Phytoremediation of Arsenic

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ABSTRACT

Arsenic is listed as the number one hazardous substance by The U.S Agency for Toxic Substances and Disease Registry. Arsenic has been used in embalming fluids, insecticides, herbicides, and defoliants. Acute exposure to arsenic through inhalation, or though consumption, can lead to an increased risk of several cancers and other diseases such as gangrene. The use of plants to remediate contaminated areas, otherwise known as phytoremediation, has been in existence for several decades. In order for plants to be effective in phytoremediation they must produce a large amount of harvestable biomass that contains high concentrations of a particular pollutant. Several plants have been discovered that have the ability to hyperaccumulate arsenic in their plant tissues. These plants are able to reduce arsenate contained in their roots to arsenite in their shoots and also contain significantly greater amounts of arsenic in their aboveground biomass in comparison to their root system. *Pteris vittata* and *Pityrogamma calomelanos* are the only two plant species that have been identified as hyperaccumulators. Several other plant species have been investigated for their potential as phytoextractors of arsenic. The actual mechanisms of arsenic uptake and the manner in which plants detoxify these pollutants are still somewhat unknown.   It can be concluded however, that several species of *Pteris* ferns as well as *Pityrogamma calomelanos* have great potential for use in phytoremediation of arsenic contaminated sites. Phytoremediation has several advantages over acid-washing and other remediation techniques: it's cheaper and it is much more environmentally friendly. There are, however, some drawbacks to phytoremediation. The first obstacle is related to the storage and subsequent disposal of the contaminated biomass. The second is identifying several other hyperaccumulators which are able to thrive in a broad range of soil conditions and climates. As research regarding this topic continues, many of these questions will be answered.

KEYWORDS

phytoremediation, hyperaccumulator, fern; *Pteris vittata* and *Pityrogamma calomelanos*, arsenic

INTRODUCTION

Arsenic. Arsenic is a heavy metal that is considered toxic when present in very low concentrations, on the order of parts per billion (ppb). Arsenic has been used in embalming fluids, herbicides, insecticides, defoliants, and is typically found in the vicinity of metal smelters. Arsenic usage dates back to the 17th and 18th centuries when it was used to kill several kings. It was also used in the 19th century by women to improve their facial complexion. Arsenic is mostly commonly associated with its poisonous effects on drinking water. It's currently estimated that over 57 million people worldwide consume water containing arsenic concentrations in excess of 10 ppb, which is the World Health Organization's standard for drinking water. Symptoms of arsenic poisoning include, but are not limited to, stomach pains, dizziness, headaches, and delirium. Arsenic is listed as the number one hazardous substance by The U.S Agency for Toxic Substances and Disease Registry. Chronic arsenic poisoning, known as Arsenicosis, can cause various forms of cancer and even gangrene. Arsenic can be fatal if 1.5 mg is ingested by a person weighing 155 pounds (Wikipedia, 2006). Research concerning arsenic poisoning has increased due to new evidence that arsenic may be toxic at levels lower than previously reported (Lombi et al., 2002).

Arsenic, found in the environment, is derived from geological and anthropogenic sources. Arsenic is typically found in the soil in the following forms: arsenate, arsenite, dimethyl arsenic acid (DMA), and monomethyl arsenic acid (MMA). All of these forms have different solubilities and mobilities; these properties affect their bioavailability to plants. Arsenic is concentrated in magmatic sulphide minerals and iron ores. The most common arsenic ores are arsenical pyrite or arsenopyrite, realgar, and orpiment. Oxidation of sulfide minerals can release large amounts of arsenic into aquifers used for drinking water sources. Arsenic is a waste product of metals; primarily tin (Fransesconi et al., 2002). Arsenic sorption on soil particles is a process that is vital to the immobilization of this metalloid. The high affinity of arsenic

for oxide surfaces is well documented and is dependent upon, but not limited to, several factors: soil pH, texture, and organic matter (Adriano, 2001). The mobility of arsenic in soil is pH dependent; arsenate is preferably absorbed at pH (4-7), while arsenite is preferably absorbed at pH (7-10) (Pierce and Moore, 1982). In natural systems, arsenic can exist in several forms of oxidation state: -3, 0, +3, +5. As (III) arsenite and As (V) arsenate are the main forms of arsenic found in the soil.

Current agricultural application of chemicals containing arsenic still exists; these chemicals are termed arsenicals. Current defoliant arsenicals include: arsenic acid, sodium arsenate, sodium arsenite, and dimethyl arsenic acid. Monosodium methyl arsenic acid, disodium methyl arsenic acid, and methyl arsenic acid are used as herbicides. Arsenic and its associations with iron and aluminum affect its behavior in the soil. Due to their lower solubility, ferric and aluminum arsenic compounds are not typically readily removed by plants (Tu and Ma, 2002a). The Arsenic toxicity threshold for most plants is 40 mg per kg and 200 mg per kg in sandy and clay-based soils (Tu and Ma, 2002a).

