A FEASIBILITY ASSESSMENT OF NATIVE FERNS FOR PHYTOREMEDATION OF ARSENIC

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Sciences in Biology.

By

Jessica Lauren Jaynes

Director: Dr. Beverly Collins
Associate Professor of Biology
Biology Department

Committee Members: Dr. Sabine Rundle, Biology
Dr. Arthur Salido, Chemistry

March 2012

ACKNOWLEDGEMENTS

This has been a very long journey and there are so many people who have helped me to achieve this goal. In particular I would like to thank:

- My advisor Dr. Beverly Collins for sticking with me through thick and thin.
- My committee members: Dr. Sabine Rundle, Dr. Arthur Salido, and Dr. Tom Martin (reader) for all of their help and understanding along the way.
- Duke Rankin, United States Forest Service, for his willingness to help and interest in my project.
- The Biology Dept. faculty for all of their support and willingness to help.
- Dr. Jerry Miller who first introduced me to the idea of phytoremediation and helped me to get started on this project.
- The graduate students of the Biology Dept. for offering their friendship and support, I couldn't ask to be surrounded by nicer people.

Lastly, I would like to thank my family and friends who have always been there and who will continue to be there. Thank all of you so much for everything you have done for me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A FEASIBILITY ASSESSMENT OF NATIVE FERNS FOR PHYTOREMEDIATION OF ARSENIC

Jessica Lauren Jaynes, M.S.

Western Carolina University (March 2012)

Director: Dr. Beverly Collins

Arsenic contamination is a world-wide concern. In the past, soil contaminated with arsenic was removed using heavy equipment resulting in the destruction of the environment. However, in recent years a new method, phytoremediation, removes arsenic and maintains the integrity of the environment. Phytoremediation is the use of plants to sequester and remove contaminants. In 2008, water samples from Poplar Cove Creek and Cloer Branch in Macon County, NC (located in the Nantahala National Forest) had levels of arsenic ranging from 13.8 to 20.6 ppb. These results are unusually high for Western North Carolina and are higher than the EPA's drinking water standard of 10 ppb. This study sought to determine if two native fern species (*Polystichum acrostichoides* (Christmas Fern) and *Thelypteris noveboracensis* (New York Fern)) accumulate arsenic and to determine if these ferns might be suitable for phytoremediation of arsenic. In the greenhouse experiment, ferns were planted in soil spiked with arsenic ranging from 0 (control) to 50 ppm. Initial and final samples were taken of fronds, roots/rhizomes, and soil to determine arsenic concentration levels. Results showed no accumulation of arsenic in the fronds of either fern species; however, arsenic accumulated in the roots of

both fern species. *T. noveboracensis* showed a stronger relationship with arsenic in the soil and ability to take up arsenic than did *P. acrostichoides*. However, despite these positive results, the amount of arsenic taken up by these native ferns was too little to make their use feasible for phytoremediation of arsenic.

INTRODUCTION

Phytoremediation

The concept of using living plants to clean up a contaminated site is known as phytoremediation (EPA, 1999). There are several advantages to this type of cleanup process. Phytoremediation is cheaper, creates less disturbance to the environment, and is aesthetically pleasing compared to the alternative cleanup method of excavation/landfill. However, phytoremediation is not a remedy for all types of contaminants. Disadvantages of phytoremediation include the time needed for the plant to grow and sequester the contaminant; the depth of the contaminant, which must be within the root zone of the plant; and the concentration of the contaminant, which cannot be so high that it kills the plants (Glass, 2000; Rock & Sayre, 2000).

The type of contamination dictates the specific phytoremediation technique. Common techniques include phytoextraction, rhizofiltration, and phytostabilization (Miller & Miller, 2007). Phytoextraction is the "use of metal-accumulating plants that can transport and concentrate metals from the soil to the roots and aboveground shoots (Ensley, 2000)." Rhizofiltration is the "use of plant roots to absorb, concentrate, and precipitate heavy metals from water (Ensley, 2000)." Phytostabilization is a "process that retards the mobility of the contaminants in the sediment and soil (Miller & Miller, 2007)." The technique of choice in this study is phytoextraction (Figure 1).

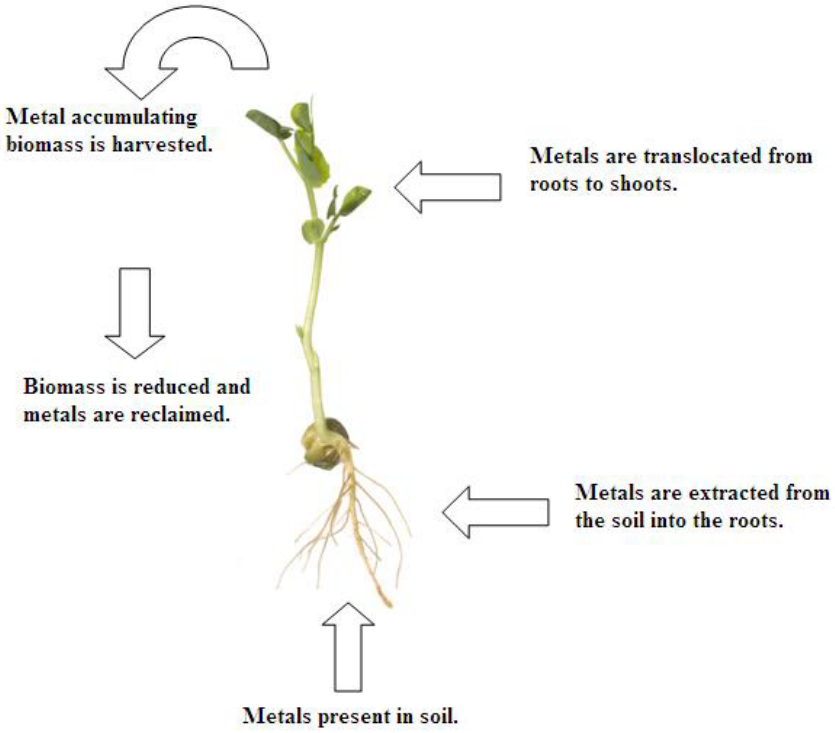


Figure 1. Schematic of phytoextraction.

Phytoextraction

In general, phytoextraction works by placing metal-accumulating plants in the contaminated site and allowing the plants to grow. After a given amount of time based upon the growth habit of the plant and the concentration of the contaminant, the plants are removed. In the best of situations the plant will translocate the contaminant from the roots to the shoots, which allows for quicker removal and less disturbance to the site. If the plant is not able to move significant amounts of contaminants to the shoots or if the overall metal concentration in the plant is high the entire plant is removed. The plant material is then reduced and the metals taken up in the plant can be reclaimed (Blaylock & Huang, 2000). The procedure of phytoextraction significantly reduces the amounts of hazardous waste created when treating contaminated sites.

Certain plant characteristics have been found desirable for phytoextraction. Plants that grow rapidly, have large biomass, can grow easily, and have the ability to take up the desired contaminant are most likely to be used in phytoextraction (Ensley, 2000). Researchers have also discovered that many plants are hyperaccumulators; in other words, they are plants with the ability to "accumulate at least 100 mg g^{-1} (0.01% dry wt.), Cd, As and some other trace metals, 1000 mg g^{-1} (0.1 dry wt.) Co, Cu, Cr, Ni and Pb and 10,000 mg g^{-1} (1 % dry wt.) Mn and Ni" (Prasad & Freitas, 2003). These desired characteristics and the nature of the contaminant can efficiently narrow down the choices of plants to be used for phytoextraction. My research reported here was based around the technique of phytoextraction and given the nature of the contaminant; I chose only one type of plant with the desired characteristics: ferns.

Ferns

Ferns can be found in almost every environment; there are around 12,000 different species of ferns present today (AFS, 2001). In 2001, at a site in Central Florida contaminated with chromated copper arsenate, researchers discovered a fern, *Pteris vittata* L. (ladder brake), that hyperaccumulates arsenic. *Pteris vittata* L. was the first plant and fern found to hyperaccumulate arsenic (Ma et al., 2001). Since that discovery, interest in ferns and other Pteridophytes (fern & fern-allies) for phytoremediation has increased tremendously. Currently, the only ferns known to hyperaccumulate arsenic are in the order Pteridales, specifically many *Pteris* L. species and *Pityrogramma calomelanos* (L.) Link (Meharg, 2003). Many of these ferns can be found in the Southeastern part of the United States and in California; however, many others are exotics to the United States (USDA & NRCS, 2012). Placing exotic plants into the environment introduces an entirely new ecological problem. Research into native ferns for phytoremediation purposes can prevent compounding ecological problems and provide valuable information to property owners and government officials who are responsible for cleaning up arsenic-contaminated sites.

Arsenic

Arsenic is a metalloid found both naturally and anthropogenically in the environment. Natural arsenic occurs in soil and minerals and is leached out into the environment through weathering. Anthropogenic arsenic occurs in the environment due to industrial usage (mining, smelting, and wood preservation) and agricultural usage (pesticides) (ATSDR, 2007). In either of these cases, arsenic that was once contained has now been released into the environment, contaminating the soil, air, groundwater, and

streams. There are several types of arsenic compounds found in the environment; however, two types pose the greatest health risk: arsenite (As^{III}) and arsenate (As^{V}) (Figure 2). These two species are toxic, abundant, and readily available in the environment (Akter & Naidu, 2006; Xie & Naidu, 2006). Arsenite reacts with dithiol groups in proteins inhibiting enzymes; this can lead to membrane degradation and cell death (Jiang & Singh, 1994; Scott-Fordsmand & Pederson, 1995). Arsenate is similar to phosphate in chemical composition and can compete with and replace phosphate (Figure 2). Arsenate prevents the formation of ATP during glycolysis (Hughes, 2002), resulting in an inadequate supply of energy at the cellular level (Scott-Fordsmand & Pederson, 1995).

