cation exchange capacity (Thomas, 1982) 4.4 Cmol(-) kg^{-1}; soil organic matter (Nelson and Sommers, 1982) 11 g kg^{-1}; total As 153 mg kg^{-1}; 880 g kg^{-1} sand; 90 g kg^{-1} silt, and 30 g kg^{-1} clay.
Experimental Design

This greenhouse study, set up as completely randomized design, assessed the influence of four physiological ages of *P. vittata* (1.5, 4, 10 and 16 months after transplant from the sporophytic phase) (Figure 4-1) on the arsenic removal of *P. vittata* from a contaminated soil. The plants of different ages were chosen based on their availability. Each treatment had four replications. Three control pots, soil without plants, were also included.

Air-dried soil (2.5 kg) was weighed into each pot and thoroughly mixed with 3.0 g of Osmocote, extended time-release fertilizer (18-6-12) (Scotts-Sierra Horticultural Products Co., Marysville, OH). After one week of equilibrium under field capacity, one plant was transplanted into each pot. The pre-experimental frond and root biomass was taken before transplantation. The plants were allowed to grow for 8 weeks. All plants were watered throughout the study to keep the soil at approximately 70% of field capacity.

![Figure 4-1. *Pteris vittata* of 1.5, 4, 10, and 16 old months before transplant.](image-url)
After 8 weeks of growth, plants were harvested and separated into roots and fronds. Plant tissues were washed thoroughly with tap water, and then rinsed with deionized water. The fronds and roots were oven-dried for 3 d at 65°C, weighed and ground with a Wiley mill to pass through a 1 mm mesh screen for chemical analysis. Soil samples from each pot were collected from the rhizosphere and bulk soil. The rhizosphere was defined as the soil attached to the roots. The rhizosphere soil was gently shaken from the roots and sieved through 2 mm mesh screen to separate it from the roots.

**Chemical Analysis**

Plants were digested using a modified version of the EPA Method 3050A for the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC). Analysis was performed with a transversely heated, Zeeman background correction equipped graphite furnace atomic absorption spectrophotometer (Perkin Elmer SIMMA 6000, Norwalk, CT). The analysis of K, Ca, Mg, Zn, Cu, Fe and Mn was performed with a flame atomic absorption spectrophotometer (Varian model FS 220, Australia) with the same digestate. Quality control of arsenic analysis was assured by including the Standard Reference Material 1547 (Peach Leaves). Total P analysis was carried out using a modified molybdenum blue method (Carvalho *et al.*, 1998). This involved reduction of the As in digestates from As (V) to As (III) with L-cysteine to minimize its interference with phosphate analysis. Tightly capped test tubes are incubated at 80°C for 5 min to allow complete reduction of arsenate into arsenite. P was determined, after cooling, by a double-beam spectrophotometer (Shimadzu UVI60U, Shimadzu Corp., Columbia, MD).
Data Analysis

All results were expressed as an average of four replicates. Treatment effects were determined by analysis of variance according to the General Linear Model procedure of the Statistical Analysis System (SAS Institute Inc., 1987). Duncan test at a 5% probability was used for post-hoc comparisons in order to separate treatment differences.

Results and Discussion

Arsenic Uptake

Arsenic uptake in *P. vittata* ranged from 3.98 to 6.18 mg plant$^{-1}$ in the fronds and from 0.94 to 2.84 mg plant$^{-1}$ in the roots (Figure 4-2). The As uptake in the fronds of 1.5-month old *P. vittata* was 36% greater than those in the 4- and 16-month old plants and similar to that of the 10-month old plants. On the other hand, the arsenic accumulation in the roots of the 1.5-month old ferns was lower than the rest. Interesting to note that, even though the 10-month old plants accumulated as much arsenic in the fronds as those in the 1.5-month old ferns, they accumulated more arsenic in the roots as compared to the plants of other ages.

Transfer factor (TF) (As in fronds/As in roots) was calculated to evaluate the relative effectiveness of plants in moving arsenic from the roots to the fronds. This characteristic of *P. vittata* in accumulating high arsenic concentrations in its fronds makes this plant suitable for phytoextraction. The TF values for 1.5, 4, 10 and 16 months’ old plants were 3.20, 2.05, 1.63 and, 1.60, respectively (data not shown). The TF reduced with plant age. The 1.5-month old plants were the more efficient in translocating arsenic from the roots to the fronds.
Figure 4-2. Arsenic accumulation in the fronds and roots of *P. vittata* of different ages after 8 weeks of growth in an arsenic-contaminated soil. Means with the same letter for the fronds or the roots are not significantly different (P < 0.05) based on Duncan’s multiple range test.

Table 4-1. Plant biomass, bioconcentration factor and translocation factor of *P. vittata* of different ages after 8 weeks of growth in an arsenic-contaminated soil.

<table>
<thead>
<tr>
<th>Plant growth stage (months)</th>
<th>Biomass DW (g pot⁻¹)</th>
<th>Bioconcentration Factor</th>
<th>Translocation Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frond</td>
<td>Root</td>
<td>Frond</td>
</tr>
<tr>
<td>1.5</td>
<td>12.7 b¹</td>
<td>6.8 b</td>
<td>2.9 a</td>
</tr>
<tr>
<td>4</td>
<td>12.0 b</td>
<td>8.8 b</td>
<td>1.9 b</td>
</tr>
<tr>
<td>10</td>
<td>20.7 a</td>
<td>16.0 a</td>
<td>1.9 b</td>
</tr>
<tr>
<td>16</td>
<td>13.1 b</td>
<td>8.9 b</td>
<td>2.0 b</td>
</tr>
</tbody>
</table>

¹ Means (3 reps.) followed by the same letter within the column are not significantly different (P < 0.05) based on Duncan’s multiple range test.

Tu et al. (2004) also reported that young fern plants were more efficient in removing arsenic from a hydroponic system than older ferns. Arsenic levels in the growth medium seem to determine the extent of arsenic accumulation by plants of different age. For a
given plant, arsenic tends to be preferentially stored in young fronds at low arsenic level and in old fronds at high arsenic level in the media (Tu and Ma, 2002; Tu and Ma, 2005).

The ability of young *P. vittata* to accumulate more arsenic may be related to several factors. The concentration of glutathione, a sulfur-containing tripeptide thiol and a precursor of phytochelatins, a very important antioxidant involved in plant detoxification (Scott et al., 1993), seems to decrease with the plant age (Hatton et al. 1996).

In *P. vittata*, the glutathione concentration tends to increase with the arsenic concentration in the plant (Cao et al., 2004). Elsewhere, it has also been reported that plant contaminant absorption decreases as plant is more mature, hence tolerance increase with age (Wilcut et al., 1989; Leah et al., 1995). Knuteson et al. (2001) reported that four–week-old parrot feather and canna were more tolerant of simazine than two-week-old plants. Additionally, the root system at different ages has different nutrient uptake activities. The average uptake rate per unit of root decreases as a plant matures (Barber, 1984).

The fact that *P. vittata* ferns are very diverse, easy to adapt to different environments and are widespread thriving in both temperate and tropical climates (Matschullat, 2000) raises the concerns on using these ferns for phytoremediation of contaminated sites as they are classified as type II invasive species. One aspect that contributes to this is their life cycle and breeding system. The large quantities of spores produced and released by the ferns are easily dispersed to long distances favoring colonization of wide ecological niches (Bondada and Ma, 2002). Therefore, the use of younger plants besides being more efficient in arsenic uptake could also minimize the risk of uncontrolled ferns propagation since younger plants could grow longer up to the phase of spore maturation in a
contaminated site. The arsenic rich biomass could be harvested before the spores maturation phase as compared with older plants, which produce spores earlier after being transplanted in a field.

**Plant Biomass**

Arsenic hyperaccumulation efficiency depends on both plant biomass production and arsenic concentration in the plant tissue (Baker et al., 1991; Salt et al., 1998; Ma et al., 2001). Therefore, the biomass of *P. vittata* was determined for all plant ages. After 8 weeks of growth, the frond and root biomass of the 10-month old plants was greater than the rest (Figures 4-3 and 4-4).

Because the initial differences in the frond and root biomass of the treatments, the similarity of final root and frond biomass among plants of different ages indicated that plant biomass output occurred at different rates. Therefore, the net biomass (final minus initial biomass) for each plant age and for each plant part (frond and root) was calculated. Even though the final biomass of the 10-month old plants was greater than the plants of other ages, the rate of increase in biomass was greater in the 1.5-month old plants, suggesting a higher metabolic activity.

**Phosphorus Distribution in the Plants**

As a P chemical analogue, arsenic is taken up by plants via the phosphate transport system (Meharg and Hartkey-Whitaker, 2002). Phosphate concentrations in *P. vittata* of different ages were determined to examine its relationship with arsenic concentrations.

During vegetative growth phase, P contents in plants usually ranges from 97 to 161 mM of plant dry matter (Marschner, 2003).
Figure 4-3. The frond biomass of *P. vittata* of different ages before and after 8 weeks of growth in an arsenic-contaminated soil. Means with the same letter are not significantly different (*P* < 0.05) based on Duncan’s multiple range test.

Figure 4-4. The root biomass of *P. vittata* of different ages before and after 8 weeks of growth in an arsenic-contaminated soil. Means with the same letter are not significantly different (*P* < 0.05) based on Duncan’s multiple range test.
In this study, P concentrations ranged from 140 to 183 mM in the fronds and from 97 to 124 mM in the roots, which were typical of most plants. Phosphate concentrations in the fronds and roots increased with plant age (Figures 4-5 and 4-6). As for arsenic, its concentrations in the roots increased with plant age whereas those in the fronds decreased. Therefore, accumulation of both arsenic and P in the roots was the highest in the 16-month old plants. On the other hand, the tissues of older *P. vittata* had lower arsenic concentrations in the fronds and higher arsenic concentrations in the roots, as compared with the younger plants. It is interesting to note that the molar ratios of P/As stayed approximately one in the roots for all plant age (Figure 4-6) whereas those in the fronds increased with plant age (Figure 4-5).

The effectiveness in arsenic hyperaccumulation by *P. vittata* is related to a series of detoxification mechanisms which are important to mitigate arsenic toxicity in the roots and fronds (Lombi et al., 2002). One of them is the ability to maintain lower concentrations of arsenic in the roots and higher in the fronds, thereby minimizing the potential damage to the roots. The lower arsenic concentrations in the roots of the younger plants suggest a more effective arsenic removal process by *P. vittata*.

Another important factor is the ability of *P. vittata* to manipulate phosphate in the plant biomass. Tu and Ma (2003c) reported that *P. vittata* maintained greater amount of phosphate in the roots than *Nephrolepis exaltata*, a non arsenic-hyperaccumulator fern. It is hypothesized that the ability of *P. vittata* in maintaining higher concentrations of phosphorus in the roots may constitute one of its mechanisms of arsenic tolerance (Tu and Ma, 2003c).
Figure 4-5. Effect of plant age on phosphorus and arsenic distribution in the fronds of *P. vittata* after 8 weeks of growth in an arsenic-contaminated soil. Bars represent standard deviations of four replicates.

**Concentrations of Other Nutrients in Plants**

The K concentrations in the fronds and in the roots of *P. vittata* were within the normal range for most plants (Havlin et al., 2005). However, the K concentration in the fronds of the 1.5-month old plants was higher than those in other ages (Table 4-2). The K distribution in the fronds and the roots in plants of different ages was similar to that of arsenic suggesting synergetic relationship of K with arsenic in the plants. Tu and Ma (2005) reported that arsenic and K had similar distribution in the fronds of *P. vittata* and speculated that K may function as a counterbalancing ion in these plants.
Figure 4-6. Effect of plant age on the phosphorus and arsenic distribution in the roots of *P. vittata* after 8 weeks of growth in an arsenic-contaminated soil. Bars represent standard deviations of four replicates.