A study conducted by Koch et al. (2000) on gold mine tailings in Yellowknife, Canada, reported finding significant amounts of inorganic arsenic (i.e. arsenate and arsenite) in several different species of plants taken from several different areas in around the Yellowknife area. Arsenic is associated with gold mining because of its arsenopyrite complex.   The results of this study indicate that plants growing in arsenic contaminated regions primarily consist of inorganic species of arsenic although, in one species, *Lemna gibba,* or duckweed, methylated arsenic was found to be residing within the plant tissue in significant amounts (Mkandawire and Dudel, 2005). The results of this study conducted by Koch et al. (2000) are important because inorganic species of arsenic are the most toxic. It was also concluded that inorganic arsenic species are the most water soluble and that the remaining arsenic contained within the plant may be complexed with lipids or attached to cell walls. This conclusion was based on their extraction efficiency data which had a median value of only 49%, which meant that a majority of the arsenic contained within the various species of plants studied was not water soluble.

Arsenate prevails under aerobic conditions, is less toxic and less mobile than arsenite, which is found in anaerobic conditions, due to its stronger sorption to soil (Gonzaga et al., 2006). Arsenate interferes with oxidative phosphorylation and arsenite exerts inhibitory effects on enzyme activity by binding to thiol groups. The toxicity and bioavailability of arsenic is dependent upon its chemical form or species. Several studies have been conducted so far which have shown that the predominant arsenic species found in most plants is of the inorganic arsenite form (Koch et al., 2000; Ma et al., 2002; Tu et al., 2002; and Tu et al., 2003). Arsenicals are converted to arsenate in well drained soils. Gaseous arsines are toxic, while arsenocholine and arsenobetaine, found in marine systems, are nontoxic (Tu et al., 2003). Typically, inorganic arsenicals are more toxic than organic ones.

There are several current methods used for remediation of arsenic-contaminated soils. Current arsenic remediation techniques can cost up to $400,000 per hectacre (Raskin et al., 1997).   Excavation involves the physical removal and disposal of contaminated soil in a specially designed landfill (USEPA, 2002). This method produces immediate results, but is very expensive. Capping, which involves placing a hard cover over the contaminated site, guards against contaminant exposure; it does nothing to remove the contaminant from the soil (USEPA, 2002). Solidification and stabilization requires that the contaminate soil be mixed with stabilizing compounds which reduce the mobility of the arsenic in the soil. Once again, this method does nothing to actually remove the contaminants from the soil and is very expensive. Injecting glass matrix forming compounds, which chemically bond to the arsenic, into arsenic contaminated sites is termed vitrification. Washing arsenic contaminated soil with an acid aqueous solution allows for extraction of water-soluble arsenic. The aforementioned arsenic remediation strategies are very expensive, environmentally disruptive, and pose many dangers to the workers carrying out these methods. Phytoremediation, which uses plants to take up arsenic from the soil, is a method which is not only much more environmentally friendly, but is also relatively cheap in comparison to other arsenic remediation methods. No single soil remediation technique is suitable for all soils (Gonzaga et al., 2006).

While phytoremediation has several advantages, it also has its own set of disadvantages. One major drawback of phytoremediation is that the contaminant is simply transferred from the soil to the plant. The contaminant taken up by the plant is not always transformed into a less toxic species either. Essentially, the

burden of contamination is shifted from the soil to the plant. The storage and designation of a disposal site for biomass containing toxic contaminants needs to be well-though out and planned. According to Tu et al. (2003), as arsenic fronds age, the arsenite contained within them oxidizes back to arsenate. Leachate samples, taken from the fronds which were rinsed with acid and allowed to decompose, contained significant amounts of arsenate (Tu et al., 2003); it is therefore vitally important that the biomass from plants used to remediate contaminated sites be stored away from drinking water supplies in order to prevent secondary contamination. Plant biomass containing arsenic should not be burned because combustion of inorganic arsenic leads to release of toxic As_2O_3 (Franseconi et al., 2002).

Arsenite and arsenate are considered to be the most toxic forms of arsenic of naturally-occurring arsenic species found in the environment. Marine systems can biotransform and detoxify inorganic arsenic. Seawater contains 1 μg per L arsenic primarily as arsenate (Franseconi et al., 2002). Arsenite added to seawater is quickly converted to arsenate, the more thermodynamically stable form. The first stage of detoxification of is the formation of arsenosugars by algae, and the final arsenic metabolite appears to be arsenobetaine, a stable, non-toxic form of arsenic found in all marine animals. Some marine unicellular algae can carry out this biotransformation at arsenate concentrations 1000-times that found in ambient levels. With this in mind, it may be possible to dispose of ferns containing large amounts of arsenic in the sea. Once in the ocean the arsenic would be degraded and transformed into non-toxic forms by natural processes. There are drawbacks to this disposal method: species changes in algae populations and presence of other toxic substances in the fern (Francesconi et al., 2002).

