Leafing Out to Remediate: Bioaccumulation of Arsenic in *Pteris vittata* Over Time

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ABSTRACT

Urban agriculture has potential to increase urban food security and improve urban air and soil quality. However, urban soils may have historic contamination, with arsenic being a pollutant of major concern. Arsenic is a carcinogenic metalloid with proven negative consequences on human health and the environment, and is thus a target of remediation efforts. The Chinese brake fern *Pteris vittata* accumulates arsenic in high concentrations, and the plant is often used in remediation efforts. While studies measuring the effectiveness of *P. vittata* in remediation exist, there is little data on the effect of age on accumulation. I conducted a controlled greenhouse experiment over 16 weeks, growing 12 *P. vittata* ferns in arsenic contaminated soil from our Berkeley field site. Frond sampling was conducted at 4 week intervals and temporal replicates were also taken to measure arsenic uptake over time. Samples were prepared and sent for arsenic concentration analysis by Brookside Laboratories in New Bremen, Ohio. I found that both concentration of arsenic by biomass and total arsenic levels increased over the 16 week period. Fronds grown after planting in contaminated soil showed arsenic levels at an order of magnitude higher than fronds present before exposure to arsenic. These findings are relevant for those interested in utilizing *P. vittata* for remediation, who should focus efforts on younger ferns that can produce many new fronds to accumulate arsenic fastest for best results.

KEYWORDS

Chinese Brake fern, hyperaccumulator, phytoextraction, phytoremediation, temporal replicate

INTRODUCTION

Urban agriculture is an increasingly popular practice taking shape in many of the nations' cities. The process of urban agriculture includes the production, distribution and marketing of food and food products within and around urban, metropolitan areas (University of California Dept. of Agriculture and Natural Resources, 2017.). It can help promote food security through decreased prices from minimal transportation fees, since the produce is grown and eaten locally (Golden 2013). It can also improve the urban environment through air quality improvement, soil structure development and biodiversity maintenance through cultivation of plants (Bellows et al. 2004). However, past and present industrial processes in urban areas can lead to historic, long-term contamination of water and soils.

Arsenic is one such contaminant of high concern. Arsenic is a metalloid that is historically prevalent in our environment, both from natural and anthropogenic sources. Arsenic is well known for its toxicity; as a proven carcinogen to humans, arsenic is linked to lung, bladder and skin cancers (Ng et al. 2003). Arsenic poisoning (arsenicosis) from contaminated groundwater can lead to lung disease, skin diseases and neuropathy (Mazumder et al. 2010). These crippling effects mean that arsenic is one of the World Health Organization's 10 chemicals of major public health concern (World Health Organization 2012). Arsenic is naturally present in the environment in trace amounts, but due to industrial applications such as coal mining, wood treatment, and arsenic-based fertilizers, dangerously high levels are found in urban soils and water (Bissen and Frimmel 2003). Chromated copper arsenate (CCA) was the world's most commonly used wood preservative and was only recently regulated by voluntary cancellations in residential usage in 2004 (U.S. Consumer Product Safety Commission 2011). Historic usages of arsenic in herbicides, insecticides, and fertilizers have also introduced the toxin to soils (Nriagu 1994). For example, inorganic lead arsenate, a form of arsenic, was the most widely-used insecticide prior to DDT and was not fully banned until the 1990's (Shepard 1951, Welch 2000). High arsenic concentrations in the environment can leach into groundwater, a source of drinking water and part of the water cycle (Welch 2000). Arsenic also interferes with essential nutrient uptake in plants, including phosphorous, an important regulator of plant processes like photosynthesis (Tu and Ma 2002). In urban agriculture, a dangerous and direct exposure comes from growing food in these arsenic contaminated soils.

Unfortunately, the presence of arsenic in the natural environment can remain for many years due to the toxin's persistence. This classifies arsenic as a "legacy pollutant": contaminants that remain in the environment long after industrial polluting facilities have been banned by regulation or relocated (Shriver et al. 2014). The previous high usage of the pollutant combined with their chemical properties make legacy pollutants especially persistent in the environment (). This adds to the severity of the risks of arsenic. A solution to this contamination is needed to prevent further damage to human health and ecosystems.