The Ca and Mg distribution in the fronds and roots of *P. vittata* were similar to that of P, and increased with plant age (Table 4-1). Also, the plants that accumulated more arsenic (1.5- and 4-month old plants) in the fronds had lower Ca. Tu and Ma (2005) reported that Ca concentrations in the fronds were inversely related to those of arsenic and were higher in older fronds growing in a soil spiked with up to 30 mg kg⁻¹ As. Due to the very low mobility of calcium in the phloem, the growth of the young parts of the plant is dependent upon the concurrent uptake of calcium.

Among other roles of calcium in plants, the ionic form of calcium is required in the vacuole of plant cells as a counter-cation for inorganic and organic anions (Marschner, 2001). As arsenic forms compounds with calcium in soil and arsenic is hypothesized to be
stored in the cell vacuoles, it would be reasonable to assume that the calcium concentration in the plant would increase with increasing arsenic concentrations in the plant tissues. However, based on the results of this study and the studies of Tu and Ma (2005) and Fayiga and Ma (2005), that does not seem to be the case. In fact, the fact that calcium concentration in the older plants increased while arsenic concentrations decreased suggests a limited role of calcium in arsenic detoxification by the plants.

Table 4-2. Macronutrients (K, Ca, and Mg) (g kg\(^{-1}\)) and micronutrients (Fe, Zn, and Mn) content (mg kg\(^{-1}\)) in the fronds and roots of *P. vittata* of different ages after 8 weeks of growth in an arsenic contaminated soil.

<table>
<thead>
<tr>
<th>Elemental content</th>
<th>Frond growth stage (Month)</th>
<th>Root growth stage (Month)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>4</td>
</tr>
<tr>
<td>Potassium</td>
<td>18.8 a</td>
<td>14.2 b</td>
</tr>
<tr>
<td>Calcium</td>
<td>13.5 b</td>
<td>15.5 b</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.80 b</td>
<td>3.40 b</td>
</tr>
<tr>
<td>Iron</td>
<td>69.2 ab</td>
<td>75.0 a</td>
</tr>
<tr>
<td>Zinc</td>
<td>43.6 a</td>
<td>37.0 a</td>
</tr>
<tr>
<td>Manganese</td>
<td>15.1 bc</td>
<td>28.6 a</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a row and within each plant part are not different by the Duncan test at \(P < 0.05\)

The concentrations of Fe, Mn and Zn were within the range of most plants (Havlin et al., 2005). While the Fe concentrations in the fronds of the 1.5-and 4-months old plants were higher, the Fe concentrations in the roots of the 16-month old plants were higher than those in other plant ages. Tu and Ma (2005) observed an inverse correlation between arsenic and Fe in *P. vittata*, but they offered no explanation. Chen et al. (2003) reported an unusual high concentration of Fe in the epidermis of *Pteris nervosa* roots, indicating the formation of Fe plaques. Other studies with non hyperaccumulator plants
also showed the formation of Fe plaques and the inhibition of arsenic uptake in roots (Otte et al., 1991; Colleen et al., 2002).
CHAPTER 5
ARSENIC ACCUMULATION AND ROOT CHARACTERISTICS OF *Pteris vittata* L.
AND *Nephrolepis exaltata* L

**Introduction**

For successful application of phytoextraction, the hyperaccumulator plants must be metal-tolerant, adapt to soil and climate environment of their intended use, and effectively absorb the metal from soils (Keller et al., 2003; Schwartz et al., 1999). Since heavy metals are present in soils predominantly as insoluble forms, their bioavailability is generally low. Therefore, the efficacy of phytoextraction largely depends on the development of extensive plant root systems in a contaminated soil (Schwartz et al., 1999).

Plant root systems perform many important functions including water and nutrient uptake, anchorage to the soil, and the establishment of biotic interactions in a rhizosphere. The characteristics of a root system are important in plant nutrient acquisition, particularly for elements of low mobility such as phosphorus and arsenic.

Plant root systems can be characterized based on length, surface area, biomass and their relationship to plant shoots (Van Noordwijk and Brouwer, 1991; Jungk, 2002). The efficiency of roots to transfer metals from a soil to the shoots can be characterized by net influx per unit root length, and root length per unit of shoot biomass. Structural root properties, such as diameter of the roots and the formation of root hairs, may also exert an influence on nutrient uptake from the immediate vicinity of a root cylinder (Waisel and Eshel, 2002).
According to Ernst (1996), most hyperaccumulators have limited root systems. However, in a number of studies, unexplained growth enhancements were observed in plants subjected to a mild stress from toxic metals. For example, the root growth of *Betula pendula* seedlings was induced by low concentrations of cadmium (Gussarsson, 1994). Also, enhanced root elongation, root biomass production, and root hair formation were found in plants of a tolerant population of *Silene vulgaris* treated with lead chloride (Wierzbicka and Panufnik, 1998). Jiang and Liu (1999) reported stimulated root growth of *Brassica juncea* plants with low concentrations of lead nitrate. Schwartz et al (1999) and Whiting et al (2000) reported a preferential root development in the Zn hyperaccumulator *Thlaspi ceaulescens* in the presence of Zn.

Little information is available on the root system of *P. vittata*. The process by which *P. vittata* mobilizes and takes up arsenic is not well known. Hyperaccumulator species may release root exudates containing chelators to enhance heavy metal uptake, translocation and resistance (Wenzel et al., 2003). The ability of a plant to exude large quantities of dissolved organic carbon (DOC) and to change the rhizosphere pH may enhance the arsenic bioavailability in soils, thereby increasing its arsenic uptake (Tu et al., 2004). The fate and bioavailability of arsenic in the rhizosphere can be different from that of the bulk soil (Fitz et al., 2003). Depending on plant and soil factors, rhizosphere pH can be up to two units different from the bulk soil (Marschner, 2003). Factors affecting the rhizosphere, such as pH, plant nutritional status, organic acids excretion, and CO$_2$ production by roots and rhizosphere microorganisms, are accountable for the differences (Marschner, 2003).
Several attempts have been made to measure the bioavailability of metals in soils. Tessier et al. (1979) used a sequential extraction technique for determining the labile metal in soils. This approach is based on the fact that metal associated with various geochemical phases varies in its chemical reactivity and bioavailability. The drawback of the method is its non-selectivity and metal redistribution among geochemical phases (Howard and Brink, 1999). Despite its limitation, fractionation provides an understanding of the relative mobility and bioavailability of metals in soils (Fitz and Wenzel, 2002; Krishnamurti and Naidu, 2002), because plant metal uptake or metal toxicity is related to those fractions (Chlopecka and Adriano, 1996; Guo and Yost, 1998). Water-extractable and exchangeable forms of metals are usually considered to be the most available to plants (Petruzzelli, 1989).

This study was conducted aiming to evaluate and compare two fern species, an As-hyperaccumulator (P. vittata) and a non As-hyperaccumulator (Nephrolepis exaltata), in terms of: 1) root system characteristics (biomass, length, surface area and diameter); 2) plant biomass and arsenic uptake; and 3) root uptake efficiency of arsenic; and 4) effects of plant arsenic uptake on arsenic distribution and bioavailability in the rhizosphere and bulk soils. The information obtained from this study should be useful for determining the plant density and effective root depth of P. vittata for phytoextraction purpose as well as for providing the critical linkage between the ability of P. vittata in solubilizing soil arsenic and arsenic hyperaccumulation.
Materials and Methods

Soil Characterization

The soils used in this study were collected (0-20 cm) from an abandoned chromated-copper-arsenate (CCA) wood preservation site (As-contaminated soil) and from a non-contaminated site (control soil) in Florida. The site was contaminated with arsenic from using CCA wood preservative between 1951-1962 (Komar, 1999). The soil analyses were performed as described in the Chapter 4 and summarized in Table 5-1.

Table 5-1. Selected properties of the soils used in this study

<table>
<thead>
<tr>
<th>Property</th>
<th>As-contaminated soil</th>
<th>Control soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:2 soil/water ratio)</td>
<td>7.30 ±0.01*</td>
<td>6.81 ±0.02</td>
</tr>
<tr>
<td>Organic matter content (g kg⁻¹)</td>
<td>11.0 ±0.30</td>
<td>13.2 ± 0.42</td>
</tr>
<tr>
<td>CEC** (cmol(+) kg⁻¹)</td>
<td>4.40 ±0.02</td>
<td>5.60 ±0.04</td>
</tr>
<tr>
<td>Total As (mg kg⁻¹)</td>
<td>101 ±5.40</td>
<td>0.41±0.01</td>
</tr>
<tr>
<td>Water-soluble As (mg kg⁻¹)</td>
<td>0.2 ±0.02</td>
<td>0.003 ±0.001</td>
</tr>
<tr>
<td>Mechlic III extractable P (mg kg⁻¹)</td>
<td>21.8 ± 0.30</td>
<td>87.2 ±0.77</td>
</tr>
<tr>
<td>Sand (g kg⁻¹)</td>
<td>882 ± 4.32</td>
<td>892 ±4.76</td>
</tr>
<tr>
<td>Silt (g kg⁻¹)</td>
<td>91±0.40</td>
<td>75 ±0.30</td>
</tr>
<tr>
<td>Clay (g kg⁻¹)</td>
<td>27 ±0.01</td>
<td>33 ±0.02</td>
</tr>
<tr>
<td>Bulk density (g cm⁻³)</td>
<td>1.29</td>
<td>1.39</td>
</tr>
</tbody>
</table>

* Values represent mean standard deviation; ** Cation exchange capacity

Experimental Set up

The study employed a completely randomized design in a 2 x 2 split plot scheme. Two fern species (*P. vittata* and *N. exaltata*) were used as the main plot and the chemistry of the rhizosphere and bulk soil as the sub-plot. Each treatment was replicated four times.
A known weight (2.5 kg) of air dried and sieved (2 mm) soil was poured into plastic bags and thoroughly mixed with 3.0 g of Osmocote extended time-release base fertilizer (18-6-12) (Scotts-Sierra Horticultural Products Co., Marysville, OH), and then poured into plastic pots (2.5-L rhizopot).

The rhizopot (16 cm in height and 15 cm in diameter; Figure 5-1) was used to grow the plants. Plastic frames (13 cm in height and 7 cm in diameter) covered with nylon mesh cloth (mesh size 45 µm) were used to separate the rhizosphere from the bulk soil in the rhizopot.

Figure 5-1. Cross section of the rhizopot used in the study.

*Pteris vittata* was propagated in our laboratory whereas *N. exaltata* was procured from a nearby nursery (Milestone Agriculture, Inc., Apopka, FL). Efforts were made to ensure visual uniformity across all plants. The average biomass per plant before the transplant was 0.70 g for the fronds and 0.45 g for the roots.
Root growth was limited within the nylon cloth in the central compartment (500 g soil). One week before the study, the soil was set to equilibrate at water holding capacity. One healthy fern of similar age with five to six fronds was transplanted into each pot.

The plants grew for 8 weeks in a greenhouse with an average night/day temperature of 14/30°C and an average photosynthetically active radiation flux of 825 µmol m² s⁻¹. The plants were watered throughout the study to keep the soil at approximately 70% of its field capacity. At the end of the experiment, the ferns were harvested and each plant was separated into roots and fronds. Rhizosphere and bulk soil were collected and used for analysis of arsenic, soil pH and dissolved organic carbon (DOC). Rhizosphere soil was defined as the soil attached to the roots, which was removed from the roots by shaking gently. The rhizosphere soil was sieved to remove the roots while keeping the roots intact as much as possible. The bulk soil was defined as the soil outside the central compartment.