Arsenic is taken up relatively readily and easily by several species of plants; this due to arsenic's chemical similarities with phosphorus, a macro-nutrient for plants (Brooks, 1998). Due to these growth characteristics, arsenic may be considered a great candidate for phytoremediation. One important item worth noting is that many plants may grow in soils containing arsenate, while arsenite is toxic to many plants and reacts with sulfydryl enzymes and is inhibitory towards cell function, which ultimately leads to death in most plants (Lombi et al., 2002). When taken up, arsenate replaces phosphate in the production of ATP (Luongo and Ma, 2005).

Phytoremediation. Phytoremediation is defined as the removal of a substance from the air, water, or soil, via either a microbial organism or plant. There are several subdivisions of phytoremediation: phytoextraction, phytovolatilization, phytostabilization, and rhizofiltration. Phytoextraction, the use of pollutant accumulating plants that are able to extract and translocate pollutants to regions that can be harvested and properly disposed of, will be the main focus of this paper. For phytoremediation to be effective, the plant used to remediate the contaminated media must be capable of producing a relatively large biomass which contains significant concentrations of the contaminant; it is also preferable to have a majority of the biomass to be located above the ground so that it may be harvested and the contaminant can be completely transferred away from the site. Phytovolatilization uses plants to volatilize pollutants and has been shown to be effective for mercury and selenium contaminated sites. Phytostabilization is the use of plants to physically stabilize polluted sites by preventing significant erosion, reducing the potential for air contamination from polluted soil, and controlling leaching of pollutants. Plants typically used for this form of phytoremediation have extensive root systems and allow for adequate soil cover. The use of aquatic plants to absorb pollutants from various bodies of water and streams is termed rhizofiltration or phytofiltration (Brooks, 1998).

There are several known plant species that have demonstrated arsenic uptake: water cress, several species of ferns, duckweed, and Indian mustard. The level of uptake by these plants varies. There are several, however, that exhibit hyperaccumulating characteristics. Current research has demonstrated that two species of ferns: *Pteris vittata* and *Pityrogamma calomelanos*, can be considered hyperaccumulators (Ma et al., 2001; Tu and Ma, 2002a; Luongo and Ma, 2005).

HYPERACCUMULATING PLANTS

The term hyperaccumulator is defined as any plant species that accumulates a particular heavy metal in excess of 100 times greater than the accumulation concentration of the highest value expected for a non-accumulating plant and also must have a relatively large ratio of biomass concentration of the contaminant to soil concentration (i.e. a plant that grows in soil with very high concentrations of a contaminant and the plant biomass therefore has a large concentration of this contaminant is not considered a hyperaccumulator based solely on this (Brooks, 1998). A hyperaccumulator can also simply be defined as plant which takes up greater than 1,000 µg per gram dry weight of a particular pollutant. To date, over 400 plant species are known as hyperaccumulators; most of these have been identified as nickel accumulators (Brooks, 1998). There have only been a handful of identified arsenic hyperaccumulators. Hyperaccumulators facilitate the disposal of contaminant rich biomass primarily because the biomass is so concentrated within a particular plant that less biomass needs to be produced to remediate a polluted site. Most plants will not accumulate arsenic in their plant tissue in any significant amount due to several possible reasons: their roots restrict uptake of arsenic, limited ability to translocate arsenic from root to frond, arsenic phytotoxicity at low concentrations in plant tissues, and low bioavailability of arsenic in the soil (Wang et al., 2002).

Pteris Vittata. *Pteris vittata* (Figure 1), a fern species that grows in the southeast and southern California and it is not native only to soil contaminated with arsenic, has been shown to hyperaccumulate arsenic in several studies (Ma et al., 2001; Tu et al., 2002a; Luongo and Ma, 2005). This fern species is a perennial that grows very rapidly and produces a significant amount of biomass. Compared to non-hyperaccumulating plants, *P. vittata* contains significantly higher amounts of phosphate in its roots and it is believed that this may play an important role in the plant's tolerance of arsenic (Luongo and Ma, 2005). Tu et al. (2002b) reported that the *P. vittata* biomass quadrupled every four weeks when grown in soil containing arsenic.   Zhang et al. (2002) reported that 95% of the arsenic taken up by *P. vittata* was located in its above ground biomass, which allowed for the biomass to be harvested and significant amounts of arsenic were transferred away from the contaminated site.   Not all species of *Pteris* fern hyperaccumulate arsenic. *Pityrogramma calomelanos* is the only known hyperaccumulator of arsenic that is not of the *Pteris* genus (Luongo and Ma, 2005).