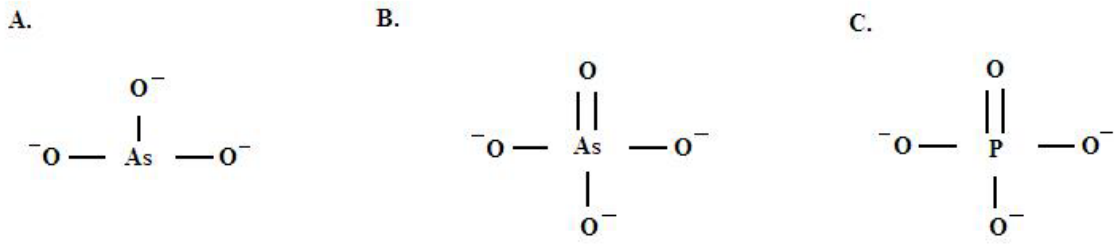


Figure 2. (A) Chemical structure of arsenite (As^{III}). (B) Chemical structure of arsenate (As^{V}). (C) Chemical structure of phosphate.

Given arsenic's toxic effects, it has become an element of concern to human health worldwide. It is a known carcinogen; long-term exposure can cause bladder, lung, and kidney cancer (EPA, 2006). These concerns have led to many studies on daily human contact with arsenic and resulted in a push for standards for allowable levels of arsenic. However, it has been found that variables such as location, occupation, diet, gender, body type, and age can affect the amount of arsenic an individual contacts and/or accumulates. Taking into consideration all of these variables, the FDA does not recommend a daily dietary intake of arsenic that is more than 2.1 ug/kg body weight for adults (ATSDR, 2007). In 2006, the Environmental Protection Agency and the World Health Organization set new standards for arsenic concentrations in drinking water to 10 ppb, before this change the standard was 50 ppb (EPA, 2006).

Uptake Of Arsenic By Ferns

Plants that uptake arsenic can be split into three strategies based on the characteristics of metal uptake: excluders, indicators, and accumulators (Baker, 1981). Excluders contain a small concentration of the metal, but do not take up the metal further, even when concentrations vary in the environment. Indicators have a linear relationship with the metal concentrations found in the surrounding environment. Accumulators and hyperaccumulators accumulate metals in greater concentrations than found in the surrounding environment (Baker, 1981). Currently, the only known arsenic hyperaccumulators are ferns, but the abilities of the ferns to hyperaccumulate varies greatly among species (Fitz & Wenzel, 2006; Meharg, 2003). The mechanism by which these plants are able to take up arsenic depends on the species of arsenic. The mechanism of arsenate (As^{V}) uptake is phosphate transporters (Figure 3 & 4). Arsenate

and phosphate are in the same chemical group and arsenate is able to mimic or act as a chemical analog of phosphate (Figure 2) (Adriano, 2001; Smith et al, 1998). When arsenate (As^{V}) enters the plant it is reduced enzymatically to arsenite (As^{III}) via arsenate reductase (Figure 4) (Briat, 2010). The enzyme, arsenate reducatase, evolved in both prokaryotes and eukaryotes to detoxify the ubiquitous amounts of arsenic found in the environment (Mukhopadhyay & Rosen, 2002). The mechanism of arsenite (As^{III}) transport is through aquaporins (Figure 3) which are intergral membrane proteins that act as primary water pores (Agre et al., 1993). In plants, aquaporins can be broken down into four subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic membrane proteins (NIPs), and small and basic intrinsic proteins (SIPs) (Chaumont et al., 2005). It is thought that NIPs, specifically, a NIP for silicic acid, are involved in arsenite transport into plants. Arsenite and silicic acid have similar molecular sizes, allowing arsenite to use this transport system and enter the plant (Figure 4) (Ma et al., 2006; Ma et al., 2008).