The plants were washed thoroughly with tap water, and then rinsed with distilled water. The fronds were oven-dried for 3 days at 65°C, weighed and ground using a Willey mill to 60-mesh fineness for chemical analysis. The roots were immediately separated and scanned to prevent dehydration. After scanning, the roots were also oven-dried, weighed and ground for chemical analysis.

**Root Measurement**

The roots were washed with water, spread out on a transparent tray and then the image was digitalized using a scanner (Envisions88005) at 400 dpi resolutions with Vistascan software (UMAX Data system, Inc). When necessary, root samples were divided into sub-samples for more accurate measurement. The digitalized images were
processed by the program GSROOTS (Guddanti and Chambers, 1993), which was programmed to yield root length data of pre-defined diameter classes (<0.10; 0.10 < d < 0.25; 0.25 < d < 0.50; 0.50 < d < 0.75; 0.75 < d < 1.0; 1.0 < d < 2.0; and >2.0 mm). Root length density ($L_v$, root length per unit volume of soil) and root area density ($A_v$, root surface area per unit volume of soil) were calculated. Unit root length and unit root area were used in all discussions unless otherwise specified. The root biomass was also determined.

**Chemical Analysis**

Plants and soils digestion and analyses were performed as described in the Chapter 4.

Rhizosphere and bulk soil samples were evaluated for water-soluble arsenic and DOC in 1:4 soil to water ratio (Olsen and Sommers, 1982), obtained after shaking (1 h), centrifuging (15 min at 3500 g) and filtering (0.45 µm syringe filter). Arsenic in solution was determined by the same method described above. The concentration of DOC was measured using a TOC-5050A TOC analyzer (Shimadzu, Japan). Quality control of arsenic analysis was included using Standard Reference Materials 1547 (Peach Leaves) and 2710 (soil) (US NIST, MD).

**Arsenic Fractionation**

The improved sequential extraction procedure (Wenzel et al. 2001) was used to fractionate arsenic into five operationally-defined fractions, including non-specifically bound (N), specifically bound (S), amorphous hydrous oxide-bound (A), crystalline hydrous-oxide-bound (C), and residual (R), as described in the Chapter 3.
Data Analysis

All results were expressed as an average of four replications. Treatment effects were determined by analysis of variance according to General Linear Model procedure of the Statistical Analysis System (SAS Institute Inc., 1987, Cary, NC). The Duncan test at 5% of probability was used for post-hoc comparisons to separate treatment differences.

Pearson correlation coefficients were calculated between the root characteristics and different plant parameters.

Results and Discussion

Root Biomass, Root Length, Root Area, and Root Diameter

Plant roots are in direct contact with, and are affected by soil constituents in addition to regulating many plant processes. Measurement of below ground plant productivity may provide information on root growth, distribution, water and nutrient supply and plant potential of removing contaminant (Van Noordwijk and Brouwer, 1991). Root-soil contact is an especially important factor for the uptake of less mobile elements such as P and arsenic from a soil. However, the available information on the root characteristics of *P. vittata* has been limited to the measurement of root biomass (Tu et al., 2002; Tu and Ma, 2003; Liao et al., 2004).

Root characteristics including root biomass, length and surface areas were determined for both plants. They are important for estimating plant water and nutrient uptake (Zhuang et al. 2001). In both soils, *P. vittata* had a more extensive root system than *N. exaltata* (Table 5-2). The root biomass of *P. vittata* was 3.0 and 2.4 times greater than that of *N. exaltata* in the As-contaminated soil and in the control soil, respectively.
The root biomass accounted for approximately 25% of plant biomass for both plant species (Tables 5-2 and 5-3).

Several studies have reported that the presence of contaminants stimulated the growth of plant roots (Gussarsson, 1994; Wierzbicka and Panufnik, 1998; Jiang and Liu, 1999). Other studies indicate a severe inhibition of root growth. For instance, Schwartz et al. (1999) reported that *Thlaspi caerulences* growing in a soil spiked with Zn had reduced root growth while it explored a large soil volume with enhanced Zn acquisition in an unpolluted soil. However, the root growth of *T. caerulescens*, a Zn hyperaccumulator, was higher than that of *Thlaspi arvense*, a non-hyperaccumulator, when grown in a Zn-amended soil (Schwartz et al., 1999; Whiting et al., 2000).

Table 5-2. The root biomass (dw), root length, and root area of *P. vittata* and *N. exaltata* after 8 weeks of growth in a As-contaminated soil (As-soil) and control soil.

<table>
<thead>
<tr>
<th>Fern species</th>
<th>Root biomass (g dw pot⁻¹)</th>
<th>Root length (cm cm⁻³)</th>
<th>Root area (cm cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>As-contaminated soil</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. vittata</em></td>
<td>1.8a</td>
<td>4.3a</td>
<td>1.3 a</td>
</tr>
<tr>
<td><em>N. exaltata</em></td>
<td>0.6b</td>
<td>1.1b</td>
<td>0.4 b</td>
</tr>
<tr>
<td><em>Control soil</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. vittata</em></td>
<td>2.2a</td>
<td>6.2 a</td>
<td>1.8 a</td>
</tr>
<tr>
<td><em>N. exaltata</em></td>
<td>0.9b</td>
<td>2.1 b</td>
<td>0.7 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column within the same soil are not significantly different at p < 0.05 (n=4).

The root length and root area of *P. vittata* were 3.9 and 3.2 times greater than that of *N. exaltata* in the As-contaminated soil, and were 2.9 and 2.6 times greater in the control soil, respectively (Table 5-2). In our study, the root length and surface area were lower in the As-contaminated soil than in the control soil for both fern species. Although
the overall chemical and physical characteristics of the two soils (Table 5-1) were similar, the higher P content and low As content of the control soil must have accounted for the better development of the plant roots in that soil.

Root diameter determines the extent of the soil volume that potentially supplies nutrients and contaminants to plants. For both soils, *P. vittata* had a greater root length for all classes of diameter than *N. exaltata* (Figure 5-2). The roots of 0.25 to 1.00 mm of diameter comprised a major proportion of *P. vittata* roots as measured by root length. For both soils and plant species, the roots < 0.50 mm diameter comprised over 40% of the total root length, while those of 0.50 to 1.00 mm diameter contributed another 25%. The fine roots (0.25 to 1.00 mm) of *P. vittata* contributed more to the total root length than did *N. exaltata*. This is important because fine roots allow the root system to exploit a soil volume more effectively while coarser roots have greater transport capacity (Forde and Lorenzo, 2001).

For all classes of root diameter, *P. vittata* had a greater root area than *N. exaltata*. The roots with diameter >1 mm represented 67 to 71% of the total root area for *N. exaltata*, whereas they only accounted for 59 to 60% for *P. vittata* (Figure 5-3). Based on the root characteristics (root biomass, length and area) of the two fern species, *P. vittata* presented a much greater potential to remove As and nutrients from soils than *N. exaltata* regardless of the soil characteristics.

**Frond Biomass, Arsenic Uptake, Bioconcentration and Translocation**

Good biomass production and high arsenic accumulation ability are two of many desirable treats of hyperaccumulator plants for a successful application of phytoextraction. Although the two ferns species had similar frond biomass
(approximately 0.7 g dw plant\(^{-1}\)) before the experiment, \textit{P. vittata} had a greater biomass (5.5 - 6.6 g) than \textit{N. exaltata} (1.8 – 2.8 g) after 8-week of growth (Table 5-3). The frond biomass of \textit{P. vittata} was 3.1 and 2.4 times greater than that of \textit{N. exaltata} in the As-contaminated soil and in the control soil, respectively.

This observation confirms the faster growing characteristic of \textit{P. vittata} when compared with other fern species, which is desirable for a plant to be used for phytoremediation purpose. Other studies have shown that \textit{P. vittata} can grow normally or even have the growth stimulated in the presence of arsenic (Tu and Ma, 2002).

The amount of arsenic taken up by \textit{P. vittata} (2.51 mg per plant) was 29 times greater than that of \textit{N. exaltata} (0.09 mg per plant) in the arsenic-contaminated soil (Table 5-3). Tu and Ma (2004) found a 5.8 fold increase in arsenic accumulation of \textit{P. vittata} over \textit{N. exaltata} in a hydroponic study. Huang et al. (2004) reported that \textit{P. vittata} and \textit{Pteris cretica}, another arsenic hyperaccumulator, removed more arsenic from water than \textit{N. exaltata}. While 92\% of the plant arsenic was accumulated in the fronds of \textit{P. vittata}, only 37\% was in the fronds of \textit{N. exaltata} (Table 5-3).

The ratio between the contaminant concentrations in the tissue relative to that in the soil (bioconcentration factor, BF) as well as the partitioning of the contaminant between the fronds and roots (transfer factor, TF) were determined aiming to gain insight of the arsenic accumulating capability of the plants.
Figure 5-2. The root length density (Lv) of *P. vittata* and *N. exaltata* as a function of root diameter in a control soil (a) and an As-contaminated soil (b) after 8 weeks of growth. Bars represent standard deviations of four replicates.
Figure 5-3. The root surface area density ($A_v$) of *P. vittata* and *N. exaltata* as a function of root diameter in a control soil (a) and an As-contaminated soil (b) after 8 weeks of growth. Bars represent standard deviations of four replicates.
Table 5-3. The frond biomass (dw), arsenic accumulation, and bioconcentration and translocation factors of *P. vittata* and *N. exaltata* after 8 weeks of growth in a arsenic-contaminated soil and control soil.

<table>
<thead>
<tr>
<th>Fern species</th>
<th>Frond biomass (g plant(^{-1}))</th>
<th>As (mg plant(^{-1}))</th>
<th>Bioconcentration factor (BF)</th>
<th>Transfer factor (TF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frond</td>
<td>Root</td>
<td>Frond</td>
<td>Root</td>
</tr>
<tr>
<td><strong>As-contaminated soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. vittata</em></td>
<td>5.5 a</td>
<td>2.30 a</td>
<td>0.21 a</td>
<td>1.7 a</td>
</tr>
<tr>
<td><em>N. exaltata</em></td>
<td>1.8 b</td>
<td>0.03 b</td>
<td>0.06 b</td>
<td>0.1 b</td>
</tr>
<tr>
<td><strong>Control soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. vittata</em></td>
<td>6.6 a</td>
<td>0.003 a</td>
<td>0.00</td>
<td>0.55 a</td>
</tr>
<tr>
<td><em>N. exaltata</em></td>
<td>2.8 b</td>
<td>0.000 b</td>
<td>0.00</td>
<td>0.03 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column within the same soil are not significantly different at p < 0.05 (n=4).

We assumed that plant root characteristics play a more important role in determining BF than TF. The fronds (1.7) and roots (0.5) BF of *P. vittata* was 17 and 1.3 time greater than that of *N. exaltata* in the arsenic-contaminated soil (Table 5-3). The fact that *P. vittata* had a more extensive and more efficient root system may have contributed to its greater BF as compared with *N. exaltata*. The transfer factor (TF=3.6) of *P. vittata* was 18 folds greater than that of *N. exaltata* (Table 5-3). Huang et al. (2004) also reported that *N. exaltata* was unable to translocate the absorbed arsenic from the roots to the fronds, which was expected for a non-hyperaccumulator plant specie. The ability of *P. vittata* to translocate arsenic from the roots to the fronds is considered to be one of the tolerance and detoxification mechanisms involved in its arsenic hyperaccumulation.

**Root Uptake Efficiency of Arsenic and Phosphorus**

Root uptake efficiency (RUE), which is defined as elemental accumulation in plant tissues (fronds or roots) per unit root biomass, length or area, can be used to
evaluate how efficient a plant is in taking up an element (Table 5-4). The arsenic RUE for the fronds of *P. vittata* measured by root biomass, root length and root area was 15-23 times greater than that of *N. exaltata* in both soils. This means that not only did *P. vittata* have a greater root biomass but also a more efficient root uptake system for arsenic. Because *N. exaltata* tended to accumulate arsenic in the roots, the differences in the arsenic RUE for the roots between the two plants were smaller, 8-10 times in the control soil and similar in the arsenic contaminated soil (Table 5-4).