Figure 1. Picture of *Pteris vittata (source: Forest and Kim Starr USGS)*

Ma et al. (2001) were the first to examine this species' ability to accumulate arsenic in unprecedented levels. Inorganic arsenic is the dominate species found in plants (Luongo and Ma, 2005). Arsenic uptake and accumulation is dependent upon several factors: plant species, soil arsenic concentration, soil pH, presence of other ions, exposure time, and the age of the plants. Under normal conditions, arsenic concentrations in most plants are typically less than 10 mg per kg dry weight (Tu et al., 2002b). Ma et al. (2001) found that *P. vittata* accumulated up to 93% of the arsenic it took up in its fronds and the remainder in its root system. *P. vittata* was clearly shown to be a hyperaccumulator by Ma et al. (2001) (see Table 1).

In this groundbreaking study, Ma et al. (2001) examined this species growing in a site contaminated with chromated copper arsentate. These results were later validated by Tu et al. (2003) when they reported that 94% of the arsenic taken up by *P. vittata* was contained in the fronds.

Table 1. Arsenic concentrations in P. vittata

Treatments	Soil Arsenic (ppm)	Plant Arsenic (ppm)	
		2 weeks	6 weeks
Control	6	755	438
As-contaminated soil*	400	3,525	6,805
Low As[1]	50	5,131	3,216
Medium As[1]	500	7,849	21,290
High As[1]	1,500	15,861	22,630

*Arsenic-contaminated soil was collected from the site where P. vittata was obtained
1 Artificially contaminated soil was spiked with three levels of water-soluble potassium arsenate
(Source: Ma et. al, 2001)

The plants studied by Ma et al. (2001) were evaluated and it was determined that As (III) was more predominant in the fronds than the root system. This led the researchers to conclude that As (V) had been converted to As (III) during translocation from the roots to the fronds; this result has been confirmed in several other studies (Tu et al., 2002b and 2003; Visoottiviseth et al., 2002; Zhang, 2002; and Luongo and Ma, 2005). This finding is very interesting because arsenite is more toxic than arsenate. Arsenate reduction is easier for *P. vittata* than phosphate reduction. It's believed that following this reduction arsenite is less toxic due to vacuolar storage of arsenic by formation of arsenite-thiol complexes with glutathione, or extrusion of arsenite from the cytoplasm, which is driven by the hydrolysis of ATP. The extruded arsenite may be accumulated near the cell wall possibly as inorganic salts or hydrolyzable organic compounds, which are less toxic to the plant (Tu et al., 2003). Not only was arsenate taken up in large amounts during the study conducted by Ma et al. (2001), it also was taken up very rapidly; when growing in soil contaminated with 1,500 ppm As (III), the ferns' As (III) concentrations increased from 29.4 ppm to over 15,000 ppm in just two weeks (Ma et al. 2001). For most species of non-accumulating plants, 5-20 mg per kg dry weight of arsenic is toxic, while hyperaccumulating plants have the ability to tolerate concentrations exceeding 10,000 mg per kg dry weight (Lombi et al., 2002).

In a study conducted by Lombi et al. (2002), it was shown that the concentration of arsenic taken up by the plant exponentially increased from the base of the plant to its extremities or pinnae. The arsenic concentration of the pinnae was reported to be between 6,000-9,000 mg per kg dry weight. Tu et al. (2002b) reported that the *P. vittata* fronds accumulated arsenic in concentrations of 7,200 mg per kg dry weight. Concentrations of arsenic in the roots were in the range of 200-300 mg per kg dry weight and remained essentially constant throughout the experiment. This indicated that almost all of the arsenic taken up by the plant was translocated to the fronds. Luongo and Ma (2005) reported that *P. vittata* was able to translocate arsenic from roots to fronds, reduce arsentate to arsenite, and maintain high concentrations of phosphate in the roots which all contributed to its arsenic tolerance and hyperaccumulation.

X-ray microanalysis, conducted by Lombi et al. (2002), displayed that arsenic was highly concentrated in the central intracellular portion of several samples of cut cells. The vacuole occupies a very large majority of the central cell and it was hypothesized by these researches that arsenic is sequestered in the vacuoles and the authors believe this may be a key to metal tolerance by this type of plant. This sequestration of arsenic in the vacuoles may serve as an efficient strategy to reduce the amount of Arsenic contained in the cytoplasm. A majority of the Arsenic found in the fronds is this study was present as As (III). The reduction of As (V) to As (III) has been reported in several other studies (Tu et al., 2002a, 2002b, and 2003; Luongo and Ma, 2005; Lombi et al., 2002; Franseconi et al., 2002; and Koch et al., 2000). This specific study was able to show that it would take four to five harvests (80-100 wk) to phytoremediate a soil which contains 100 mg Arsenic per kg. This time frame is much shorter than the typical 10-year period suggested for phytoremediation by Robinson et al. (1997), and shows that using *P. vittata* to remediate arsenic contaminated soil is a viable option.