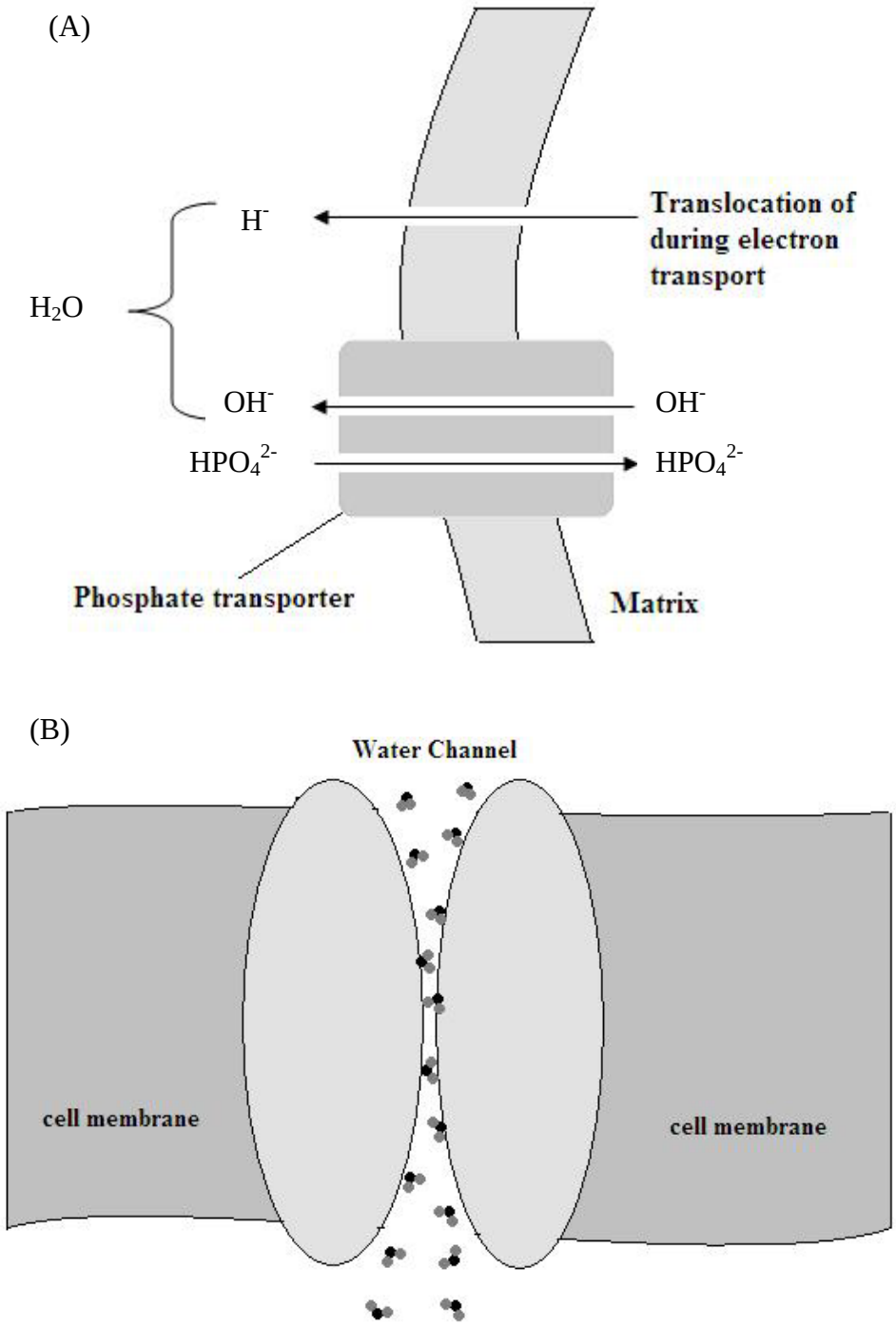


Figure 3. (A) Phosphate transporter schematic (B) Aquaporin schematic

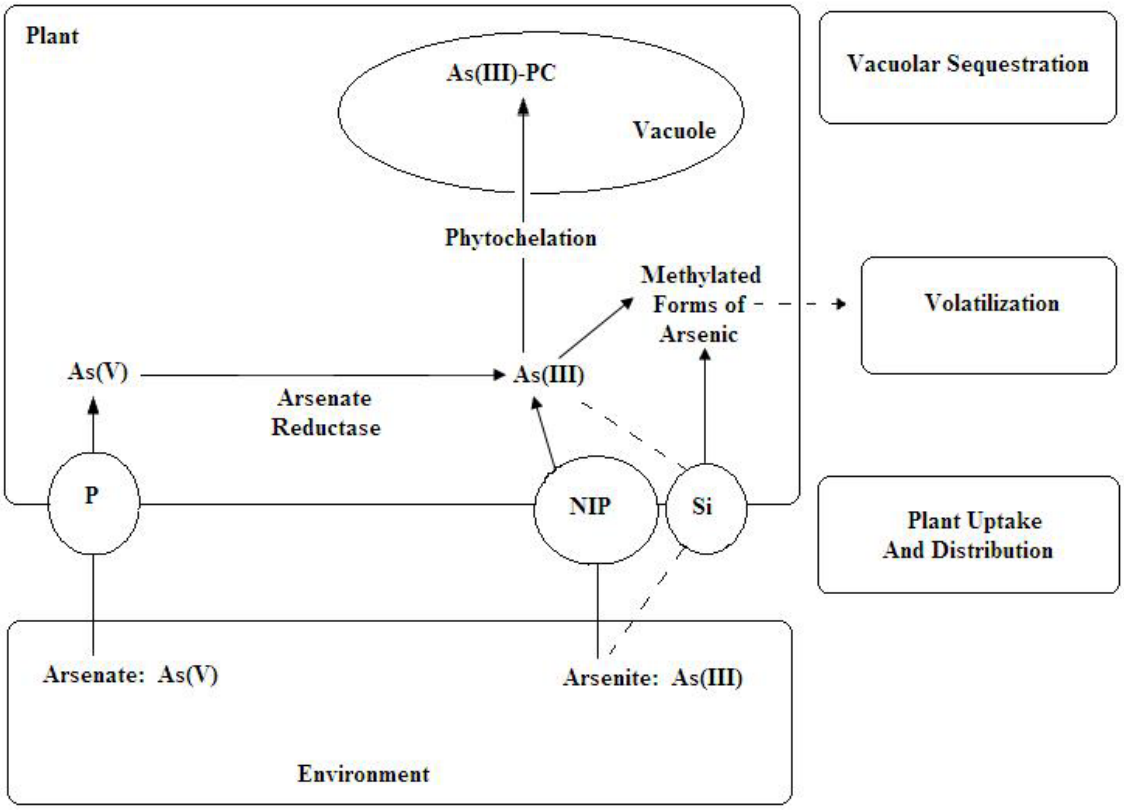


Figure 4. Schematic of mechanisms of transport from the environment to a plant for both arsenate (As^{V}) and arsenite (As^{III}). Arsenate (As^{V}) enters through phosphate transporters and is then reduced enzymatically to arsenite by arsenate reductase. Arsenite (As^{III}) enters through aquaporins known as NIPs and has been linked to NIPs that transport silicon (Si). Arsenite (As^{III}) can then be detoxified by methylating the arsenic and volatilizing it out of the plant or by using phytochelation and sequestering the arsenic in a vacuole (Briat, 2010).

Phytoremediation In Action

Since its utility for phytoremediation was discovered in 2001, the fern *P. vittata* L. (ladderbrake) has been used in multiple phytoremediation projects with success. In 2004, *P. vittata* L. was planted in Washington D.C. at Spring Valley, an area once used for chemical testing in World War I, to help clean up approximately 600 acres of contaminated land (Ruder, 2004). Also, in 2004 the fern was tested in Albuquerque, New Mexico for its abilities to clean drinking water through hydroponic systems. The ferns significantly reduced the amount of arsenic present in the drinking water; however, the system would have to be used for small scale water sources (Ruder, 2004). In 2009, the EPA implemented the use of *P. vittata* at Ryeland Road in Heidelburg Township, Pennsylvania to clean up a wetland that was contaminated with arsenic from a pesticide manufacturing company. The EPA reports that the site is showing significant decreases in arsenic as each growing season passes (EPA, 2011). A current project using *P. vittata* for phytoremediation is the Crozet site in Crozet, Virginia. This site was contaminated with arsenic through the use of pesticides and was deemed an EPA Superfund site. *Pteris vittata* was planted in 30 by 30 foot plots where levels of arsenic were highest. The project has gone through at least one growing season, but will require multiple growing seasons to reduce the arsenic to safe levels (EPA, 2011). The EPA currently has 867 sites in their database that are contaminated with arsenic (EPA, 2012).

Arsenic In The Local Environment

My research was inspired by a local stream: Poplar Cove Creek; located in Macon County, North Carolina in the Nantahala National Forest (Figure 5). The site is on U.S. Forest Service land and has been used for timber production in the past. Poplar Cove

Creek was showing signs of distress and contamination (i.e. no plant or animal life present) creating cause for concern. Water samples from Poplar Cove Creek and Cloer Branch, a nearby stream that showed no distress, were taken to determine if any contaminants were present. These samples were sent to the University of Georgia Lab for Environmental Analysis and were tested for heavy and trace metals using an ICP-MS (inductively coupled plasma – mass spectrometry). The analyses revealed elevated levels of arsenic in not only Poplar Cove Creek, but also in Cloer Branch which was meant to be the control (Table 1). The levels ranged from 13.8 to 20.6 ppb arsenic, these levels are not extreme, but are higher than the 10 ppb EPA standard for drinking water (EPA, 2006). The source of the arsenic contamination is unknown; however, several non-point sources such as roads (i.e. runoff from vehicles and erosion), past land usage (i.e. pesticides used when it was timber land), and bedrock could contribute arsenic to the stream and surrounding ecosystem. Interest in the potential for ferns, which are part of the local flora, to remediate arsenic in contaminated ecosystems such as Poplar Cove Creek spurred my research.

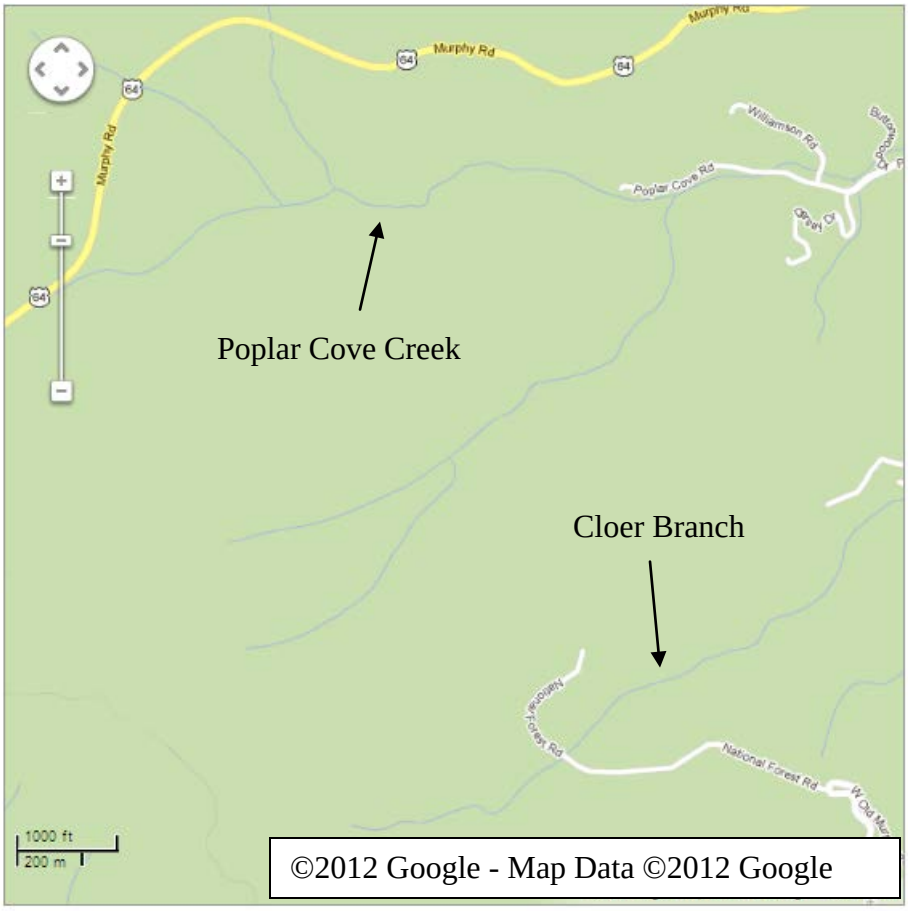


Figure 5. Map of Poplar Cove Creek and Cloer Branch (Google Maps, 2012).

Table 1. Summary of arsenic concentration for Poplar Cove (downstream and upstream of the Forest Service road crossing), Cloer Creek, and the Safe Drinking Water Act Maximum Contaminant Level.

	As (ppb)
Cloer Branch	18.2
Poplar Cove (DS)	20.6
Poplar Cove (US)	13.8
SDWA MCL	10

Objectives And Hypotheses

The objective of my research was to determine if native ferns could extract arsenic from the soil and thus could be used to remediate contaminated ecosystems such as the Poplar Cove Creek site. Two fern species that are native to Western North Carolina occur in the local flora around Poplar Cove Creek. These ferns have life-history characteristics suited to phytoremediation. The ferns chosen were: *Thelypteris noveboracensis* (L.) Nieuwl (New York fern), which can spread quickly via rhizomes, and *Polystichum acrostichoides* (Michx.) Schott (Christmas fern), which is evergreen. I asked the following specific questions:

1. Will these ferns extract arsenic?

2. Where does the arsenic accumulate (roots, shoots, throughout the plant)?

3. Are these ferns feasible for phytoremediation purposes?

To address these questions a greenhouse experiment was conducted in which the two fern species were planted in soil that was watered initially with an arsenic/water solution at 0 (Control), 5, 10, or 50 ppm. These arsenic concentrations were chosen to display ranges that would test the ferns for high and low concentrations and to exceed the detection limits of the ICP-OES (0.1 ppm). I hypothesized the native ferns would sequester arsenic in both roots and shoots acting as indicators, linear relationship, to the arsenic concentrations in the soil.

MATERIALS AND METHODS

Greenhouse Experiment

Bare root plants of *T .noveboracensis* and *P. acrostichoides* were purchased from TN Nursery Wholesale Nursery Company in March, 2010. Plants were placed in water and allowed to grow outdoors. In June, 2010, 40 bare-root plants of each species were planted in 6-8 inch diameter pots with Garden Magic Topsoil (Michigan Peat Company), which was screened for a uniform consistency. Ferns were grown for approximately one month in an outdoor environment that mimicked their natural habitat.