Despite the need for effective removal, traditional methods of remediation are often problematic. Methods like excavation, extraction, or thermal treatment can be prohibitively invasive and expensive (Kopittke et al. 2010). In addition to high cost, these methods can be disruptive to the people living in or near the contaminated sites. Large-scale removal can further damage the environment, make arsenic airborne and releasing pollutants from machinery, making life difficult for the people suffering from the initial arsenic contamination. For urban agriculture, preserving soil structure for cultivation is imperative, and requires non-destructive remediation solutions (Brussaard et al. 2007). It is crucial then, to formulate solutions to this global problem that are also sustainable and considerate to local communities and the environment.

Phytoremediation is one approach to removing contaminants without the risks of more invasive procedures. Phytoremediation is defined as the direct use of living plants for *in situ*, or in place, removal of pollutants from the soil and/or water bodies (Kertulis-Tartar et al. 2006). Phytoremediation of arsenic using *Pteris vittata* ferns is a relatively new method of arsenic removal, discovered in 2001 by Lena Ma and her lab at the University of Florida. *P. vittata* removes arsenic by phytoextraction, where plants take up and translocate metal contaminants from soil to the above ground biomass, like fronds and leaves (Mirza et al. 2014). The toxin can then be harvested from above-ground biomass and be properly disposed of as hazardous waste (Xie et al. 2009). It is cost-effective and relatively simple, as well as sustainable: it utilizes the natural characteristics and systems of the plant to accumulate and degrade arsenic (Anjum et al. 2012). In addition, it is a less invasive, more sustainable method of remediating polluted sites, as plants revegetate and can keep remediating after removal or senescence of their fronds. There is no need for constant replanting or disturbance of soil structure, reducing risks of arsenic exposure. (Kopittke et al. 2010). Rather than relocate homes or deal with the repercussions of a

huge arsenic extraction, people can instead utilize plants for the same purpose, leading to a less disruptive solution.

However, the accuracy of arsenic concentrations measured in these studies can be influenced by many factors, including the age of the fern and time passed since the start of phytoremediation (Gonzaga et al. 2007). While many studies explore the effect of *P. vittata* in remediation efforts, no studies specifically consider the effect of age of the fronds on the amount of arsenic accumulated. Observations on where arsenic is stored within the fern are also limited. This information would be useful in helping determine the accuracy of arsenic concentration measurements done on fronds. Additionally, it can help identify trends in arsenic contamination over the life span of the fern, which may be useful when researchers conduct their own arsenic phytoremediation projects using *P. vittata*, and when and where to target fern harvests for best results. I aim to answer the following questions related to phytoremediation with *P. vittata*:

How does the amount of arsenic accumulation in the arsenic hyperaccumulator plant *Pteris vittata* change over time*?*

More specifically, (1) How does arsenic uptake change over time in "transplanted" ferns, ferns repotted in arsenic? (2) In transplanted ferns, is there selectivity in where arsenic is stored: within fronds grown before or after exposure to historically arsenic-contaminated soil?

I expected time will influence arsenic accumulation in fronds; the first 8 weeks in arsenic contaminated soil will show the least amount of arsenic concentration, while the last 8 weeks will have accumulated the most total arsenic, and arsenic per unit biomass.

METHODS

Sites

Santa Fe Right-of-Way (SFROW)

The Santa Fe Right-of-Way is the field site and prior location of the ferns and soil before transplant and soil excavation. It is located at 1417 Derby St., Berkeley, California, 94704 (37.8585229, -122.28170339999997). Our research plot within the site is 24m x 6m large. The

site was originally planned as a community orchard. However, initial testing found arsenic contamination (100 parts per million) in the soil that deemed it unsafe to grow food in for the orchard (Ecology Center, 2013). This is due to the history of SFROW as a former railroad right-of-way. Since then, there has been university and community partnership along with funding from the Chancellor's Community Partnership Grant Program in sustainably remediating the soil using *P. vittata* under Celine Pallud's lab.