According to Tu et al. (2002), plants have two strategies in dealing with arsenic: accumulation and exclusion. The exclusion strategy is used by plants such as carrots, grass, and tomatoes; these plants do not contain significant amounts of arsenic and the arsenic they do contain is located in their root system. The accumulation strategy involves "bioconcentration" of the arsenic is the plant's fronds. The older the *P. vittata* fronds, the higher the concentration of arsenic they contained. Aside from taking up large amounts of contaminants, effective phytoextraction plant species candidates should also translocate these contaminants to their shoots; this allows for the contaminated material to be completely removed from the soil. According to Lombi et al. (2002), arsenic uptake of 38 mg of arsenic per plant was observed during their study with 90% of the arsenic stored in the above ground biomass.

Luongo and Ma (2005) believe that ligand chelation is also a detoxification mechanism employed by plants to prevent phytoxicity from arsenic. Arsenic may exist in organic forms as organoarsenic compounds, such as arsenosugars or methylated species, or arsenic-biomolecule complexes, which may decompose into simple inorganic arsenic species during the course of extraction or separation; this makes detection of theses complexes very difficult. Arsenic methylation typically only occurs under phosphate or nitrogen deficient conditions. Arsenic species in water are stable for up to four days. This complexation may be necessary to enable the plant to accumulate extremely high concentrations of arsenic while at the same time avoiding high concentration of free arsenic in cytoplasm, which causes disruption of cell function and even cellular death (Luongo and Ma, 2005). Arsenite, MMA, DMA are oxidized and biochemically oxidized in the soil. In a study by Tu et al. (2003), these compounds were not found to exist in the soil that was spiked with them after 18 weeks. The fact that a majority of the studies conducted found no methylated arsenic species in the plants suggests that these compounds are either metabolized after uptake or are converted to inorganic compounds before plant uptake (Tu et al., 2003). Phytochelatins (PC), a family of peptides, have been reported to form upon exposure to arsenic by some plants (Zhang et al., 2002). Results of study a conducted by Zhang et al. (2002), suggest that the formation of stable arsenic-PC complexes does not occur in the *P. vittata* in any significant quantity. Research on heavy metal hyperaccumulating pants indicate that some organic and amino acids may also be involved in heavy metal detoxification.

Tu and Ma (2002b) reported that at low-water soluble arsenic levels in soils, arsenic concentrations increased from old to young ferns. At moderate to high water-soluble arsenic levels in soils, arsenic concentrations increased from young to old fronds. At low levels, arsenic is taken up as a nutrient and was thus more prevalent in the young ferns. At high levels, arsenic uptake in older fronds was predominant because it was translocated to all the ferns without regard to age and thus older fronds contained more arsenic because they have been exposed to the contaminant for a longer period of time.

Zhang et al. (2002) also reported that older fronds contained more arsenic than younger fronds, and had subsequently taken up more arsenic in the fronds than their roots. It is believed that transport of arsenic from the roots to the fronds is via the xylem sap in these plants. The increased amount of arsenic located in older ferns is most likely due to a greater cumulative amount of transpiration stream that has passed through the older plant. Dead leaves contain significantly less Arsenic than living ones (84 and 428 µg/g for young and old fronds vs. living with 4893 µg/g and 7575 µg/g) (Zhang et al., 2002).

The bioconcentration factor (BF) or ratio of arsenic in the plant tissue to that contained in the soil can be used to evaluate the effectiveness of the plant in taking up arsenic from the soil and transferring it to its biomass. For ferric and aluminum arsenic complexes, the BF factor is substantially lower than those composed of elements such as calcium, sodium, and potassium (Tu and Ma, 2002b). Zhang et al. (2002) reported that the BF for *P. vittata* was 193, which indicates very efficient uptake of arsenic from the soil by the plant.

The translocation factor (TF) or ratio of arsenic concentrations in the fronds to that of the roots is used to evaluate translocation effectiveness. According to Tu and Ma 2002b, the TF factor for *P. vittata* is 4 to 25 times that of most other plants because most other plants do not translocate arsenic to their fronds. Luongo and Ma (2005) also reported finding a high TF for *P. vittata*. Tu and Ma (2002b) reported that the TF did decrease after the soil concentration of arsenic was increased beyond 50 mg per kg. The TF also increased with increasing water-solublility of the compounds. Soil contaminated with 100 mg arsenic per kg resulted in the greatest arsenic accumulation into the aboveground biomass with 13 mg per plant, which accounted

for 10% of the initial concentration (Tu and Ma, 2002b). At soil arsenic concentrations greater than 100 mg per kg, the *P. vittata* begins to show signs of arsenic poisoning (Tu et al., 2002a). The effects of the different forms of arsenic on phytoextraction by the *P. vittata* are a function of their water solubility and not of their molecular form. In order for a plant to be considered a hyperaccumulator of arsenic the BF > 1 and the TF > 1 (Gonzaga et al., 2006).