In July, 2010, 1.42 liters of Garden Magic Topsoil were placed in 8-in pots and watered to saturation with deionized water spiked with 0 (Control), 5, 10, or 50ppm disodium arsenate (Na_2HAsO_4 x $7H_2O$) . Soil samples were taken to determine initial arsenic values; these samples were placed in the greenhouse to air-dry pending chemical analyses. Also, a 100ml sample of each concentration of the arsenic/water solutions was taken and filtered through Whatman 40 filter paper to determine the accuracy of the arsenic concentrations

Based on growth of mature fronds and development of fiddleheads, 40 *T. noveboracensis* and 28 *P. acrostichoides* ferns were chosen from the stock of 40 plants. Initial frond and root samples were taken to identify a baseline for each plant before the arsenic treatments. These samples were air-dried in the greenhouse prior to chemical analyses. The ferns were then planted in the pots treated with arsenic. *Thelypteris noveboracensis* had ten replicates for each arsenic level treatment and *P. acrostichoides* had seven replicates for each treatment. The ferns were allowed to grow in the treated

soil in the Western Carolina University greenhouse for three weeks. They were checked daily and watered as needed. After the three week period, ferns were removed from the pots and washed with deionized water to remove soil. Final samples were divided into three parts: frond (above-ground), roots and rhizome (below-ground), and soil. These samples were air-dried in the greenhouse for approximately one month.

In August, 2010, tissue and soil samples were ground using a mortar and pestle or an electric grinder and stored in plastic bags. In October, 2010, each sample was weighed and 15% of that total weight was used as the component for digestions.

Digestions Of Frond And Root/Rhizome Samples

Frond and root/rhizome samples were digested using a Kjehldahl distillation unit. To digest the plant material, 4ml HNO_3 (nitric acid) and 4ml H_2O_2 (hydrogen peroxide) were used. Weighed tissue samples were placed into the Kjehldahl flasks; HNO_3 was added; and samples were allowed to sit for one hour. The H_2O_2 was then added and the samples were boiled for approximately 5 to 8 minutes. Ultra pure water (25ml) was added to each flask; the samples were then filtered through Whatman 40 filter paper and brought up to a final volume of 100ml using ultra pure water. Reagent blanks were also created by following the same procedure minus the tissue sample. All materials that were used repeatedly were washed between each use in a 10 % HNO_3 acid bath.

Soil Samples

Soil samples were processed by using a sequential extraction procedure (Porter, 2003). Initial soil samples were pooled by treatment, because, procedurally, the samples should have contained the same amount of arsenic. Final soil samples were tested

individually to show differences among plants. All soil samples consisted of one gram and were placed in VWR 50 ml Centrifuge Tubes.

To test for plant-available arsenic, 25 ml ultra-pure water was added to the soil samples and they were shaken in a New Brunswick Scientific G24 Environmental Incubator Shaker at 250 rpm for 30 minutes. These samples were then centrifuged in a Beckman Coulter Allegra X-15R Centrifuge at 3,000 rpm for 15 minutes. The supernatant was then collected and filtered through Whatman 40 filter paper. Solids were retained for extraction of arsenic attached to organics.

To test for arsenic attached to organics in the soil, 25 ml 12N HCl was added to the soil samples and they were shaken in a New Brunswick Scientific G24 Environmental Incubator Shaker at 250 rpm for 30 minutes. These samples were then centrifuged in a Beckman Coulter Allegra X-15R Centrifuge at 3,000 rpm for 15 minutes. The supernatant was then collected and filtered through Whatman 40 filter paper and brought up to a final volume of 100 ml using ultra-pure water.

Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES)

Using the ICP-OES programming, a method was created to identify arsenic on four possible wavelengths. This method was used for all the samples. Calibration standards of 0.5, 1, 5, and 10 ppm arsenic were created by diluting a 100 ppm stock solution of arsenic. These calibration standards were run through the ICP-OES to determine the calibration curve used for all samples. The standard used was a sample of ultra pure water. To ensure quality control, calibration standards of either 1 or 5 ppm were tested in the ICP-OES every 10 to 15 samples.

Data Analysis

Data from the ICP-OES were converted from mg/L to ug/g to show the amount of arsenic present relative to sample mass. The data were analyzed using a multi-factorial ANOVA using R (R Development Core Team, 2011). Average arsenic concentrations were compared between fern species and among arsenic treatments. Separate analyses were performed for soil and for fern frond and root/rhizome tissue. Linear contrasts were performed to compare average arsenic concentrations in the 5, 10, and 50 ppm treatments with those in the control. Concentration data were log transformed (ln(x)) to better fit model assumptions.

RESULTS

Soil Samples – Water Extraction

Soil samples for both *P. acrostichoides* and *T. noveboracensis* were below detection limits of the ICP-OES (0.1 ppm), meaning samples were not discernable from zero, for the Control, 5 ppm, and 10 ppm arsenic treatments. Arsenic was present at the 50 ppm treatment level in both initial and final soil samples for both species of ferns. The amount of arsenic present in the soil increases with increasing treatment concentration as expected (Table 2). A plot of the means for initial (Figure 6) and final (Figure 7) soil samples for both species also shows the presence of arsenic in the soil increases with the treatment level.

Table 2. Multifactorial ANOVA for final soil samples (water extraction) arsenic concentrations for *P. acrostichoides* and *T. noveboracensis* comparing species, treatment, and the interaction between species and treatment.

	Sum Sq	**Df**	**F Value**	**Pr (>F)**
Species	0.0208	1	0.6149	0.43602
Treatment	17.9005	3	176.2759	$< 2x\ 10^{-16}$
Species:Treatment	0.2448	3	2.4109	0.07566
Residuals	2.0310	60		

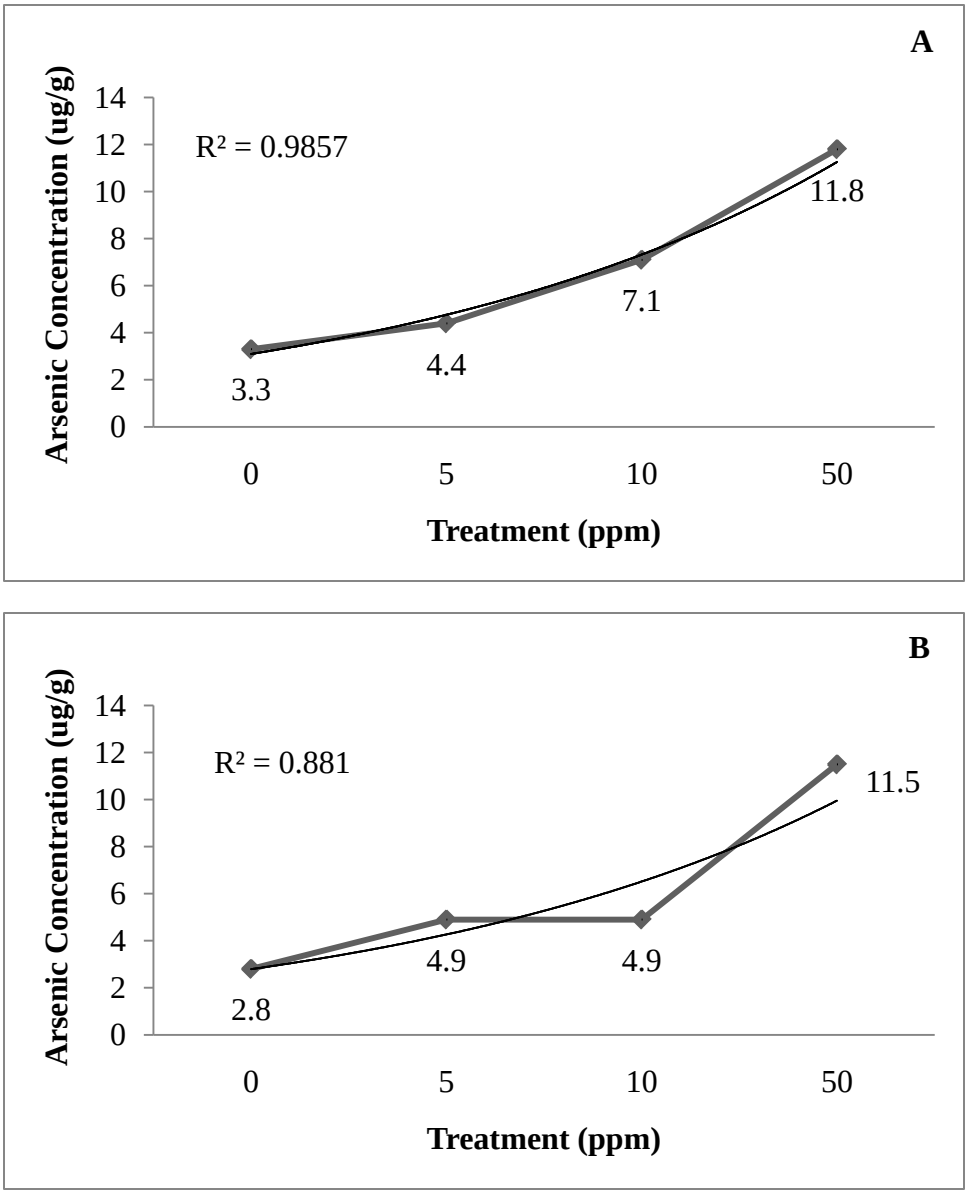
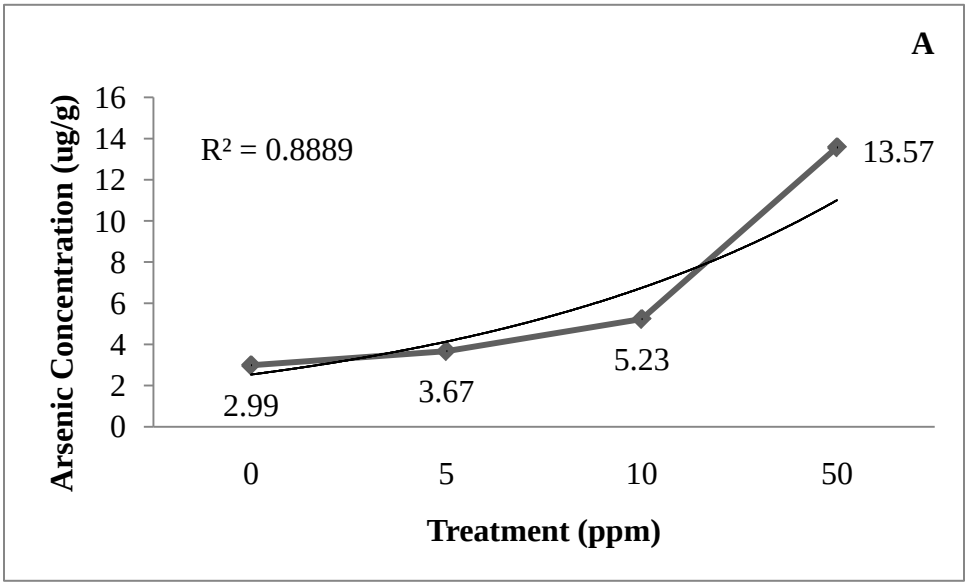