Oxford Tract Insectary Greenhouse

The experiment is conducted in Insectary Greenhouse (IGH) 11 at the UC Berkeley Oxford Tract, at 1751 Walnut Street, Berkeley, California 94720 (37.875639, -122.267537). It is a university research space owned by the College of Natural Resources allowing for plant, insect and other biological research.

Study design

The *P. vittata* ferns were purchased from Edenspace Systems Corporation by the Pallud Lab at UC Berkeley about 3 years prior to this study. Our 12 ferns were subdivided from these initially purchased ferns. The ferns were harvested for senesced fronds in order to view new frond growth. I chose the 12 ferns first on similar length of the existing fronds between the ferns, then on number of existing fronds. The 12 chosen range from 7-11 fronds. The ferns were transferred from their prior location from SFROW into 1 gallon pots for the study.

Arsenic-contaminated soil was excavated from SFROW in mid-November 2016. Gathering a composite sample was difficult due to the uneven soil moisture of the field site that impeded digging, and the presence of dense weeds and greenhouse structures made it impossible to sample certain areas. Therefore, we accommodated and decided on a spot on the northern side of the growing area, where the soil was soft and not densely covered with weeds. Using pickaxes and shovels, lab members and I first removed the mulch, grass and organic matter top layer to a depth of about 15 cm to expose the soil. Then we dug another 15 cm for a total depth of 30 cm and collected 25 gallons of soil. After separating the soil into 5-gallon buckets, the soil was

sieved using a double layer of two 4-mm mesh sieves. This allows the largest of soil particles to pass through (sand at 2mm), and also larger soil aggregates, organic materials and possibly small rocks, but removes larger rocks, glass, etc. This is necessary to replicate realistic but optimal soil structure when growing the ferns in the greenhouse. After sieving, the soil was mixed for uniformity by placing all of the soil into the mixer and turning the soil counterclockwise and clockwise ten times each. To profile the SFROW soil, 1 cup subsamples each for nutrient content, water content, pH, and arsenic concentration (in mg/kg) were made. After a second sieving at 2mm to remove >2mm rocks and break down large aggregates, the nutrient subsamples were sent for analysis at University of Massachusetts at Amherst. The arsenic was done separately at University of Brookside Laboratories in New Bremen, Ohio. Arsenic analysis is done using EPA Method 200.7: Inductively Coupled Plasma-Atomic Emission Spectrometry. Results from both facilities were in mg/kg. The arsenic concentration in soil was assumed to be constant in the well-mixed soil. Soil texture was determined by the hydrometer method to be loamy sand.

Transplant of the ferns into pots involved first weighing soil to be nearly exact in each pot. Soil net weights per pot ranged from 3260.2 g - 3262.2g. The ferns were transplanted into the new pots and covered to the root crown with remaining soil up to ~3260 g. The repotted ferns were watered manually and left to equilibrate for a week prior to the start of observations on December 6th, 2016. Lastly, each 2 ferns were placed into trays to capture water draining out of pots, and given an ID number from 1-12. All experimental work was done using techniques appropriate for handling hazardous materials.

For optimal growth, I housed the ferns in a greenhouse at the UC Berkeley Oxford Tract. Growing conditions can be controlled and kept relatively constant so as to eliminate it as a confounding factor. *P. vittata* grows well in temperature ranges throughout day from 14°C (night) to 30°C (day) (Gonzaga et al. 2007), which were similar to those in the Oxford Tract greenhouse. The plants were watered by drip irrigation in the greenhouse, delivered at 1ml water per fern per day. Soils were fertilized once at the beginning of the study with 14-14-14 (N-P-K ratio) Osmocote slow release fertilizer at about 1 tbsp. per pot.