Luongo and Ma (2005) concluded that available phosphate is critical to the growth of *P. vittata* at toxic concentrations of arsenic (400 mg arsenic per kg). A minimum ratio of phosphorus to arsenic of 1.2 in the fronds is required for normal growth of P. vittata. Upon exposure to 10 mg arsenic per L, phosphate concentrations in the *P. vittata* increased by 91.8% in the fronds and 45% in the roots in comparison to the control plants. The TF for phosphate was much lower upon exposure to high concentrations of arsenic than the other twelve ferns studied. This showed that under high arsenic concentrations *P. vittata* was able to keep much more of the phosphate in its roots than the others. Arsenic translocation occurs in the roots in order to minimize the damage done to the roots (Luongo and Ma, 2005).

In a study conducted by Fayiga and Ma (2005), the effect of phosphate addition to a soil spiked with arsenic and several other metals resulted in an increase in the arsenic uptake from 608 to 1,046 mg per kg of *P. vittata* fronds on a dry weight basis. From the results of their study, Fayiga and Ma (2005) were able to conclude that the presence of additional phosphate enhances arsenic uptake by *P. vittata*. The enhancement of arsenic uptake via the presence of available phosphate could be due to several factors: the phosphate increases the bioavailability of arsenic by desorbing arsenate from the soil and increasing its water solubility; and the phosphate is able to reduce the toxic effects of arsenate in the plant tissues. Similar findings have been found in several other studies. For example, Biosson et al. (1999) reported an increase of arsenic uptake after applying hydroxyapaptite, a substance derived from bone, to a soil contaminated with arsenic and other metals.

Elless et al. (2005) conducted a study using *P. vittata* to remediate arsenic contaminated drinking water in New Mexico. The plants were grown hydroponically and the experiment was conducted to investigate phytofiltration of arsenic contaminated waters. The ferns used during the study initially contained less than 20 mg/kg dry weight. At the end of the study, the ferns contained anywhere from 66 to 407 mg arsenic per kg dry weight depending on their placement from the inlet stream. The system was able to treat 1900 L/day and produced water which contained less than 2 ppb of arsenic. The inflow concentration of Arsenic was varied over the course of the study from 6.6 and 14 ppb arsenic. It was determined that storage and uptake of arsenic by these plants are independent of one another and therefore capacity of arsenic does not become an issue in the roots.

Srivastava et al. (2006) studied several other *Pteris* species. They reported that *P. biaurita*, *P. quadriaurita*, and *P. ryukyuensis* accumulated arsenic in sufficient amounts given the experimental conditions that they could be classified as hyperaccumulators; they accumulated over 1000 mg As per kg dry weight and their BF and TF were found to be greater than one. These plants were also shown to translocate arsenic contained in their roots to their shoots and transform arsenate taken up in the roots to arsenite in their aboveground plant tissue.

Pityrogamma calomelanos. *P. calomelanos* grows in Thailand around the tin mining belt. This plant is known as the silver fern, and prefers tropical and sub-tropical climates with lots of rainfall. Most of the 32 species of plants collected from this particular area in the study contained 4-79 µg arsenic per gram on a dry weight basis which is in agreement with previous studies for plants collected from other arsenic contaminated areas (Ma et. al, 2001 and Francesconi et al., 2002). The soil contained 510 µg arsenic per gram and the concentration of arsenic was as follows for the *P. calomelanos*: (2760-8350 µg per gram) for the fronds, (150-380 µg per gram) for the stalk, and (88-310 µg per gram) for the rhizoids (root). The senescent leaves contained 10-fold lower concentrations of arsenic than those of the younger leaves. The data suggests that *P. calomelanos* is actively retaining arsenic by translocation from the senescent leaves to the younger parts of the plant. Again, arsenate was predominant in the roots and arsenite was predominant in the fronds. *P. calomelanos* are food items in Thailand and may be fried and used in salads. Fronds from a *P. calomelanos*, which was purchased from a market in Bangkok, were determined to contain 60 µg Arsenic per gram DW, a value well in excess of many countries standards for arsenic in food items

(Franseconi et al., 2002). This example readily shows the importance of identifying arsenic accumulators and ensuring that these species are not consumed and are instead disposed of properly.