Figure 6. Mean arsenic concentrations in initial water-extracted soil samples (thick line) with an exponential trendline (thin line). (A) *Polystichum acrostichoides.* (B) *Thelypteris noveboracensis.* Treatments = 0 (control), 5 ppm, 10 ppm, 50 ppm.



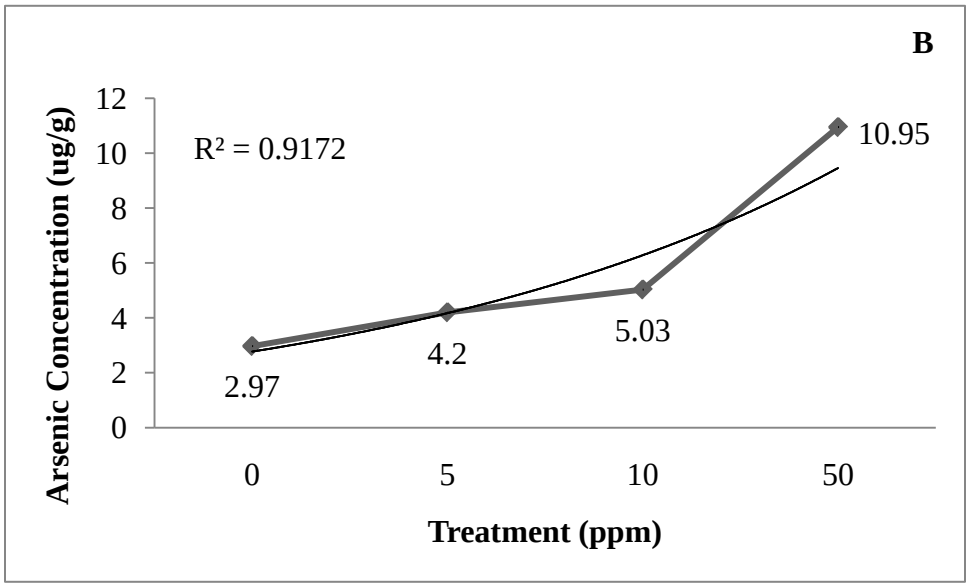


Figure 7. Mean arsenic concentrations in final water-extracted soil samples (thick line) with an exponential trendline (thin line). (A) *Polystichum acrostichoides.* (B) *Thelypteris noveboracensis.* Treatment = 0 (control), 5 ppm, 10 ppm, 50 ppm.

Soil Samples – Acid Extraction

Soil samples for both *P. acrostichoides* and *T. noveboracensis* were below the detection limits of the ICP-OES (0.1 ppm), meaning samples were not discernable from zero, for the Control, 5 ppm, and 10 ppm arsenic treatments. Arsenic was present at the 50 ppm treatment level in initial soil samples for both species, but was only present in *P. acrostichoides* final samples. Statistical analysis of these samples shows that the treatment is significant; arsenic concentration increases with the treatment level as expected (Table 3). Arsenic increased in the soil as the treatment level increased (Figure 8 & 9).

Table 3. Multifactorial ANOVA for final soil samples (acid extraction) arsenic concentrations for *P. acrostichoides* and *T. noveboracensis* comparing species, treatment, and the interaction between species and treatment.

	Sum Sq	**Df**	**F Value**	**Pr (>F)**
Species	0.0294	1	1.5324	0.22057
Treatment	4.0456	3	70.3714	< 0.0001
Species:Treatment	0.1331	3	2.3145	0.08489
Residuals	1.1498	60		

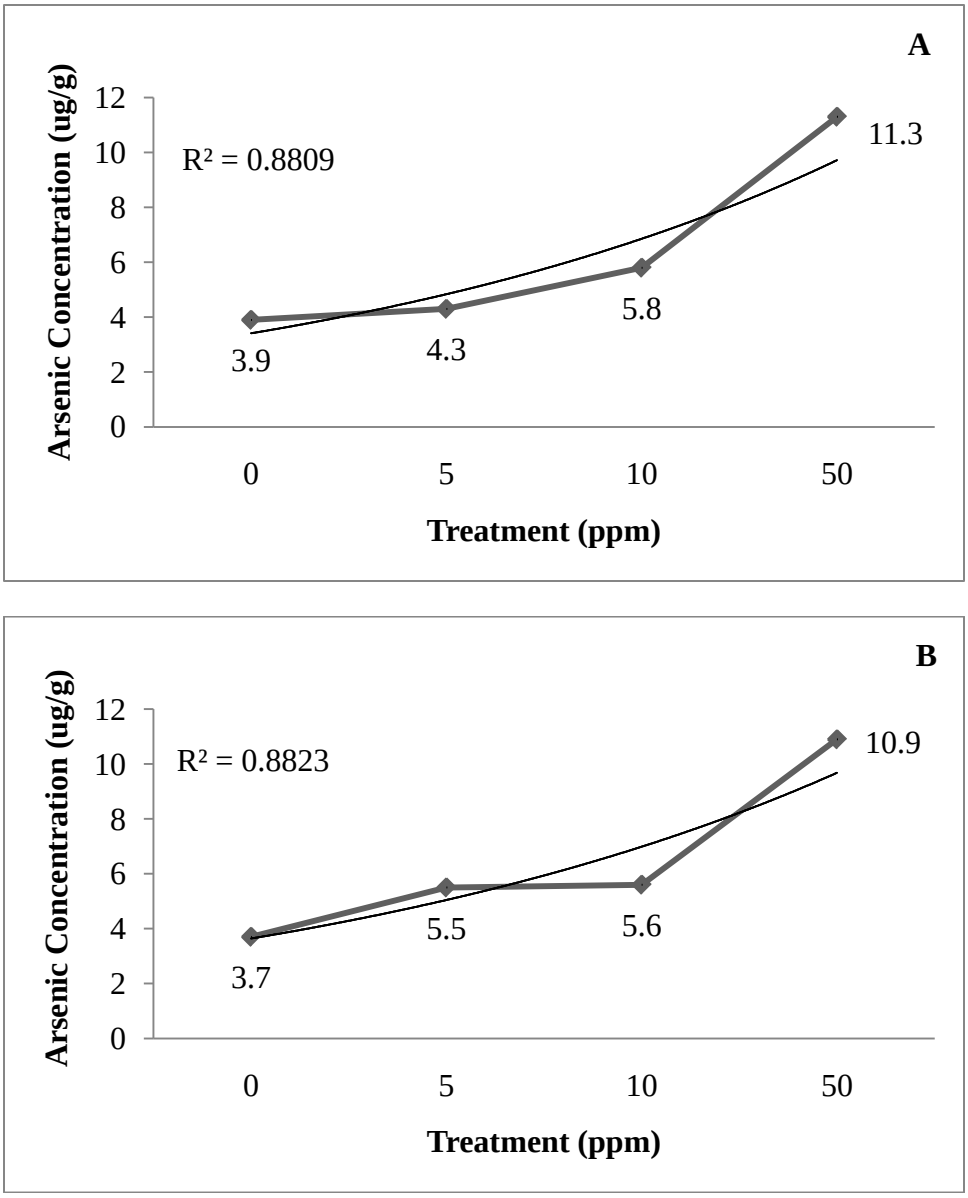


Figure 8. Means of arsenic concentrations in initial acid-extracted soil samples (thick line) with an exponential trendline (thin line). (A) *Polystichum acrostichoides* (B) *Thelypteris noveboracensis.* Treatment = 0 (control), 5 ppm, 10 ppm, and 50 ppm.