Data Collection

Because each fern will vary in frond growth, the beginning point of frond emergence must be identified and logged to conduct the rest of the observations. After the plants equilibrated for a week after transplant, on December 6th I examined each fern for emerging fronds (EF) around 2cm in length. This length was determined as it signified the beginning of the young fiddlehead stage (Gonzaga et al. 2007). Any frond larger may have emerged prior to transplant into arsenic contaminated soil and may not be reliable to observe full growth in study soil over time. Instead, those larger fronds were counted and tagged with small pieces of floss so as to remain separate from the emerging fronds. During analysis, we referred to fronds already present *before* repotting in arsenic contaminated soil as "Pre-Transplant" fronds. Likewise, fronds emerged *after* repotting were referred to as "Post-Transplant", or emerging frond (EF). Post-Transplant fronds were logged with a unique 3 part ID composing of the fern ID # from which it came, a number corresponding to emergence relative to other EFs on the same fern, and the date. This data is visualized in Table 1. Relative emergence was determined by comparing the length in cm of the EFs to each other. The larger EF was determined to have emerged first and would be labeled 1. EFs were too fragile to be measured using any tools, so relative emergence was measured using eyeball estimates. This was simple to determine initially since no fern had more than 2 EFs.

Table 1. Ferns – Initial Emerging and Pre-transplant Frond Counts

Fern ID #	# of emerging fronds (EF) + ID (Fern ID # - EF # - Date emerged)	# of pre-transplant fronds (emerged prior to repotting in As-contaminated soil)
1	0	10
2	• 2 EF • 2-1-12/6/16 • 2-2-12/6/16	9
3	• 2 EF • 3-1-12/6/16 • 3-2-12/6/16	9
4	• 1 EF • 4-1-12/6/16	8
5	• 1 EF • 5-1-12/6/16	9
6	• 1 EF • 6-1-12/6/16	9
7	0	8
8	• 1 EF • 8-1-12/6/16	8
9	• 1 EF • 9-1-12/6/16	8
10	• 1 EF • 10-1-12/6/16	7
11	• 1 EF • 11-1-12/6/16	11
12	• 0	9

From this point each EF was monitored, as was the rest of each fern for any new EFs. Each new EF is ID'd using the same method above. To keep track of each unique EF's location and identify them, a waterproof label taped to floral wire was wrapped around each EF. I continued logging new EFs until 8 weeks into the greenhouse study, which would allow for an 8 week old temporal replicate frond sample when the 16 week sample was due.

This controlled greenhouse study is conducted over the course of 16 weeks, from December 6th, 2016 to March 28th, 2017. I measured fern growth at 4 time points of 4, 8, 12, and 16 weeks to see growth within specific, even time intervals. Observations began on December 6th and ended April 5th.

For a representative sample, the 12 ferns were randomly divided into three groups consisting of four ferns each using a random number generator at random.org. This was done by inputting numbers 1-12 into the generator to produce a new random sequence, then dividing the sequence into 4 groups of 3. The groups were as follows:

- Ferns sampled at 1st Time Point of 4 weeks after transplant: #2, 9, 10
- Ferns sampled at 1st Time Point of 8 weeks after transplant: #4,6,8
- Ferns sampled at 1st Time Point of 12 weeks after transplant: #3, 11, 1
- Ferns sampled at 1st Time Point of 16 weeks after transplant: #12, 5, 7

All groups began observations at the same time. To measure arsenic concentration over time, samples occurred at each of the 4 time points for the designated randomly assigned group of ferns (Table 2). 24 samples were taken, at 2 frond samples for each of the 12 ferns. This amount varied according to the 0.5 gram weight requirement by Brookside Lab's arsenic analysis requirements. For the 8 week time point, I combined fronds sampled with fronds of identical age from ferns outside of the random assignment to meet the requirement. We deemed this not detrimental since we were measuring general nature of arsenic uptake over time and not specific to individual ferns.

In addition to samples based on age of fern, a Pre-Transplant frond from the randomly assigned group for the time interval was also sampled to see if any changes in arsenic concentration occur in fronds already present prior to exposure to contaminated soil. Temporal replicates, or samples corresponding to a frond aged at the previous 4 week interval, were conducted in addition to the frond age matching the time point after week 8 (i.e. at 12 week sample, also sampled a frond aged at 8 weeks old). This totals to 3 replicates for 8 week fronds sampled at 8 weeks, 12 weeks, 16 weeks. This is to increase the statistical power of results with a limited number of ferns.

Table 2. Sampling process.