In a study conducted by Visoottiviseth et al. (2002), thirty-six different plant species were collected from two different sites that had been contaminated by arsenic which had leached from mine tailings. It was determined that *Pityrogamma calomelanos* was a hyperaccumulator because leaf samples were determined to contain concentrations of arsenic in excess of 8,000 mg per kg dry weight of the plant. This concentration was significantly greater than that found in the soil from which these plants were collected from. It was also concluded from this study that there was a substantial difference in how the arsenic was distributed within the *P. calomelanos*. Once again, the fronds contained significantly higher concentrations of arsenic than the roots (see Figure 2). The senescent fronds contained less arsenic than the young and mature fronds. It was also reported that arsenate had been reduced to arsenite within the plant tissue.
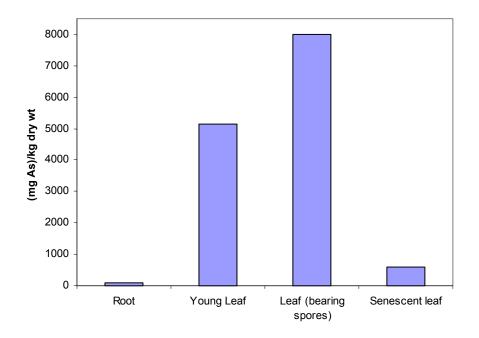

Figure 2. Concentration of arsenic per kg dry weight of *P. calomelanos* for different regions of the plant (Visoottiviseth et al., 2002).

It can be concluded that the actual mechanism of arsenic uptake and tolerance is not yet fully known. According to Agely et al. 2005, it's reasonable to expect that mycorrhizal symbiosis may be involved in arsenic uptake by ferns. This is due to arbuscular mycorrhizal (AM) fungi's well documented role in phosphate (P) uptake and other poorly mobile nutrients. Both arsenic and phosphorus belong to the same chemical group and thus have similar geochemical behavior. AM plants are better at adsorbing minerals in the soil than are nonmycorrhizal plants, because they have a greater total absorptive surface area and different uptake kinetics. Ferns are well colonized by AM fungi. Colonization of *P. vittata* by AM fungi significantly increased the biomass of the Chinese brake fern at high arsenic levels. This suggests this colonization may be a viable technology to increased arsenic uptake efficiency. Plants growing in highly arsenic contaminated soils are typically mycorrhizal. The primary function of mycorrhizal fungi is to obtain phosphorus for their host; however, this may become a problem in soil that has a high concentration of arsenic. Addition of arsenic affected phosphorus plant concentration and this supports prior evidence that arsenic uptake is via phosphate transport systems (Agely et al. 2005).
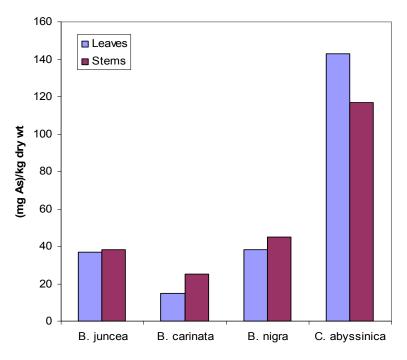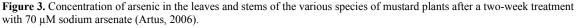
NON-HYPERACCUMULATING PLANTS

Lupinus Albus. *Lupinus Albus*, commonly known as White Lupin, can typically be found in the Mediterranean area. It is an annual legume that is considered an acidophilus species that has the ability to

grow in areas with very poor soil conditions. This plant species is able to grow in these areas because of its nitrogen fixation ability, strong root system, excretion of citrate through proteoid roots, and high resistance to soils containing cadmium and arsenic (Huyghe, 1997).

Due to the characteristics of White Lupin, Vazquez et al. (2006) chose to study this plant's potential as a phytoremediator of arsenic contaminated soil. When grown in soil spiked with arsenic, this plant species was shown to increase the soil pH, decrease the solubility of arsenic, and accumulated most of the arsenic in its root system. It was therefore concluded that White Lupin should be used as a phytostabilizer, and not as a phyoextractor due to very low concentrations of arsenic found in its aboveground biomass. The plant's ability to accumulate arsenic is in its root nodules and strongly bound the arsenic to its cell walls, extensive root system, and acidic soil pH enhancement ability make White Lupin a great candidate as a phytostabilizer of arsenic polluted soils. By secreting citrate from its roots, White Lupin can serve as a buffer to soil acidification. Citrate has a pKa of 6.3 and therefore is considered a weak acid. Due to its high pKa value, citrate can react with any free hydrogen protons in the soil at low pH and thereby raise the pH of the soil as was seen in this study (Vazquez et al., 2006).

Mustard Plants. A study was conducted by Artus (2006), which investigated the potential use of mustard plants as phytoextractors of arsenic contaminated sites. This study compared the effects of sodium arsenate at 0, 70, and 140 µM on several mustard plant species: *Brassica juncea*, *Brassica carinata*, *Brassica nigra*, and *Crambe abyssinica*. Figure 3 displays the results after the plants were treated with 70 µM sodium arsenate. From the figure it's clear that the *Crambe abyssinica* accumulated more arsenic in its leaves and stems than the other three species (Artus, 2006). This finding is noteworthy in light of a study conducted by Pickering et al., 2000, which concluded that *Brassica juncea* accumulated substantially greater amounts of arsenic in its roots than its stems and leaves. Using a plant that only significantly accumulates a particular pollutant in its root system makes it difficult to use as a phytoextractor, but may it be used as a phytostabilizer. It is apparent that the mechanism of arsenic uptake is therefore different for *Crambe abyssnica* than that of *Brassica juncea*.