Because arsenic is an analogue to phosphorus, we also determined the P RUE for the fronds and roots of both plants. The P RUE in the control soil and for roots in the arsenic contaminated soil was similar between the two plant species (Table 5-4). However, the P RUE for the fronds was greater in *N. exaltata* than *P. vittata* in the arsenic-contaminated soil.

These RUE results are rather interesting because they indicate that despite the similarity between P and arsenic, both *P. vittata* and *N. exaltata* root systems tended to remove P from the soil preferentially and more efficiently than arsenic.

Recently, it has been found that some natural hyperaccumulators proliferate their roots positively in patches of high metal availability. In contrast, non-accumulators actively avoid these areas, and this is one of the mechanisms by which hyperaccumulators absorb more metals when grown in the same soil (Schwartz et al., 1999; Whiting et al., 2000).

In the greenhouse study it was observed that most of the roots of *P. vittata* tended to concentrate in the top 7 cm of the pot (data not shown). The observation gathered in this study points to the need to evaluate the behavior of *P. vittata* root system under field
conditions in an arsenic-contaminated soil as well as the effective root depth of the plant. The answer to this question will help to determine the effective depth that the plant will be able to clean up arsenic-contaminated soil.

Table 5-4. The root uptake efficiency (RUE) as measured by As and P uptake (mg plant$^{-1}$) by the fronds and roots of *P. vittata* and *N. exaltata* per unit of root parameters (root dry weight-RB, length-Lv, and surface area-Av) in a As-contaminated soil and control soil.

<table>
<thead>
<tr>
<th>Root characteristics</th>
<th>As-contaminated soil</th>
<th>Control soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. vittata</em></td>
<td><em>N. exaltata</em></td>
</tr>
<tr>
<td>Frond</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As-RB*</td>
<td>1277 a</td>
<td>61 b</td>
</tr>
<tr>
<td>As-Lv**</td>
<td>552 a</td>
<td>31 b</td>
</tr>
<tr>
<td>As-Av***</td>
<td>1913 a</td>
<td>85 b</td>
</tr>
<tr>
<td>P-RB*</td>
<td>5400 b</td>
<td>10500 a</td>
</tr>
<tr>
<td>P-Lv**</td>
<td>2300 b</td>
<td>5600 a</td>
</tr>
<tr>
<td>P-Av***</td>
<td>8200 b</td>
<td>14500 a</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As-RB</td>
<td>116 a</td>
<td>92 b</td>
</tr>
<tr>
<td>As-Lv</td>
<td>50 a</td>
<td>47 a</td>
</tr>
<tr>
<td>As-Av</td>
<td>176 a</td>
<td>124 a</td>
</tr>
<tr>
<td>P-RB</td>
<td>2400 a</td>
<td>1700 a</td>
</tr>
<tr>
<td>P-Lv</td>
<td>1100 a</td>
<td>900 a</td>
</tr>
<tr>
<td>P-Av</td>
<td>3700 a</td>
<td>2400 a</td>
</tr>
</tbody>
</table>

*: ug plant$^{-1}$ (As or P)/g root; **: ug plant$^{-1}$ (As or P)/cm cm$^{-3}$ root; ***: ug plant$^{-1}$ (As or P)/cm$^2$ cm$^{-3}$ root. Means followed by the same letter in a row within the same soil are not different at $P < 0.05$ (n=4).

**Plant Nutrient Uptake**

Balanced supply of essential nutrients is one of the most important factors in increasing crop yields (Fageria, 2001). The frond P (1.8 –3.5 g kg$^{-1}$), K (1.28 - 2.51 g kg$^{-1}$), Ca (1.26-1.58 g kg$^{-1}$), and Mg (2.8-4.8 g kg$^{-1}$) concentrations of both fern species
(Table 5-5) were within the normal concentration range for most plant species (Marschner, 2003). They were also similar to those reported by Tu and Ma (2005).

For the As-contaminated soil, the P and Mg concentrations in the fronds of *N. exaltata* were greater than that of *P. vittata* (Table 5-5). Positive interaction between P and Mg are expected since Mg is an activator of kinase enzymes and activates most reactions involving phosphatase transfer (Faglia, 2001). In most plants, P is generally concentrated in the upper parts or reproductive organs (Marschner, 2003). However, in this study, the frond P concentration of *P. vittata* (1.8 g kg\(^{-1}\)) was lower than that in the roots (2.4 g kg\(^{-1}\)) in the arsenic-contaminated soil. This result agrees with Tu and Ma (2004) who found similar trend for P concentration in fronds and roots of *P. vittata* and *N. exaltata* growing in a hydroponic solution. Arsenic hyperaccumulation by *P. vittata* may be facilitated by its high arsenic influx rate and its high molar P/As ratio in the roots resulting from both high arsenic TF and low P TF (Tu and Ma, 2004).

In the control soil, *N. exaltata* had a greater K and Mg concentration in the fronds (1.7-1.9 fold) as well as Mg in the roots than *P. vittata*. Plant potassium content influences the plant biomass and arsenic content (Komar, 1999). In the present study neither plant biomass nor arsenic uptake of *N. exaltata* was related with the status of K in the plant. It is unclear why *N. exaltata* took up more K in both soils, especially in the fronds.

**Arsenic Reduction and Distribution in the Soil**

Whereas they caused no significant change in total arsenic concentration in the bulk soil (Table 5-6), *N. exaltata* and *P. vittata* reduced arsenic concentrations in the rhizosphere soil by 28.6 and 40.7% (Table 5-7).
Table 5-5. Nutrients concentrations (g kg\(^{-1}\)) in the fronds and roots of *P. vittata* and *N. exaltata* after 8 weeks of growth in an As-contaminated soil and a control soil.

<table>
<thead>
<tr>
<th>Fern species</th>
<th>As-contaminated soil</th>
<th>Control soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fronds P K Ca Mg P K Ca Mg</td>
<td>fronds P K Ca Mg P K Ca Mg</td>
</tr>
<tr>
<td><em>P. vittata</em></td>
<td>1.8b 13.3a 15.8a 3.2b 2.4a 8.9a 17.3a 3.0a</td>
<td>3.5a 12.8b 15.0a 2.8b 2.8a 7.6b 16.0a 2.5a</td>
</tr>
<tr>
<td><em>N. exaltata</em></td>
<td>3.4a 25.1a 14.9a 4.8a 1.7a 7.8a 17.2a 2.8b</td>
<td>3.0a 21.7a 12.6a 4.4a 2.5a 10.3a 13.3a 2.6a</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column within the same soil are not significantly different at \(p < 0.05\) (n=4).

To achieve the arsenic reduction from 105 to 75.0 mg kg\(^{-1}\) for *N. exaltata* and 105 to 62.3 mg kg\(^{-1}\) for *P. vittata* in the rhizosphere soil, the soil mass required to contribute the 0.09 and 2.51 mg arsenic (Table 5-3) constituted only 3 and 61g out of 2.5 kg, respectively.

Similar to total arsenic, no change was observed for different fractions of arsenic in the bulk soil with or without a plant (Table 5-6). Most of the arsenic was present in the A fraction (61.5%) and C fraction (22.2%), contributing a total of 83.7% (Table 5-6). The N and R fractions accounted for only 4.63 and 2.93%.

Unlike the bulk soil, the decrease in arsenic concentrations was observed across all five arsenic fractions in the rhizosphere soil (Table 5-7). *Nephrolepis exaltata* and *P. vittata* reduced arsenic concentrations in the A fraction (As-A) from 66.7 to 42.0 and 66.7 to 30.9 mg kg\(^{-1}\), respectively, accounting for 76 and 67% arsenic decrease in the rhizosphere.
Table 5-6. Arsenic distribution in different fractions in the bulk soil after 8 weeks of plant growth.

<table>
<thead>
<tr>
<th>As fractions</th>
<th>No plant</th>
<th><em>P. vittata</em></th>
<th><em>N. exaltata</em></th>
<th>As distribution***</th>
</tr>
</thead>
<tbody>
<tr>
<td>N**</td>
<td>5.00 a</td>
<td>5.30 a</td>
<td>5.40 a</td>
<td>4.63</td>
</tr>
<tr>
<td>S</td>
<td>9.80 a</td>
<td>9.50 a</td>
<td>8.60 a</td>
<td>9.07</td>
</tr>
<tr>
<td>A</td>
<td>66.4 a</td>
<td>63.8 a</td>
<td>63.1 a</td>
<td>61.5</td>
</tr>
<tr>
<td>C</td>
<td>24.0 a</td>
<td>24.4 a</td>
<td>22.4 a</td>
<td>22.2</td>
</tr>
<tr>
<td>R</td>
<td>3.20 a</td>
<td>3.20 a</td>
<td>3.70 a</td>
<td>2.96</td>
</tr>
<tr>
<td>Sum</td>
<td>108</td>
<td>106</td>
<td>103</td>
<td>100</td>
</tr>
<tr>
<td>Total As</td>
<td>105</td>
<td>99</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>103</td>
<td>107</td>
<td>106</td>
<td></td>
</tr>
</tbody>
</table>

* Means with the same letter in each row are not significantly different according to Tukey – HSD test at 5 % level.
** Fractions N, S, A, C and R stand for non-specifically bound, specifically bound, amorphous Al and Fe hydrous-oxide bound, crystalline hydrous-oxide bound, and residual arsenic fraction;
*** Based on the treatment with no plant.
Table 5-7. Arsenic distribution in different fractions in the rhizosphere of *P. vittata* (PV) and *N. exaltata* (NE) after 8 weeks of plant growth.

<table>
<thead>
<tr>
<th>As fractions</th>
<th>Soil As concentrations (mg kg⁻¹)</th>
<th>% As in each fraction**</th>
<th>As reductions *** (mg kg⁻¹)</th>
<th>% of total As reductions ****</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No plant PV NE</td>
<td>No plant PV NE</td>
<td>No plant PV NE</td>
<td>No plant PV NE</td>
</tr>
<tr>
<td>N*****</td>
<td>4.30 a 2.0 b 4.10 a*¹</td>
<td>4.63 4.18 6.03</td>
<td>2.30 0.20</td>
<td>4.38 0.62</td>
</tr>
<tr>
<td>S</td>
<td>10.7 a 3.9 c 6.50 b</td>
<td>9.07 8.16 9.56</td>
<td>6.80 4.18</td>
<td>13.0 12.9</td>
</tr>
<tr>
<td>A</td>
<td>66.7 a 30.9 c 42.0 b</td>
<td>61.5 64.6 61.8</td>
<td>35.2 24.6</td>
<td>67.0 76.2</td>
</tr>
<tr>
<td>C</td>
<td>16.0 a 9.7 b 13.7 ab</td>
<td>22.2 20.3 20.1</td>
<td>6.40 2.30</td>
<td>12.2 7.12</td>
</tr>
<tr>
<td>R</td>
<td>2.80 a 1.3 b 1.70 b</td>
<td>2.96 2.09 2.50</td>
<td>1.50 1.10</td>
<td>2.86 3.41</td>
</tr>
<tr>
<td>Sum</td>
<td>101 a 47.8 c 68.0 b</td>
<td>100 100 100</td>
<td>52.5 32.3</td>
<td>100 100</td>
</tr>
<tr>
<td>Total As</td>
<td>105 a 62.3 c 75.0 b</td>
<td></td>
<td>40.6 28.5</td>
<td></td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>95.7 76.7 90.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Means with the same letter in each row are not significantly different according to Tukey – HSD test at 5 % level.
** Based on total arsenic of 101, 47.8 and 68.0 mg kg⁻¹.
*** Based on no-plant treatment for each fraction.
**** Based on total reduction of 52.5 and 32.3 mg kg⁻¹.
***** Fractions N, S, A, C and R stand for non-specifically bound, specifically bound, amorphous Al and Fe hydrous-oxide bound, crystalline hydrous-oxide bound, and residual arsenic fraction;
Compared to *N. exaltata*, *P. vittata* removed more arsenic from each fraction. For example, reductions in arsenic concentration in the A, S and C fractions were 24.6, 4.18 and 2.30 mg kg\(^{-1}\) for *N. exaltata* and 35.2, 6.80, and 6.40 mg kg\(^{-1}\) for *P. vittata* (Table 5-7). However, plant uptake had little effect on the relative arsenic distribution among the five fractions in the rhizosphere of two plant species, with 61.8-64.6% in the A fraction, 20.1-20.3% in the C fraction and 8.16-9.54% in the S fraction. This was because arsenic in all fractions was reduced, with an average reduction of 52.7% and 27.0% for *P. vittata* and *N. exaltata*, respectively (Table 5-7).