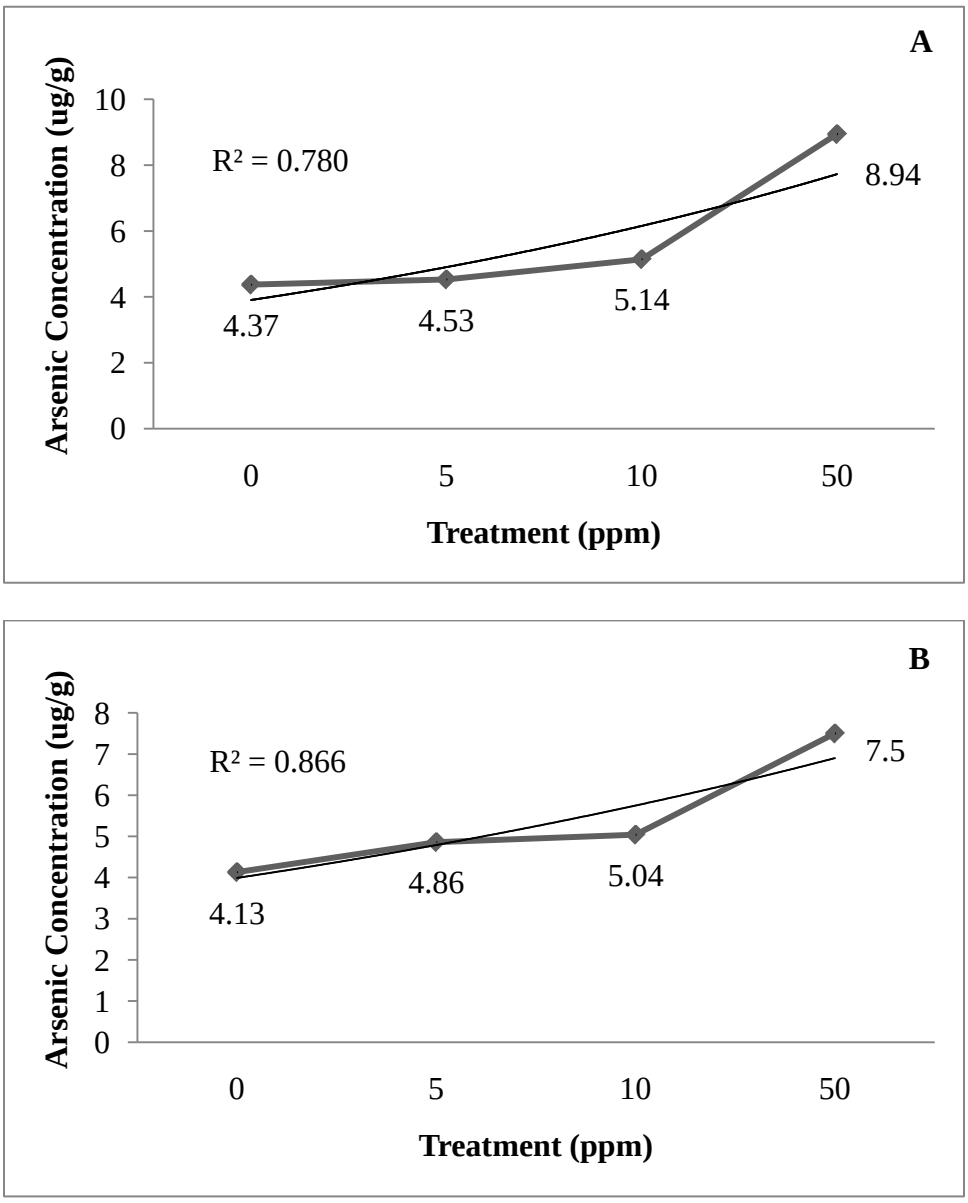


Figure 9. Means of arsenic concentrations in final acid-extracted soil samples (thick line) with an exponential trendline (thin line). (A) *Polystichum acrostichoides.* (B) *Thelypteris noveboracensis.* Treatment = 0 (control), 5 ppm, 10 ppm, and 50 ppm.

Frond Samples

All initial and final frond samples of both *P. acrostichoides* and *T. noveboracensis* show arsenic accumulation below the detection limit of the ICP-OES (0.1 ppm), meaning the samples were not discernable from zero. No trends were observed, which could be the result of large variation among samples and/or low power, due to missing samples (which occurred if the fern produced no fronds or the fronds died during the experiment) (Table 4).

Table 4. Mean initial and final frond arsenic concentration values including standard deviations for *P. acrostichoides* and *T. noveboracensis* for all treatments (Control, 5, 10, and 50 ppm).

		Control		**5 ppm**		**10 ppm**		**50 ppm**	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
P. acrostichoides	Mean	264.35	261.51	89.77	1880.33	45.27	75.22	63.97	687.17
	Std. Dev.	408.81	604.23	94.06	3526.13	38.40	101.04	85.02	1556.53
T. noveboracensis	Mean	209.68	66.88	193.46	75.1	259.18	282.09	291.32	190.69
	Std. Dev.	269.45	51.31	141.55	24.21	338.2	493.7	235.52	156.19

Root/Rhizome Samples

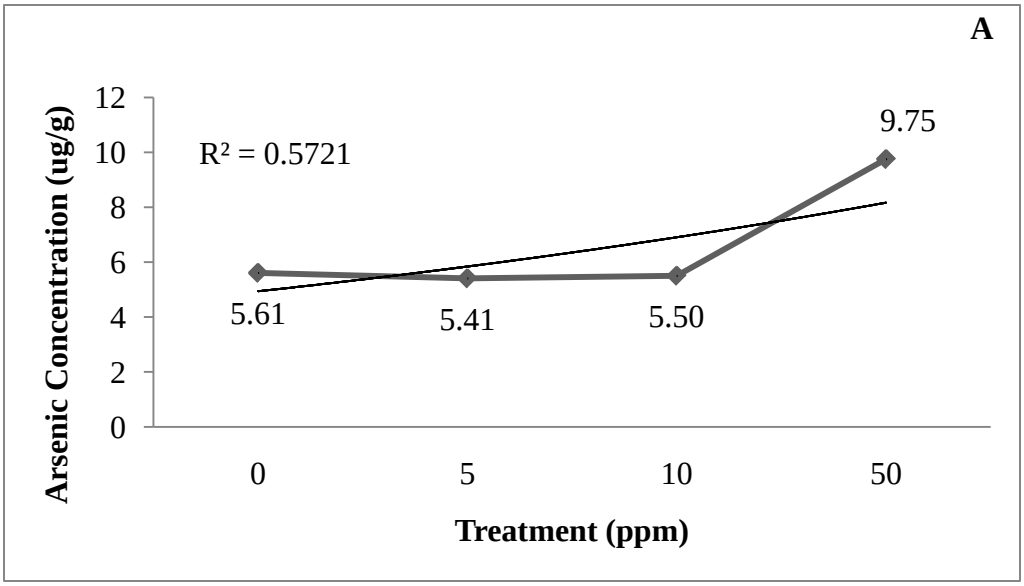
Final root/rhizome samples for both *P. acrostichoides* and *T. noveboracensis* show uptake of arsenic at the 10 and 50 ppm treatment levels (Table 5). Arsenic levels were below the detection limit for the ICP-OES (0.1 ppm), meaning samples were not discernable from zero, for all initial samples at all treatment levels and for final samples in the control and 5 ppm treatments. Analyses of the final root/rhizome samples of the 10 and 50 ppm treatment levels show significant interaction between the fern species and arsenic treatment level (Table 6). Further analysis of this interaction revealed *T. noveboracensis*, not *P. acrostichoides*, a significant response to the treatment (p – value = 2.239×10^{-06}). A plot of the means for both species shows that *T. noveboracensis* has a much stronger reaction at 50 ppm than *P. acrostichoides.* *T. noveboracensis* shows an exponential increase in uptake of arsenic at the 50 ppm treatment level (Figure 10).

Table 5. Linear contrasts comparing arsenic treatments (5, 10, 50 ppm) to the control (0 ppm arsenic).

Treatment	Estimate Std.	Error	t- value	Pr (>|t|)
5 ppm	0.3377	0.2006	1.683	0.2357
10 ppm	0.6161	0.2006	3.071	0.0111
50 ppm	1.2769	0.2006	6.366	< 0.001

Table 6. Multifactorial ANOVA for final root/rhizome arsenic concentrations for both *P. acrostichoides* and *T. noveboracensis* comparing the two species, treatment levels, and the interaction between the species and treatment levels.