Figure 3. Concentration of arsenic in the leaves and stems of the various species of mustard plants after a two-week treatment with 70 µM sodium arsenate (Artus, 2006).

Root growth was found to be inhibited for all four species at sodium arsenate concentrations of 140 µM. Shoot growth was found to be inhibited for all four species at sodium arsenate concentrations of 140 µM.

Although *Crambe abyssinica* produced the greatest amount of biomass for all four species studied, it did show the greatest amount of shoot growth inhibition when grown under the condition of 70 µM Sodium Arsenate. Much of the current phytoremediation research using mustard plants has centered on *Brassica juncea* because of its previously demonstrated ability to take up heavy metals cadmium and lead; it can be easily genetically engineered; and its high amount of biomass (Zhu et al., 1999). *Crambe abyssinica* accumulated more arsenic in its plant tissues and produced more biomass than the other three mustard plant species. The results of this study indicate that, while although *Crambe abyssinica* is not a hyperaccumulator, it may be better suited than *Brassica juncea* for further research into its arsenic phytoremediation capabilities.

In the study conducted by Pickering et al. (2000), arsenate was reduced to arsenite within the plant tissue of *Brassica juncea* which also occurs for the *Pteris* ferns and *Pityrogamma calomelanos*. One approach which may increase the amount of the arsenic transport to the aboveground biomass of *Brassica juncea* is the use of chelation agents. The use of which has been shown to increase the plant accumulation of lead, gold, and uranium. The results from a study conducted by Pickering et al. 2000 using Brassica juncea showed that the addition of chelating agents greatly increased the amount of arsenic accumulation in the stems and leaves of the plant.

***Lemna gibba*.** In a study by (Mkandawire and Dudel, 2005) *Lemna gibba* (duckweed), which is an aquatic plant species that grows very rapidly, was found to be growing in wetland tailing waters in Germany. It was shown that these waters contained significant amounts of arsenic. The predominant arsenic species contained within these waters that were sampled were arsenate and arsenite. The results from Mkandawire and Dudel (2005) conclusively show that *Lemna gibba* accumulates arsenic in direct proportion to the amount present in the water and inversely proportionately to the concentration of phosphate with which it is subjected to. The only drawback to using *Lemna gibba* as a phytoextractor of arsenic is the plant's floating capability and the fact that it may be readily transferred to uncontaminated areas by flowing water. *Lemna gibba* may therefore better serve as an indicator for arsenic as opposed to a phytoextracter (Mkandawire and Dudel, 2005).

***Lepidium sativum*.** *Lepidium sativum*, or watercress, is consumed as a vegetable in many parts of New Zealand. Robinson et al. (2003) conducted a study using this species and used water from the Waikato River which contained significant amounts of arsenic (0.041 mg/L), which is four times greater than the World Health Organization's limit of 10 ppb. The source of the arsenic contained within this river is due to geothermal activity. During this study, it was shown that watercress was, on average, able to decrease the arsenic concentration of the water by 7.3%. When grown in water containing arsenic concentrations of 0.4 mg/L and greater, the plant's arsenic concentration exceeded 2 mg/kg, which is the WHO limit for food items. It was concluded from this study that watercress should not be taken from any area which may contain arsenic due to the plant's ability to significantly accumulate this pollutant.

CONCLUSIONS

Throughout this paper the potential for using various plant species in the remediation of arsenic-contaminated soils has been examined. A majority of these plants are able to reduce arsenate taken up by their roots systems to arsenite, which is rather interesting due to its greater toxicity. The exact mechanisms regarding this phenomenon are still being investigated. Most of the plant species discussed in this paper have the ability to translocate a significant majority of the arsenic taken up by their root system to their leaves and stems. It's clear that several species of *Pteris* ferns and *P. calomelanos* show a large amount of potential for extracting large amounts of arsenic from regions that are highly contaminated with arsenic. The rest of the plants presented here are capable of tolerating arsenic contaminated soil, but do not accumulate arsenic in large enough concentrations to be considered viable for phytoremediation purposes. The primary difficulty with using *Pteris* ferns or *P. calomelanos* for phytoremediation of arsenic is their climate limitations. While many of the plants studied to this point have not been shown to transform the arsenic taken up by their root systems to less toxic forms of arsenic, they still provide an economical and environmentally-friendly option to remediate contaminated areas. Further research regarding phytoremediation of arsenic will produce a more complete answer to the following question: Why do

plants transform arsenate into the more toxic arsenite? This is a mysterious finding that's needs to be answered in order to truly understand the mechanism of arsenic uptake by many plants.

Finally, it's anticipated that further research into this issue will uncover several other plant species that are capable of large-scale phytoextraction of arsenic and are capable of growing over a wide range of climates and soil conditions.

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