The results for arsenic distribution in different fractions obtained in our study were consistent with the data reported by Wenzel et al. (2001) where they determined arsenic fractionations in 20 Austria soils with differing levels of arsenic contamination (96–2,183 mg kg\(^{-1}\)). Arsenic in those soils was also mostly present in the A fraction (42.3%) followed by C fraction (29.2%), accounting for a total of 71.5% or 12.2% less than that obtained in our study. However, they reported concentration of 0.24 and 17.5% in the N (most available) and R (least available) fractions. Comparatively, the soil used in our study had 4.39% more arsenic in the most available fraction and 14.5% less arsenic in the least available fraction, indicating that arsenic in the soil used in our study had greater availability than in the soils from their study.

The decrease in arsenic concentration in the five arsenic fractions of the rhizosphere soil did not follow arsenic availability according to the sequential extraction, rather it was positively correlated to the arsenic concentrations in each fraction (\(r = 0.995\) for both plants, \(n=4\)). This means that the fraction having the highest arsenic concentration, i.e. the A fraction, had the greatest reduction in its concentration. The fact
that arsenic concentration in the N fraction, the most available, was lower with *P. vittata* (2.0 mg kg\(^{-1}\)) than *N. exaltata* (4.1 mg kg\(^{-1}\)) was consistent with the greater ability of *P. vittata* to take up arsenic. The results also imply that arsenic transfer from less-available fractions to the N fraction was slower than the arsenic depletion by *P. vittata*. Fitz et al. (2003) reported a much lower arsenic transformation in *P. vittata* rhizosphere in a soil with a higher buffer capacity. The high rate of arsenic mobilization (reduction in the A fraction) observed in the rhizosphere of *P. vittata* can also be attributed to the sandy nature of the soil (88% sand) (Table 5-1), i.e. low ability to retain arsenic, which was supported by low oxalate extractable Fe and Al (267 and 260 mg kg\(^{-1}\)) (Table 5-1).

The mass balance of the total arsenic in the soil, as determined by the sum of the fractions, was evaluated (Table 5-6 and 5-7). Good recoveries of total soil arsenic (103–107% in the bulk soil and 77-96% in the rhizosphere) indicated that the difference between the two methods ranged from 3 to 24%. Despite all the criticism attributed to sequential extraction, the procedure was able to provide some insight into the mobilization and availability of arsenic in contaminated soils. Additionally, the sum of the fractions was in good agreement with the total arsenic values.

**Influence of Plants on Water-Soluble Arsenic, Soil pH and DOC**

The water soluble arsenic in the rhizosphere of *P. vittata* (0.7 mg kg\(^{-1}\)) was two times lower than that in the *N. exaltata* (1.4 mg kg\(^{-1}\)), which was similar to the no plant treatment (1.7 mg kg\(^{-1}\)). Compared to the bulk soil, reduction in water-soluble arsenic in the rhizosphere soil by *P. vittata* and *N. exaltata* were 58.9 and 17.6% (Fig 5-4). The high water-soluble arsenic in the *N. exaltata* rhizosphere was consistent with the results found for arsenic in the N fraction, the most available arsenic.
Concentration of water-soluble metal is a good indicator of bioavailability for plant uptake in soils (McBride, 1994) since plants preferentially take up nutrients from soil solution (Linehan et al., 1985). The depletion or the enrichment of an element in the rhizosphere is determined by the capacity of a soil to replenish the soluble or exchangeable forms of the element (Hinsinger, 1998).

The supply of elements such as P and As to the plant rhizosphere is limited by diffusion. When plant outpaces the soil supply capacity, the depletion occurs in the rhizosphere, as it was the case of *P. vittata* (Table 5-7). When the ability of the plant removes arsenic from the soil is lower than the arsenic diffusion rate, arsenic accumulates in the rhizosphere, as it was the case of *N. exaltata* (Table 5-7). Thus, lower water-soluble arsenic in the rhizosphere of *P. vittata* was due to the higher ability of the plant to deplete the arsenic from the rhizosphere as compared with *N. exaltata*. These results are consistent with the more extensive root system of *P. vittata*, as well as with the arsenic reduction in its rhizosphere.

The changes in rhizosphere pH and DOC may have a great effect on soil arsenic bioavailability. The influence of plant growth on soil pH was reflected by the pH difference in the bulk and rhizosphere soil. Plants had no effect on the bulk soil pH for all treatments (Figure 5-5).
Figure 5-4. Comparison of water-soluble arsenic in the bulk and rhizosphere soil of *P. vittata* and *N. exaltata* after 8 weeks of growth in an arsenic-contaminated soil. Bars represent standard deviations of four replicates.

However, the rhizosphere pH of *P. vittata* (7.66) and *N. exaltata* (7.18) were 0.4 units higher and -0.13 lower, respectively, than that in the no plant treatment.

Youssef and Chino (1989) and Luo et al. (2000) also reported an increase of pH in the rhizosphere of *Triticum aestivum* and *Thlaspi cearulences*. Yet Fitz et al. (2003) found no change in the rhizosphere pH of *P. vittata*. Since the soil used in their experiment had high carbonate content and high buffer capacity, their results were not unexpected. The increase in soil pH observed in the rhizosphere of *P. vittata* in our study was more likely due to the low buffer capacity of the sandy soil as well as the cation/anion balance caused by high excretion of hydroxyl groups in response to high absorption of arsenic. The pH buffering capacity of the soil and the initial soil pH are the main factors determining the extent to which plant roots can change rhizosphere pH.
(Marschner, 2003). Thus, the high rhizosphere pH measured in the *P. vittata* rhizosphere not only made $\text{HAsO}_4^{2-}$ the prevailing form in the system but also increased the negative surfaces charges of soil minerals (iron and aluminum oxides).

Similar to pH, plant growth had no effect on DOC concentration in the bulk soil. However, the rhizosphere of *P. vittata* and *N. exaltata* had about 44 and 33% higher DOC concentration than in the bulk soil (Fig. 5-6). The DOC content in the rhizosphere soil of *P. vittata* was approximately 9% higher than that of *N. exaltata*. Some components of DOC are active substances exhibiting strong affinities with trace elements (Phillippe, 1999). Thus, increased DOC content may facilitate the change of otherwise stable arsenic fractions.

The small difference in DOC concentration in the rhizosphere between the two ferns suggests that DOC-induced mobilization of arsenic in the rhizosphere may not play a significant role in the arsenic hyperaccumulation by *P. vittata*. Fitz et al. (2003) reported an increase of 86% in DOC in the rhizosphere of *P. vittata* growing in a soil containing 2,270 mg kg$^{-1}$ As with higher pH and higher clay content. Wenzel et al. (2003) also found a significantly higher DOC concentration in the rhizosphere than in the bulk soil of the Ni hyperaccumulator *Thlaspi goesingense* after growing in soil containing 2,580 mg kg$^{-1}$ Ni. Tu et al. (2004) reported DOC concentration of *P. vittata* twice as high as *N. exaltata* in a hydroponic nutrient solution. However, data obtained from hydroponic studies do not account for plant-soil interactions and the potential role of microorganisms in the rhizosphere (Wenzel et al., 2003).
It has been proposed that hyperaccumulator species may enhance metal solubility in the rhizosphere via root exudation as it is known for other plants (Knight et al., 1997). However, the separation of plant root exudation from those derived from microbial metabolites in the rhizosphere is a difficult task since the root exudates are hard to collect. Moreover, the rates of rhizo-deposition even for a given plant species vary greatly and can be 2-4 times higher for soil-grown plants than for plants grown in nutrient solution (Trofymow et al., 1987). This is because rhizosphere microorganisms increase rhizodeposition (Meharg and Killham, 1991).
The different results from the above mentioned studies are likely due to differences in soil characteristics, plant species, growing medium (soil x nutrient solution), metal concentration in the soil, different microbial population and activity, experimental period and climate conditions, plant age, and stress factors (Uren, 2000). Also, the methods used to extract DOC affect the amount extracted.
CHAPTER 6
ARSENIC ACCUMULATION BY TWO HYPERACCUMULATOR Pteris SPECIES FROM TWO ARSENIC CONTAMINATED SOILS

Introduction

Arsenic (arsenic) pollution has become a major public concern in many countries. Phytoextraction is an in situ, low cost and environmentally sustainable emerging technique (McGrath, 1998). This technology relies on the use of hyperaccumulator plants that can produce high biomass and achieve sufficiently high metal concentrations in the shoots (Raskin and Ensley, 2000).

Many studies have been conducted to identify plant species capable of accumulating undesirable toxic elements. For instance, Reeves and Baker (2000) compiled an exhaustive list of plant species that hyperaccumulate metals. Ma et al., (2001) reported that Pteris vittata L, an arsenic-hyperaccumulator fern, accumulated concentrations as high as 23 000 mg kg\(^{-1}\) As in the fronds. Later research has identified several other arsenic-hyperaccumulator fern species belonging to the order Pteridales, including Pteris cretica, Pteris longifolia and Pteris umbrosa (Zhao et al., 2002). A screening experiment carried out in a hydroponic system revealed that another fern species Pteris biaurita was capable of accumulating as much arsenic as P. vittata when growing in solution containing 50 mg L\(^{-1}\) of arsenic (Srivastava et al., 2006). The ability of this new fern specie in accumulating arsenic from contaminated soils with different arsenic levels is still
unknown as well as its growth and development. This could be important when choosing a given plant specie to phytoremediate contaminated soils.

The geographical distribution, growth and physiology of pteridophytes species such as *P. biaurita* and *P. vittata* are highly influenced by abiotic factors such as sunlight, available water, substrate conditions, soil fertility and etc (Smith et al., 1997). Some ferns are found only in sunny and disturbed habitats, others are in shady regions (Greer et al., 1997). Shade leaves are thinner with more leaf surface area, have higher chlorophyll contents and less dry mass per unit leaf area than sunny leaves (Brach et al., 1993). Hill (1972) reported that fern species from open, sunny habitats have higher maximum photosynthesis rates than the shade species.

Due to the diversity of ecosystems that contamination occurs, effort has to be made not only to identify new plants with the ability to accumulate the contaminant but also to compare the performance of hyperaccumulator plants in different environments. In the present study we selected two arsenic hyperaccumulators, *P. vittata*, a plant adapted to wide range of ecosystem, specially in sunny, alkaline and well drained environment, and *P. biaurita*, a plant adapted to low temperature, high moisture and shady conditions (Jones, 1997). The objective of this study was to compare the two plants in terms of: i) plant arsenic accumulation and distribution, ii) plant biomass; iii) chemical changes in the rhizosphere; and iv) plant elemental composition.