	Sum Sq	Df	F Value	Pr (>F)
Species	30.8342	1	141.1600	< 2.2e-16
Treatment	3.1936	3	4.8734	0.004242
Species:Treatment	6.4227	3	9.8010	2.358e-05
Residuals	13.1061	60		



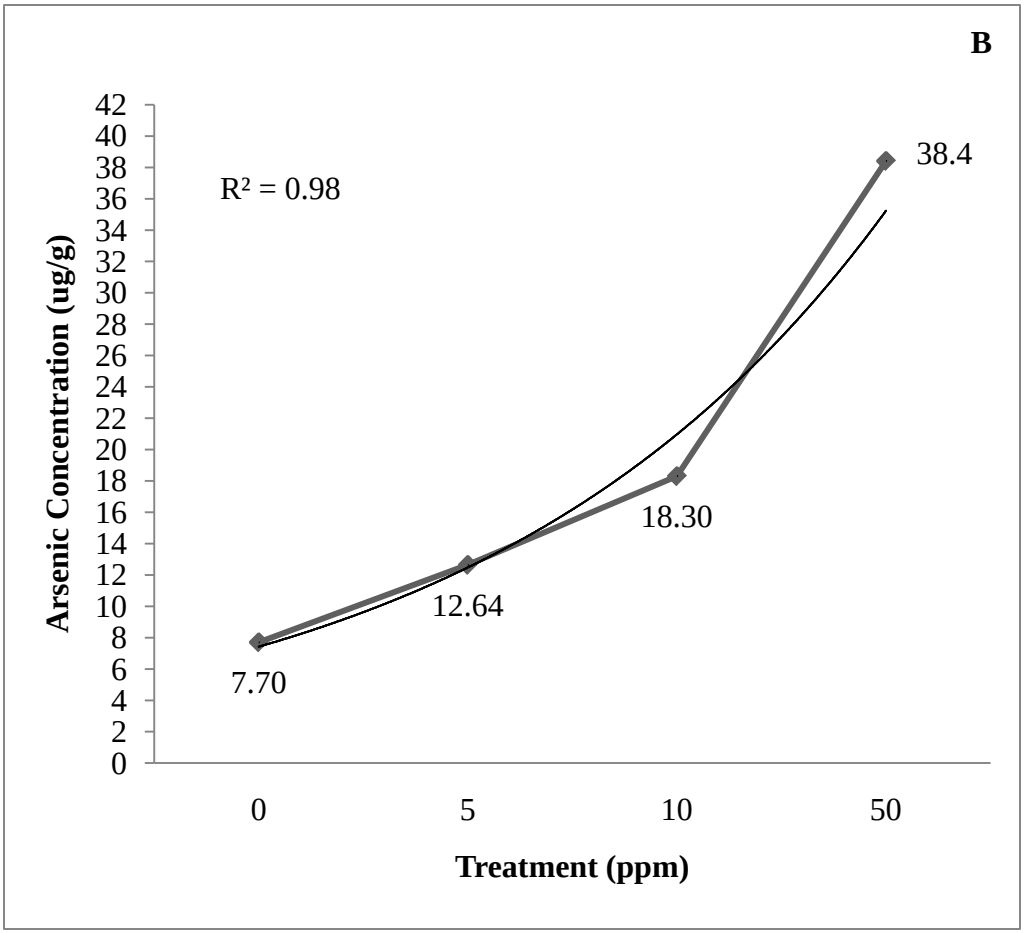


Figure 10. Mean arsenic concentrations in final root samples with an exponential trend line. (A) *Polystichum acrostichoides.* (B) *Thelypteris noveboracensis.* Treatments = 0 (control), 5 ppm, 10 ppm, 50 ppm.

Root/Rhizome And Soil Sample Interactions

A scatter plot of the final 50 ppm root/rhizome samples against the final 50 ppm soil samples (water extraction) shows a linear relationship between soil arsenic root/rhizome arsenic concentrations in *T. noveboracensis*. The relationship presents evidence that as soil arsenic values increased the root arsenic values increased in *T. noveborancensis* (Figure 11).

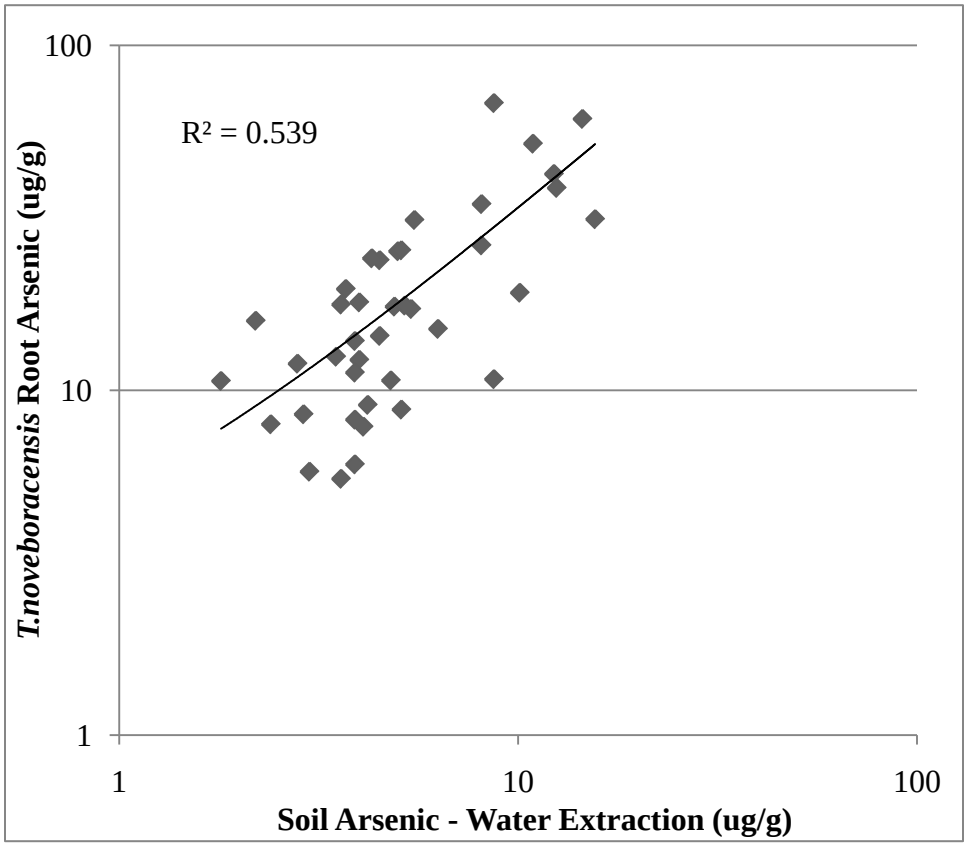


Figure 11. Scatter plot of water-extracted soil arsenic concentration vs. *Thelypteris noveboracensis* root arsenic concentrations with a linear trend line.

DISCUSSION

Question 1: Do Two Native Fern Species Take Up Arsenic?

The answer to this question is both yes and no. Both *P. acrostichoides* and *T. noveboracensis* had elevated arsenic levels in the highest (50 ppm) arsenic treatment. But, *P. acrostichoides* showed no significant uptake of arsenic in the fronds in this study. Meharg (2003) studied other *Polystichum* spp. and found uptake of arsenic in fronds ranging from 1.2 to 8.3 mg kg^{-1}. Though these values are higher than found in this study the values are still low and the ferns are not considered to be accumulators. Arsenic was detected at the 50 ppm treatment level in *P. acrostichoides* root/rhizome samples; however, the mean value was only 9.75 ug/g. Other treatment levels (Control, 5, and 10 ppm) were below detection limits of the ICP-OES (0.1 mg/L). *Thelypteris noveboracensis* also showed no significant uptake of arsenic in the fronds. However, *T. noveboracensis* did show consistent uptake of arsenic in an exponential relationship across treatment levels in the final root/rhizome samples. Also, there is a linear relationship between arsenic concentrations in the final root/rhizome samples and final soil arsenic concentrations (water extraction). Although these positive results were found for *T.noveboracensis*, the findings must be taken with caution as the majority of the samples (28 out of 40) were below detection limits of the ICP-OES (0.1 mg/L) resulting in a lack of replication and many samples not significantly different from zero. A recent study of *Thelypteris palustris* showed uptake of arsenic in both fronds and roots, however, the results varied widely and showed no significant difference across treatments. It was determined that *T. palustris* would not be a likely candidate for

phytoremediation (Anderson & Walsh, 2006). Similarly, this study found that both *P. acrostichodies* and *T. noveboracensis* could take up arsenic, but the results varied widely and the levels of uptake were not high enough for either one of these ferns to be likely candidates for phytoremediation purposes.

Question 2: Where Does Arsenic Accumulate?

In this study the ferns were analyzed in two parts: above-ground and below-ground growth. Above-ground parts included the entire frond (pinnules and stem). Below-ground parts included the roots and rhizomes (an underground stem). Both *P. acrostichoides* and *T. noveboracensis* had arsenic in some root/rhizomes samples, whereas no significant amounts of arsenic were found in frond samples. When these results are compared to the abilities of *P. vittata* the short comings of these native ferns for phytoremediation are clear. *Pteris vittata* was able to hyperaccumulate arsenic in its fronds 126-fold in two weeks in the control treatment (Ma et al., 2001). The roots of *P. vittata* showed less accumulation in comparison with its fronds; however, this indicates this fern translocates the arsenic into the fronds, making it extremely useful for phytoremediation purposes (Ma et al., 2001). How *P. vittata* is able to hyperaccumulate arsenic at such high levels has yet to be completely elucidated. Wang et al. (2002) found that *P. vittata* extracts arsenate via phosphate transporters (Meharg & Hartley-Whitaker, 2002) similar to all plants studied thus far. It has also been found that *P. vittata* is able to exceed many plants in its abilities to tolerate arsenic. Many plants exhibit signs of phytotoxicity to arsenic between 40 and 200 ppm (Sheppard, 1992). *Pteris vittata* was able to withstand arsenic levels of 500 ppm (Tu & Ma, 2002). My research tested levels of arsenic up to 50 ppm which is well below and within the range of values or arsenic

toxicity for most plants. Wang et al. (2002) note four possible reasons that many plants do not accumulate arsenic: "(a) low bioavailability of As in soil, (b) restricted uptake by plant roots, (c) limited translocation of As from roots to shoots, and (d) As phytotoxicity at relatively low concentrations in plant tissues (Wang et al., 2002)." Some of these reasons may explain why *P. acrostichoides* and *T. noveboracensis* simply did not uptake arsenic at rates that would warrant them to be accumulators.