**Materials and Methods**

**Soil Collection and Characterization**

Two soil samples were collected from the CCA site as described in the Chapter 5. Surface soil (20 cm) was sampled, air-dried and sieved (2 mm mesh).
The soil analyses were performed as described in the Chapter 4. The soil samples had different arsenic levels (153 and 266 mg kg\(^{-1}\)), and thereafter they are referred to as soil-153 and soil-266 (Table 6-1). In addition, water extractable arsenic was measured using a 1:4 soil to water ratio after shaking for 2 h at 25 ± 1°C. Extractable P was extracted with the Mehlich-3 extractant, and determined using a modified molybdenum blue method to minimize arsenic interference (Carvalho et al., 1998). Select physical and chemical properties of the soil are summarized in Table 6-1.

**Table 6-1. Selected properties of the soils used in this study**

<table>
<thead>
<tr>
<th>Property</th>
<th>Soil-153</th>
<th>Soil-266</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:2 soil/water ratio)</td>
<td>7.32 ± 0.02*</td>
<td>7.37 ± 0.04</td>
</tr>
<tr>
<td>Organic matter content (g kg(^{-1}))</td>
<td>10 ± 0.08</td>
<td>13 ± 0.40</td>
</tr>
<tr>
<td>Cation exchange capacity (cmol(-) kg(^{-1}))</td>
<td>4.40 ± 0.02</td>
<td>5.20 ± 0.03</td>
</tr>
<tr>
<td>Total As (mg kg(^{-1}))</td>
<td>153 ± 5.30</td>
<td>266 ± 5.45</td>
</tr>
<tr>
<td>Mehlich III-As (mg kg(^{-1}))</td>
<td>43.7 ± 2.60</td>
<td>61.9 ± 3.60</td>
</tr>
<tr>
<td>Mehlich III-P (mg kg)</td>
<td>28.8 ± 0.3</td>
<td>26.9 ± 0.7</td>
</tr>
<tr>
<td>Total Zn (mg kg(^{-1}))</td>
<td>0.88 ± 0.3</td>
<td>0.90 ± 0.5</td>
</tr>
<tr>
<td>Total Cd (mg kg(^{-1}))</td>
<td>0.08 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Total Cu (mg kg(^{-1}))</td>
<td>8.50 ± 2.2</td>
<td>14.0 ± 1.2</td>
</tr>
<tr>
<td>Total Cr (mg kg(^{-1}))</td>
<td>40.8 ± 4.2</td>
<td>70.5 ± 3.3</td>
</tr>
<tr>
<td>Sand (g kg(^{-1}))</td>
<td>882 ± 4.32</td>
<td>884 ± 5.76</td>
</tr>
<tr>
<td>Silt (g kg(^{-1}))</td>
<td>91 ± 0.04</td>
<td>90 ± 0.07</td>
</tr>
<tr>
<td>Clay (g kg(^{-1}))</td>
<td>27 ± 0.01</td>
<td>26 ± 0.01</td>
</tr>
</tbody>
</table>

*Mean ± standard deviations, n = 3

**Experimental Design**

This greenhouse experiment was set up as a completely randomized design in a 2 by 2 factorial scheme consisting of two arsenic levels (153 mg kg\(^{-1}\) and 266
mg kg\(^{-1}\)) with two fern species (*P. vittata* and *P. biaurita*) (Figure 6-1). Each treatment was replicated three times.

Air-dried soil (2.5 kg) was weighed into each pot and thoroughly mixed with 3.0 g of Osmocote, an extended time-release fertilizer (18-6-12) (Scotts-Sierra Horticultural Products Co., Marysville, OH). After one week of equilibrium at field capacity, one plant was transplanted into each pot.

The rhizopot used in this study was 16 cm in height and 13 cm in diameter and composed of two compartments separated by a plastic frame covered with a nylon mesh cloth (mesh size 45 µm). Root growth was limited to the central compartment of 7 cm of diameter (Figure 5-1). Pre-experimental biomass of fronds and roots of the two fern species was also determined. The average dry frond and root biomass were 5.60 g plant\(^{-1}\) and 1.85 g plant\(^{-1}\), respectively.

The plants were allowed to grow for eight weeks in a greenhouse (average temperature was 14\(^{\circ}\)C at night to 30\(^{\circ}\)C during the day, with an average photosynthetically active radiation flux of 825 \(\mu\text{mol m}^{-2} \text{s}^{-1}\)). The plants were watered throughout the study to keep the soil at approximately 70% of field capacity.

After 8 weeks of growth, the plants were harvested and separated into roots and fronds. Plant tissues were washed thoroughly with tap water, and then rinsed with deionized water. The fronds and roots were oven-dried for 3 d at 65\(^{\circ}\)C, weighed and ground with a Wiley mill to pass through a 1 mm mesh screen for chemical analysis. Soil samples from each pot were collected from the rhizosphere and bulk soil. The rhizosphere is defined as the soil attached to the roots.
Rhizosphere soil was shaken gently from the roots and passed through a 2 mm sieve to separate it from the roots.

**Chemical Analysis**

Plants and soils digestion and analyses were performed as described in the Chapter 4.

Rhizosphere and bulk soil were evaluated for water-soluble arsenic and phosphorus, and dissolved organic carbon (DOC) from the supernatant of a 1:4 soil to water ratio solution (Olsen & Sommers, 1982), obtained after shaking (1 h), centrifuging (15 min at 3500 g) and filtering (0.45 µm syringe filter). The determination of water-soluble arsenic was performed by the same procedure described above. Concentrations of DOC were measured using a TOC-5050A Total Organic Carbon analyzer (Shimadzu, Japan). The pH in the rhizosphere and in the bulk soil was also measured using a 1:2 soil to water ratio.

**Data Analysis**

Plant and soil effects were determined by analysis of variance according to the linear model procedure of the Statistical Analysis System (SAS Institute Inc. 1987). Treatment means were separated by Duncan’s multiple range tests using a level of significance of $P<0.05$.

**Results and Discussion**

**Arsenic Accumulation and Distribution in the Ferns**

Plant arsenic concentration and biomass are two important traits in selecting an ideal plant species to be used for phytoextraction purpose. We compared the arsenic accumulation ability of *P. biaurita*, a newly reported arsenic
hyperaccumulator (Srivastava et al., 2006) with *P. vittata*, one of the most studied arsenic hyperaccumulators. Regardless of the soil arsenic levels (153 and 266 mg kg\(^{-1}\)), *P. vittata* was more efficient in taking up arsenic from the two soils than *P. biaurita* (Table 6-2).

Figure 6-1. *Pteris biaurita* and *Pteris vittata* growing in an arsenic-contaminated soil.

Frond and root arsenic concentrations of the two fern species were proportional to the soil arsenic concentration. The correlation between the soil and frond arsenic concentrations has been well documented (Tu and Ma, 2003; Luongo and Ma, 2005; Al Agely et al., 2005).

After 8 weeks of growth, with soil arsenic concentrations increasing from 153 to 266 mg kg\(^{-1}\), arsenic concentrations in the fronds of *P. vittata* increased from
758 to 3759 mg kg\(^{-1}\) (5 times greater) while those in \(P.\ biaurita\) increased from 468 to 2549 mg kg\(^{-1}\) (5.4 times greater). The arsenic concentration in the fronds of \(P.\ biaurita\) was 1.6 and 1.5 times lower than that of \(P.\ vittata\) in the soil-153 and soil-266, respectively (Table 6-2). Even though \(P.\ biaurita\) accumulated large amount of arsenic in its fronds, \(P.\ vittata\) was more efficient.

Table 6-2. Comparison of the arsenic concentration (mg kg\(^{-1}\)), translocation factor (TF) and bioconcentration factor (BF) of \(P.\ vittata\) and \(P.\ biaurita\) after 8 weeks of growth in two As-contaminated soils.

<table>
<thead>
<tr>
<th>Variable</th>
<th>(P.\ biaurita) soil 153</th>
<th>(P.\ vittata) soil 153</th>
<th>(P.\ biaurita) soil 266</th>
<th>(P.\ vittata) soil 266</th>
</tr>
</thead>
<tbody>
<tr>
<td>As in soil</td>
<td>153</td>
<td>266</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As in fronds</td>
<td>468 b(^*)</td>
<td>758 a</td>
<td>2549 b</td>
<td>3759 a</td>
</tr>
<tr>
<td>As in roots</td>
<td>284 a</td>
<td>162 b</td>
<td>1024 a</td>
<td>378 b</td>
</tr>
<tr>
<td>BF in fronds</td>
<td>3.05 b</td>
<td>4.95 a</td>
<td>9.60 b</td>
<td>14.1 a</td>
</tr>
<tr>
<td>BF in roots</td>
<td>1.85 a</td>
<td>1.06 b</td>
<td>3.85 a</td>
<td>1.42 b</td>
</tr>
<tr>
<td>TF</td>
<td>1.64 b</td>
<td>5.11 a</td>
<td>2.51 b</td>
<td>9.98 a</td>
</tr>
</tbody>
</table>

\(^*\) Means followed by the same letter in a row for the same soil are not statistically different by the Duncan test at \(P < 0.05\).

On the other hand, regardless of the soil arsenic concentration, the arsenic concentrations in the roots of \(P.\ biaurita\) were much greater than in the roots of \(P.\ vittata\), further confirming the greater effectiveness of \(P.\ vittata\) in translocating arsenic from the root to the fronds. The arsenic concentration in the fronds of \(P.\ biaurita\) was only 1.6 and 2.5 times greater than the arsenic concentration in the roots in soil-153 and soil-266, respectively, while the arsenic concentration in the frond of \(P.\ vittata\) was 4.7 and 10 times greater than in the roots in the soil-153 and
soil- 266, respectively. The large arsenic concentration in the roots of *P. biaurita* may be a limiting factor for its use in phytoextraction.

Arsenic accumulation by *P. vittata* in the soil-153 was lower than previously reported (Ma et al., 2001; Tu and Ma, 2002; Wang et al., 2002; and Fayiga et al., 2004), suggesting that *P. vittata* was not functioning in its full potential. Using the same CCA soil containing 131 mg kg\(^{-1}\) As, Fayiga et al. (2004) reported arsenic concentrations in the fronds and roots of 4,200 and 130 mg kg\(^{-1}\) after 8 weeks of growth. Tu et al. (2002) reported 1,280 and 22.3 mg kg\(^{-1}\) after growing in the same CCA soil containing 98 mg kg\(^{-1}\) for 8 weeks.

However, in the four studies carried out in this research using CCA soil, the highest arsenic concentration in the fronds of *P. vittata* was 3,759 mg kg\(^{-1}\) after growing in a CCA soil containing 266 mg kg\(^{-1}\) (Table 6-3). In the other three studies, the arsenic concentration in the fronds ranged from 325 mg kg\(^{-1}\) to 747 mg kg\(^{-1}\). Even though the CCA soil samples were taken from the same CCA contaminated site for this study and for the studies of Fayiga et al. (2004) and Tu et al (2002), the arsenic concentrations were different for all samples due to the heterogeneity of the site.

Based on the results gathered in this research, it is suggested that the differences observed among the different studies are likely due to plant age and seasonal effects, among others. It is not possible to make considerations about age of the plants, seasonal effects etc, for someone else’s studies, however, in this research, the studies of the chapters 4, 5 and 6 were carried out from late January to March. Therefore, it is possible that the temperature and light affected the growth
Based on bioaccumulation factor (BF), defined as the ratio of arsenic concentration in the plant tissue to that in the soil (Tu and Ma, 2002), both plant species were efficient in accumulating arsenic from the soil-266 (BF>1) (Table 6-2). However, the BF in *P. biaurita* frond was approximately 1.4 times lower than that of *P. vittata* in the soil-266 and 1.6 times lower in the soil-153. In this regard, *P. biaurita* was not as efficient as *P. vittata* in accumulating arsenic from either soil.

Plants used for phytoextraction must be able to transport most of the contaminant to the fronds, which facilitates contaminant removal (Raskin and Ensley, 2000). In both plants we observed preferential partitioning of arsenic to the fronds, with transfer factor (TF) (the ratio of contaminant concentration in the fronds to that in the roots) also > 1, typical for hyperaccumulator plants (Baker, 1981; McGrath and Zhao, 2003). However, the ability of *P. vittata* in transferring arsenic from the root to the frond was much greater than that of *P. biaurita*, in both soils (Table 6-2). According to Ma et al. (2001), the high efficiency of arsenic accumulation by arsenic-hyperaccumulator fern species is associated with their ability to rapidly translocate absorbed arsenic from roots to shoots, although they differ in efficiency.

**Plant Biomass of *P. biaurita* and *P. vittata***

Total dry matter is another key component in determining the effectiveness of contaminant removal from a site by a hyperaccumulator plant. After eight weeks
of growth, there were no significant differences between the two fern species in terms of biomass production (Figure 6-2). Frond biomass of *P. biaurita* and *P. vittata* increased nearly 3-fold as compared to their original biomass, with the exception of the frond biomass of *P. biaurita* in the soil-266, which increased approximately two-fold. In contrast, the root dry matter accretion of both fern species was slightly smaller in the soil-266 (Figure 6-2). Many hyperaccumulator ferns, including *P. vittata*, have shown a substantial biomass production either in greenhouse or field conditions (Komar et al. 1998; Francesconi et al. 2002). For instance, Fayiga et al. (2004) reported a much higher increase (12 times) in the plant biomass of *P. vittata* after eight weeks of growth in arsenic contaminated soil (131 mg As kg$^{-1}$).

![Figure 6-2](image)

**Figure 6-2.** The frond and root biomass (mg kg$^{-1}$ DW) of *P. vittata* and *P. biaurita* after 8 weeks of growth in two arsenic contaminated soils (Soil 1: 153; Soil 2: 266). Results are means ± standard deviations (n = 3).

The final frond and root biomass were similar for the two fern species growing in the soil-153. The small root biomass of both ferns, especially at the
higher arsenic level, can be a limiting factor for both plant species in plant arsenic uptake, since a smaller volume of soil was exploited.

**Rhizosphere Chemistry**

Plant cultivation effects can be understood by observing the changes in the rhizosphere such as soil pH and DOC, which may be important for the mobilization of soil arsenic. Therefore, we measured the concentrations of the water-soluble arsenic and P in the bulk soil and rhizosphere of the two plant species.

The rhizosphere soils were more alkaline than the bulk soils, with the difference for *P. vittata* being 0.28 in the soil-153 and 0.51 units in the soil-266. The pH difference for *P. biaurita* was 0.22 and 0.47 in the two soils (Figure 6-3). No change in pH was observed in the bulk soil. This clearly showed that plant roots induced the changes in soil pH. The preferential uptake and exudation of either cations or anions by plant roots occurs in order to maintain their charge balance (Hinsinger et al., 2003), with consequent changes of rhizosphere pH of up to 1-2 units.

Similar results were observed in the chapter 5 but with greater differences between bulk and rhizosphere soils after growth of *P. vittata*. The fact that both *P. vittata* and *P. biaurita* increased the rhizosphere pH in this study while *N. exaltata* even reduced the rhizosphere pH in the chapter 3 study suggests that soil pH may play a role in the arsenic accumulation by hyperaccumulator ferns.
Figure 6-3. The bulk soil and rhizosphere pH of *P. vittata* and *P. biaurita* after 8 weeks of growth in two arsenic contaminated soils. Results are means ± standard deviations (n = 3).

The DOC concentrations in the rhizosphere of both fern species were approximately twice as high as those in the bulk soil (Figure 6-4), with the exception of *P. biaurita* in the soil-266, where the DOC content was nearly three times higher than the bulk soil. It has been reported that *P. vittata* releases organic acids of low molecular weight such as phytic and oxalic acid as well as small quantities of ascorbic, succinic and fumaric acid via root exudation (Tu et al. 2003). The presence of these acids may have a significant influence on arsenic mobilization in the plant rhizosphere. It is known that root exudation varies with species, age and relative stress (Uren, 2000). For example, in the soil-153, *P. biaurita* had 15% less DOC in its rhizosphere than *P. vittata*. But in the soil-266
the situation reversed, with *P. biaurita*’s rhizosphere having 30% more DOC than *P. vittata*’s. The change in rhizosphere DOC concentration may have further implications with respect to the availability of nutrients and soil microbial population (Kullberg et al. 1993). In general, DOC readily forms both aqueous and surface inner-sphere complexes with cationic metals and metal oxides, which, in turn, may associate strongly with other dissolved anions by metal-bridging mechanisms (Thanabalasingam and Pickering, 1986), diminishing the tendencies of such anions to form surface complexes. Thus, the reaction of DOC with arsenic is expected and has the potential to influence on arsenic sorption and mobility in soils.

![Figure 6-4. The bulk soil and rhizosphere dissolved organic carbon (DOC) of *P. vittata* and *P. biaurita* after 8 weeks of growth in two arsenic contaminated soils. Results are means ± standard deviations (n = 3).](image-url)
Water-soluble (WS) metal is a good indicator of bioavailability for plant uptake from a soil (McBride, 1994), since plants preferentially take up their nutrients from a soil solution (Linehan et al., 1985). The concentrations of water-soluble arsenic and P were significantly higher in the rhizosphere than in the bulk soil for both ferns (Figure 6-5 and 6-6). The increase in the rhizosphere WS-P and WS-As (greater than the control and bulk soil) indicated that mobilization of P and arsenic was plant driven. *Pteris biaurita* presented 27% less WS-P in its rhizosphere in the soil-153 than *P. vittata*. In contrast, the WS-P concentration in *P. biaurita* rhizosphere was 37% greater than that in *P. vittata* rhizosphere in the soil 266. It is very likely that higher DOC concentration in *P. biaurita* rhizosphere in the soil-266, compared to that in the soil-153, may have contributed to the high P as well as high arsenic mobilization in the soil.

Figure 6-5. The water-soluble P concentrations in the bulk soil and rhizosphere of *P. vittata* and *P. biaurita* after 8 weeks of growth in two arsenic contaminated soils. Results are means ± standard deviations (n = 3).
The concentrations of WS-arsenic in the rhizosphere of *P. biaurita* in the soil-266 was greater than that of *P. vittata*, likely due to both lower arsenic uptake and higher DOC exudation, showing that *P. biaurita* was not as efficient in arsenic uptake as *P. vittata*. In general, it is hypothesized that, as plant uptake depletes WS-arsenic, the plants are capable of releasing more arsenic into the soil solution, one of the characteristics of hyperaccumulators. This did not seem to apply in our study since the fern with more WS-arsenic in its rhizosphere was not the one that took up more arsenic, suggesting that other external factors contributed to the higher WS-arsenic in the rhizosphere.

![Figure 6-6](image)

**Figure 6-6.** The water-soluble As concentrations in the bulk soil and rhizosphere of *P. vittata* and *P. biaurita* after eight weeks of growth in two arsenic contaminated soils. Results are means ± standard deviations (n = 3).

A higher WS concentration of low mobility elements (As and P) in the rhizosphere is an indicator of either low uptake and/or high solubilization of the elements by plants. It is possible that the arsenic was being solubilized at a faster rate.
pace than the capacity of the plants to take it up, which resulted in accumulation of arsenic in the rhizosphere.

The combined data of WS-P and WS-As may partially explain the low plant arsenic uptake in the soil-153 (Figs. 6-5 and 6-6). Compared to the soil-266, the WS-P was greater but its WS-As was lower in the soil-153. In other words, the molar ratio of WS-P to WS-As in the soil-153 was 8.5 compared to 0.69 in the soil 266. The high WS-P in the soil-153 may have severely reduced the plant’s ability to take up arsenic via competition. However, the greater P concentration in the soil-153 did not translate into high P accumulation in the fronds nor in the roots of *P. vittata* (Table 6-3). The P concentration in the fronds of *P. vittata* was 0.46% in the soil-153 compared to 1.08% in the soil-266 though the root P concentration was the same (0.15%).

**Elemental Composition**

Proper plant nutrition may enhance arsenic accumulation and the potential for soil remediation by phytoextraction. The P concentrations in the fronds of both species were 2.5 (*P. vittata*) and 1.6 (*P. biaurita*) times higher in the soil-266 than in the soil-153 (Table 6-3). Soil arsenic concentrations influenced frond P concentrations but not root P in both fern species. Phosphorus concentration in the fronds were within the normal range for most plants (0.1 to 0.5 %) (Marschner, 2003) in the soil-153, which is consistent with the results of Tu and Ma (2005). However, significantly greater P concentration was observed in the fronds in the soil-266 (1.08 % for *P. vittata* and 0.87 % for *P. biaurita*).
Table 6-3. The macronutrients (P, K, Ca, Mg) (g kg\(^{-1}\)) and micronutrients (Fe, Zn, Mn) content (mg kg\(^{-1}\)) in the fronds and the roots of *P. vittata* and *P. biaurita* after 8 weeks of growth in two arsenic-contaminated soils.

<table>
<thead>
<tr>
<th>Elemental content</th>
<th><em>P. vittata</em> Frond</th>
<th><em>P. vittata</em> Root</th>
<th><em>P. biaurita</em> Frond</th>
<th><em>P. biaurita</em> Root</th>
<th><em>P. vittata</em> Frond</th>
<th><em>P. vittata</em> Root</th>
<th><em>P. biaurita</em> Frond</th>
<th><em>P. biaurita</em> Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus</td>
<td>4.60 A 1.50 a</td>
<td>5.50 A 1.30 a</td>
<td>10.8 A 1.50 a</td>
<td>8.70 A 1.70 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>24.8 A 4.90 a</td>
<td>27.6 A 2.70 a</td>
<td>33.5 A 2.80 a</td>
<td>23.2 B 2.70 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>7.10 A 46.9 a</td>
<td>5.50 B 3.20 b</td>
<td>6.20 A 32.6 a</td>
<td>5.90 A 21.3 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>3.40 A 2.50 b</td>
<td>3.30 A 3.70 a</td>
<td>4.20 A 3.00 a</td>
<td>3.50 A 1.80 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>160 A 1828b</td>
<td>72.0B 2625 a</td>
<td>75.0 A 2005 a</td>
<td>80.0 A 483 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>52.3 A 89.0 a</td>
<td>44.0 A 84.0 a</td>
<td>53.7 A 131 a</td>
<td>38.3 B 117 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>23.7 A 39.3 b</td>
<td>27.0 A 53.0 a</td>
<td>26.3 A 51.7 a</td>
<td>22.3 A 41.0 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers with same uppercase letter or lowercase letter in a row are not statistically different by the Duncan test at \( P < 0.05 \) for the same soil or plant, respectively.
Phosphate is critical for plant growth especially when arsenic is at toxic levels (arsenic concentration was 73% greater in the soil-266 than the soil-153). Both fern species had the same P concentrations when they were grown in the same soil. However, the frond P concentrations in both ferns were greater in the soil containing more arsenic.

Luongo and Ma (2005) studied the characteristics of several *Pteris* and non-*Pteris* ferns and observed that, for all ferns except *P. vittata*, with increasing arsenic concentrations, phosphate concentrations in the fronds and roots decreased. In their study, phosphate concentrations in *P. vittata* increased by 91.8% in the fronds and 455% in the roots when growing in a solution containing 10 mg L\(^{-1}\) As, as compared to the control.