Question 3: Are These Ferns Feasible For Phytoremediation Purposes?

Plants selected for phytoremediation possess certain characteristics; they grow rapidly, have large biomass, are easy to grow, and have the ability to take up the desired contaminant (Ensley, 2000). Both species of ferns tested in this study have most of those characteristics. *P. acrostichoides* can grow to be quite large, grows year round, tends to grow in colonies, and grows quite easily in its natural environment. *T. noveboracensis* can grow to be large, has the ability to spread rapidly, and can easily be grown in its natural environment (personal observation). Based on the data found in this study, however, neither fern would be a good choice for phytoremediation purposes. *P. acrostichoides* simply does not accumulate arsenic and would not prove to be helpful in extracting arsenic from the soil. *P. acrostichoides* might best be described as an excluder. Excluders contain a small concentration of the metal, but do not take up the metal further, even when concentrations vary in the environment (Baker, 1981). *T. noveboracensis* has the ability to uptake arsenic when concentrations in the soil are high enough, but has no response at lower arsenic concentrations levels. *T. noveboracensis* might best be described as an indicator, as it did show a linear response at the 50 ppm

arsenic treatment level in this study. Indicators have a linear relationship with the metal concentrations found in the surrounding environment (Baker, 1981).

Conclusions

This research revealed that *P. acrostichoides* and *T. noveboracensis* are not good options for phytoremediation of arsenic contamination. The ferns simply do not remove enough arsenic to make them viable options for phytoremediation. As for the future of phytoremediation of arsenic in Western North Carolina, there are many more native ferns that could and should be tested. I suggest investigating the Pteridaceae Family (maidenhair fern family) for native accumulators. Pteridaceae is the same family in which *Pteris vittata* L. (ladderbrake), a known hyperaccumulator, is found. Also, research into the physiology of hyperaccumulators and non-accumulators could prove most useful and could possibly identify what makes a plant a hyperaccumulator or excluder.

WORKS CITED

Adriano, D.C. Trace elements in the terrestrial environment. New York: Springer, 2001.

AFS. The American Fern Society - A Brief Introduction to Ferns. 17 April 2001. Retrieved on 20 June 2008. <http://amerfernsoc.org/>.

Agre, P., S. Sasaki and and M.J. Chrispeels. "Aquaporins - a family of water channel proteins." American Journal Physiology. 265.3 (1993).

Akter, K. and R. Naidu. "Arsenic speciation in the environment." Managing Arsenic in the Environment: From soil to human health. Ed. Ravi Naidu, et al. Collingwood: CSIRO Publishing, 2006. 59-74.

Anderson, L. and M.M. Walsh. "Arsenic uptake by common marsh fern *Thelypteris palustris* and its potential for phytoremedation." Science of the Total Environment. 379 (2007): 263-265.

ATSDR - U.S. Dept. of Health and Human Services; Public Health Service; Agency for Toxic Substances and Diease Registry. "Toxicological Profile for Arsenic." 2007.

Baker, A.J.M. "Accumulators and excluders - strategies in the response of plants to heavy metals." Journal of Plant Nutrition. 3 (1981): 643-654.

Blaylock, M.J. and J.W. Huang. "Phytoextration of Metals." Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. Ed. Ilya Raskin and Burt D. Ensley. John Wiley & Sons, Inc., 2000. 53.

Briat, J.F.. "Arsenic tolerance in plants: "Pas de deux" between phytochelatin synthesis and ABCC vacuolar transporters." Proceedings of the National Academy of Sciences. 107.49 (2010): 20853-20854.

Chaumont, F., M. Moshelion and and M.J. Daniels. "Regulation of plant aquaporin activity." Biology of the Cell. 97.10 (2005): 749-764.

Ensley, B.D. "Rationale for Use of Phytoremediation." Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. Ed. Ilya Raskin and Burt D. Ensley. John Wiley & Sons, Inc., 2000. 5.

EPA. Arsenic in Drinking Water. 14 September 2006. Retrieved on 28 February 2008 <http://www.epa.gov/safewater/arsenic/index.html>.

EPA. Celebrating Success: Ryeland Road Arsenic Site Heidelburg Township, Pennsylvania. September 2011.

EPA. Crozet Phytoremediation. Retrieved on 21 February 2012 <http://www.epa.gov/superfund/misc/crozet_phyto.htm>.

EPA. "Phytoremediation Resource Guide." 1999.

EPA. Superfund Site Information. Retrieved on 21 February 2012. <http://cumulis.epa.gov/supercpad/cursites/srchrslt.cfm?start=1&CFID=8631962&CFTOKEN=86896566&jsessionid=e0301117c1d9338b7bea2e6930412d2d196c>.

Fitz, W.J. and W.W. Wenzel. "Sequestration of arsenic by plants: Arsenic uptake mechanisms and rhizosphere effects." Managing Arsenic in the Environment: From soil to human health. Ed. Ravi Naidu, et al. Collingwood: CSIRO Publishing, 2006. 209-222.

Glass, D. J. "Economic Potential of Phytoremediation." Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. Ed. Ilya Raskin and Burt D. Ensley. John Wiley & Sons, Inc., 2000. 16-18.

Google Maps. "Map of Poplar Cove Creek and Cloer Creek". Retrieved on 23 March 2012. <http://www.maps.google.com>.

Hughes, M.F. "Arsenic toxicity and potential mechanisms of action." Toxicology Letters. 133 (2002): 1-16.

Jiang, Q.Q. and B.R. Singh. "Effect of different forms and sources of arsenic on crop yield an arsenic concentration." Water, Air, Soil Pollution. 74 (1994): 321-343.

Ma, J.F., K. Tamai, N. Yamaji, N. Mitani, S. Konishi, M. Katsuhara, M. Ishiguro, Y. Murato, and M. Yano. "A silicon transporter in rice." Nature. 440 (2006): 688-691.

Ma, J.F., N. Yamaji, N. Mitani, X.Y. Xu, Y.H. Su, S.P. McGrath, and F.J. Zhoa. "Transporters of Arsenite in Rice and Their Role in Arsenic Accumulation in Rice Grain." Proceedings of the National Academy of Sciences. 105.29 (2008): 9931-9935.

Ma, L.Q., K.M. Komart, C. Tu, W. Zhang, Y. Cai, and E.D. Kenelley. "A fern that hyperaccumulates arsenic." Nature. 409 (2001): 579.

Prasad, M.N.V. and H. Freitas. "Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology". Electronic Journal of Biotechnology. 15 December 2003, vol. 6, no.3. Retrieved on 23 March 2012. <http://www.ejbiotechnology.info/content/vol6/issue3/full/6/#173> ISSN: 0717-3458.

Meharg, A. A. "Variation in Arsenic Accumulation: Hyperaccumulation in Ferns and Their Allies." New Phytologist. 157.1 (2003): 25-31.

Meharg, A.A. and J. Hartley-Whitaker. "Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species." New Phytologist. 154 (2002): 29-43.

Meharg, A.A. and L. Jardine. "Arsenite Transport into Paddy Rice (*Oryza sativa*) Roots." New Phytologist. 157 (2003): 39-44.

Miller, J.R. and S.M. Orbock Miller. Contaminated Rivers: A geomorphological - geochemical approach to site assessment and remediation. Springer, 2007.

Mukhopadhyay, R. and B.P. Rosen. "Arsenate Reductases in Prokaryotes and Eukaryotes." Environmental Health Prespectives. 110(suppl 5) (2002): 745-748.

Porter, K.M. Speciation of Lead & Arsenic in Contaminated Soil. MS Thesis. Cullowhee: Western Carolina University, 2003.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org/.

Rock, S. and P.G. Sayre. "Regulation Considerations for Phytoremediation." Phytoremediation of Toxic Metals: Using Plants to Clean Up the Enivronment. Ed. Ilya Raskin and Burt D. Ensley. John Wiley & Sons, Inc., 2000.

Ruder, K. "Ferns Remove Arsenic from Soil and Water." 6 August 2004. Genome News Network. Retrieved on 21 February 2012 <http://www.genomenewsnetwork.org/articles/2004/08/06/fern.php>.

Scott-Fordsmand, J.J. and M.B. Pederson. "Soil Quality Criteria for Selected Inorganic Compounds." Working Report No. 48, for Ministry of Environment and Energy, Denmark. EPA Denmark. (1995).

Sheppard, S.C. "Summery of phytotoxic levels of soil arsenic." Water, Air, and Soil Pollution. 64.3-4 (1992): 539-550.

Smith, E., R. Naidu, and A.M. Alston. "Arsenic in the soil environment: a review." Advanced Agronomy. 64 (1998): 149-195.

Tu, C. and L.Q. Ma. "Effects of Arsenic Concentrations and Forms on Arsenic Uptake by the Hyperaccumulator Ladder Brake." Journal of Enivronmental Quality. (2002): 641-647.

USDA & NRCS. The PLANTS Database. Retrieved on 15 February 2012. <http://plants.usda.gov>.

Wang, J., F.J. Zhoa, A.A. Meharg, A. Raab, J. Feldmann, and S.P. McGrath. "Mechanisms of Arsenic Hyperaccumulation in Pteris vittata. Uptake Kinetics, Interactions with Phosphate, and Arsenic Speciation." Plant Physiology. 130 (2002): 1552-1561.

Xie, Z.M. and R. Naidu. "Factors influencing bioavailability of arsenic to crops." Managing Arsenic in the Environment: From soil to human health. Ed. Ravi Naidu, et al. CSIRO, 2006. 223-234.