Phosphate plays an important role in arsenic detoxification in ferns. Arsenate interferes with phosphate metabolism by replacing phosphate in ATP synthesis and/or in various phosphorolysis reactions (Tu and Ma, 2003b). Therefore, it may exist a proportion of P in ferns that may be replaced by arsenic without causing significant changes in plant metabolism.

Another interesting observation from these results is that the P concentrations in the roots were lower than those in the fronds (Table 6-3). Compared to non arsenic hyperaccumulators, more P tends to be concentrated in the roots in hyperaccumulator plants (Tu and Ma, 2003c). Luongo and Ma (2005) also reported that higher level of arsenic in solution reduced P translocation in *P. vittata*, which was able to keep much more P in the roots than other ferns. The same results were observed in Chapter 5 of this research when *P. vittata* and *N. exaltata* were grown in the control soil (0.4 mg kg\(^{-1}\) As). Both plants concentrated more P in the fronds than in the roots. On the other hand, *P. vittata* concentrated more P in the roots than in the fronds in the arsenic contaminated
soil, agreeing with the results of Tu and Ma (2003c). Therefore, the greater accumulation of P in the fronds of *P. vittata* and *P. biaurita* instead of in the roots likely had a negative effect on the arsenic uptake by these plants by interfering with the arsenic tolerance mechanisms. The slow growth of the plants during the experimental period and the yellowish of the leaves after 6 weeks of growth, mostly in *P. biaurita*, were signals that indicated the poor conditions of the plants.

The ability of *P. vittata* in maintaining high concentrations of P in its roots is reported to be one of its mechanisms of arsenic tolerance (Tu and Ma, 2003c). This is because arsenate uptake is intimately linked to phosphate nutrition, with increased phosphate uptake reducing arsenate uptake, via suppression of a high-affinity phosphate/arsenate uptake system (Meharg and Macnair, 1992).

The phosphate/arsenate transporter has a higher affinity for phosphate, and if external phosphate status is high, phosphate will be taken up preferentially to arsenate. This is the case for both plants. Take the soil-266 for example, the molar ratios of WS-P to WS-As were 0.69 and 0.81 for *P. vittata* and *P. biaurita*, respectively (Figures 6-5 and 6-6). On the other hand, the molar ratio of P to As in the fronds of *P. vittata* and *P. biaurita* were 7.0 and 8.0, respectively (Tables 6-2 and 6-3). This indicates that the plants preferentially take up more P than arsenic though the WS-As concentrations were greater than WS-P concentrations in the soil. Compared to *P. biaurita*, *P. vittata* not only took up more arsenic (Table 6-2) but also more P (Table 6-3). It is possible that the P uptake system in *P. vittata* was more efficient than *P. biaurita*.

In this study, the plants absorbed more phosphate to cope with the greater level of arsenate in the soils. Considering the ability of a plant to take up P as one of the
mechanisms of arsenic detoxification, both *Pteris* ferns behaved the same way in this study, that is, absorbed more P from the high arsenic soil. However, they concentrated more P in the fronds than in the roots, which was unexpected since more P in the roots is hypothesized to be required to minimize the arsenic toxicity effect by improving phosphate nutrition in the roots (Sneller et al., 1999; Tu and Ma, 2003b; Luongo and Ma, 2005). Al Agely et al. (2005) reported similar results when growing *P. vittata* in the presence and absence of mycorrhizal fungi. They observed that phosphorus concentrations were greater in the fronds than in the roots of plants. The phosphorus concentrations in the fronds of *P. vittata* grown in arsenic -contaminated soils (As concentrations ranging from 69 to 28, 522 mg kg⁻¹), under field conditions, were also higher than those in the roots (Liao et al., 2004). Therefore, the interaction between P and arsenic still remains unclear in term of arsenic hyperaccumulation and detoxification by these plants.

The nutritional status of the two plants was similar. However, in the soil-153, *P. vittata* accumulated more Ca in the fronds than *P. biaurita* (Table 6-3). Furthermore, the greater influx of Ca in *P. vittata* could be explained by the need for balancing the anions phosphate and arsenate taken up by the plants. It is hypothesized that Ca is related to the arsenic hyperaccumulation in ferns (Chen et al. 2003). The oxidative stress caused by metal toxicity can increase Ca concentrations in plant cells, where Ca stimulates the induction of free radical scavengers (Price et al. 1994) to maintain the cellular unity of a plant. In this study, frond Ca contents of both fern species did not differ and were in the normal range for most plants (0.2 to 1.0 %) (Marschner, 2003).
Our results are similar to those of Li et al. (2005), who observed Ca concentrations in the fronds of *P. vittata* ranged from 0.28 to 0.32 %. However, Ca concentrations of both fern species were much higher in the roots than in the fronds. We observed Ca enrichment in the roots of both fern species, especially in the roots of *P. vittata* in the soil-153 (4.69 %). Under high arsenic conditions, the roots accumulated Ca to maintain the cellular integrity. Liao et al. (2004) also reported Ca enrichment in the roots of *P. vittata* under field conditions. In contrast, *P. biaurita* had more root Mg than *P. vittata*.

In addition, results for Fe, Zn and, Mn were obtained for both species (Table 6-3). Zinc and Mn concentrations did not differ between the two fern species, but frond Fe concentrations of *P. vittata* were significantly higher (2.2 fold) than that of *P. biaurita* in the soil-153. However, no difference was found in the soil-266, the values were still within the normal range for plants (50 to 250 mg kg$^{-1}$; Marschner, 2003). The iron content in the roots of both fern species was greater than those in the fronds. In the soil-153, *P. biaurita* had 30% more Fe in the roots than those in *P. vitatta*. In contrast, in the soil-266, *P. biaurita* had 76 % less Fe as compared to *P. vittata*. Wei et al. (2005) reported greater Fe concentrations in *P. vittata* and *P. cretica* when compared with other plants. Wenzel et al. (2003) observed an increase in Fe concentrations in the rhizosphere of *P. vittata*, which they related to the dissolution of soil minerals caused by root activity. Under these experimental conditions, both acidification and the release of DOC into the rhizosphere may have contributed to the mobilization of iron, which was taken up by the roots.
CHAPTER 7
CONCLUSIONS

Phytoextraction is a new technology and its success depends on both plant and soil factors such as soil suitability for plant growth, depth of contamination, depth of the plant root system, level of contamination, and urgency in cleaning up. Furthermore, there is need for a better understanding of the physiology, biochemistry, and metal uptake of the plants.

The ability of *P. vittata* to grow and take up arsenic from different soils with different levels of arsenic was investigated. The ferns grew well in all soils showing little toxicity symptom. However, both the biomass production and arsenic concentrations in the plants depended on the growing season, with best results observed during warmer months. After three harvests, the plants removed 6.4 to 13% arsenic from the soils, but failed to re-grow in all soils. Plant growth influenced arsenic mobilization from different fractions of the soils as evaluated by a sequential extraction procedure, showing that plants took up arsenic from the less available arsenic pools. It is likely that the exudation of organic acids in the rhizosphere played a role in this process, as evidenced by the amounts of arsenic extracted from the soils using the organic acids and its positive relationship with the amounts of arsenic taken up by the plants. In almost all soils studied, arsenic uptake by *P. vittata* was similar to the amounts of arsenic extracted by the organic acids. Therefore, in evaluating the bioavailability of arsenic to *P. vittata*, the use of organic acids may be a good approach than the total and Mehlich III arsenic.
The influence of *P. vittata* age on the arsenic removal from a contaminated soil was also investigated. Younger plants accumulated more arsenic in the fronds as a result of their higher metabolic activity, higher rate of biomass accretion and lower P concentration in the fronds and roots. On the other hand, plants with lower biomass accretion, thus, lower nutritional requirement, had higher arsenic and P concentration in the roots, and lower arsenic concentration in the fronds. Therefore, in establishing a program of arsenic phytoextraction using *P. vittata*, the use of young plants has the benefits of higher rate of arsenic uptake, higher arsenic translocation to the fronds and consequently higher efficiency of arsenic removal. Furthermore, the use of younger plants offers the possibility for managing harvests before spores maturation, therefore avoiding the risks of uncontrolled ferns propagation.

The differences between the root system of a hyperaccumulator and non-accumulator species may account for some of its elemental scavenging ability of the species. This was observed through a comparison of the root system characteristics (biomass, length, area and diameter), plant biomass and arsenic uptake and root uptake efficiency of *P. vittata* with a non-arsenic accumulator fern species *N. exaltata*. The study revealed that *P. vittata* had a more extensive root system (root biomass, length and area) and possessed a greater proportion of fine roots (root diameter) than *N. exaltata*. *Pteris vittata* was more efficient in removing arsenic from an arsenic-contaminated soil than *N. exaltata*. Also, plant growth significantly impacted the total arsenic, water soluble arsenic, as well as arsenic distribution in different fractions in the rhizosphere soil, but not the bulk soil. *Pteris vittata* had a greater effect on arsenic mobilization from different pools than *N. exaltata*. The greater decrease in the soil pH and increase in DOC
concentrations in the rhizosphere of *P. vittata* may also have enhanced its ability to take up more arsenic.

*Pteris vittata* was compared with another arsenic hyperaccumulator fern of the same genus, *P. biaurita*, in terms of its arsenic accumulation, biomass production and changes in the rhizosphere in two soils with different arsenic contents. Regardless of the levels of arsenic contamination, *P. vittata* removed more arsenic than *P. biaurita* from both soils. Although the two ferns have different growth requirements, in general, both ferns seemed to have similar arsenic hyperaccumulation and growth characteristics. However, *P. biaurita* was more sensitive when exposed to higher soil arsenic level, showing some arsenic toxicity symptoms in the fronds. Therefore, *P. vittata* continues to be a better choice for the application to arsenic phytoextraction in subtropical ecosystems.

A relatively small group of plants are capable of sequestering arsenic in their shoot tissues at high concentrations with the majority of them belonging to the Pteridophytes. Efficient reproduction of these plants for a large scale phytoremediation can be a challenge. Cultivation of arsenic hyperaccumulators has to be optimized in order to provide an effective alternative to common engineering-based remediation technologies.

In recent years, major scientific progress has been made in understanding the physiological mechanisms of arsenic uptake and transport in these plants. However, little is known about the molecular basis of arsenic hyperaccumulation. Therefore, future research should focus on molecular genetic technologies and, possibly, transgenic plants with increased resistance and uptake not only of arsenic but also other heavy metals. Discovering the molecular mechanisms underlying arsenic tolerance and accumulation in
the arsenic hyperaccumulator ferns can be facilitated by identifying genes that are both necessary and sufficient for these properties.

Although phytoextraction is a promising remediation technology, it is still in its early stage of development and little information is available related to site cleanup from start to finish. This is probably due to the fact that few, if any, sites that have yet to be completely remediated using this technology. It is the case for arsenic phytoextraction as the first arsenic hyperaccumulator plant was only discovered in 2001.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Maria Isidoria Silva Gonzaga (named after her grandmother) was born on December 14, 1970, in Sao Felipe, Bahia, Brazil, to Mr. Henrique F. Carvalho and Mrs. Ines A. Silva. After receiving her high school diploma in 1989, Maria attended the School of Agronomy at the Federal University of Bahia, Brazil, receiving a degree in agronomy in 1995. During her study, Maria also worked there, in the Soil Physics Laboratory, lecturing and doing soil physical analyses.

In 1999, Maria received her Master of Science degree in agrarian sciences from the Federal University of Bahia, Bahia. Her master’s thesis was titled “Effects of industrial residues from cellulose production on Calopogonium mucunoides growth and on its soil physical and chemical attributes”.

In the Fall 2002, Maria joined the Soil and Water Science Department at the University of Florida, Gainesville, as a Ph.D. candidate to study the use of ferns to phytoremediate arsenic-contaminated soils.