Time	New fronds sampled after this amount of time of growth in As soil (starting from emergence/fiddlehead)	Pre-Existing fronds sampled after this amount of time exposed to As soil
4 weeks	None (too small)	Pre-existing fronds from 16 week plants
8 weeks	8 week old fronds from 8 week plants	Pre-existing fronds exposed to As for 8 weeks, cut from 4 week plants
12 weeks	1. 12 week old fronds from 12 week plants 2. 8 week old fronds from 8 week plants	Pre-existing fronds exposed to As for 12 weeks, cut from 8 week plants
16 weeks	1. 16 week old fronds from 16 week plants 2. 12 week old fronds from 12 week plants 3. 8 week old fronds from 8 week plants	Pre-existing fronds exposed to As for 16 weeks, cut from 12 week plants

*If at any time there were not enough fronds from the designated ferns to make up the 0.5 g required sample volume, fronds will be selected from other ferns and combined to yield 3 replicate samples.

During harvests, fronds from each group were selected for sampling using the frond that emerged first; if there were fronds of identical age available for sampling, a random number generator of inputted frond IDs was used. The selected fronds were logged with ID and dates. Whole fronds were harvested using sterile scissors and placed in paper bags labeled with the corresponding log data and dried at 100°C for a week in the Oxford Tract drying room. Net dry weights were measured in grams, subtracting the bag weight from the total weight of the frond in the paper bag. Then using a mortar and pestle, the dried frond was ground and sealed in small envelopes in preparation for shipment and analysis by Brookside Laboratories in New Bremen, Ohio.

Data Analysis

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Statistically significant differences in arsenic concentration by biomass and total arsenic (both over time) were measured using a multi-factor Analysis of Variance (ANOVA) and linear model estimating effects of time since transplanting, frond age, and individual fern on 1) arsenic concentrations (mg/kg) and 2) total arsenic (mg) contained in the ferns. The multiple independent factors are the 4 time points since transplanting: 4 weeks, 8 weeks, 12 weeks, and

16 weeks; frond age (pre-transplant, 8 weeks, 12 weeks, and 16 weeks; and individual fern (1-12). The dependent continuous variable is arsenic in mg/kg. Analyses were conducted in R.

RESULTS

Study site soil results

The arsenic concentration in our contaminated soil from our study site at SFROW, represented by three subsamples, ranged from 118.9 – 176.0 mg arsenic per kg soil (mg/kg) (Table 3). Averaged arsenic for the three subsamples was 138.5 mg/kg. This amount was assumed constant for the entirety of the study.

MDL (method detection limit) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, meaning the concentrations reported are highly accurate. The subsample MDL measurements ranged from 1.946-2 mg/kg for an average of 1.971 mg/kg.

Table 3. Arsenic concentration in soils from SFROW. 3 subsamples were analyzed.

Sample #	Arsenic Concentration (mg/kg)	MDL (mg/kg)
1	118.8715	1.945525
2	121.4	2
3	175.0984	1.968503

Biomass, arsenic concentration by biomass and total arsenic for Pre-Transplant fronds is listed below (Table 4). Total arsenic was calculated by converting biomass measurement (g) to kg, then multiplying by As concentration by biomass (mg As/kg biomass) to get total As in mg. Arsenic concentration in Pre-Transplant fronds for the first timepoint was 4.14 mg/kg for Fern #5, 11.83 mg/kg for Fern #7, and 3.95 mg/kg for Fern #12. This calculated to an average of 6.64 mg/kg arsenic concentration by biomass for Pre-Transplant fronds from the 4 week timepoint sample (Figure 1). Total As was 0.00244 mg, 0.00766 mg, and 0.00312 mg for Ferns #5, #7, and #12 respectively.

The 8 week timepoint sample had As concentration results of 14.33 mg/kg for Fern #2, 57.78 mg/kg for Fern #9, and 126.25 mg/kg for Fern #10. 12 week Pre-Transplant data is not present due to a missed sample. The 16 week timepoint had As concentration results of 172.72 mg/kg, 120.07 mg/kg, and 245.634 mg/kg for Ferns #1, #3, and #11.

Total As for the Pre-Transplant 4 week timepoint was calculated to be 0.0024 mg for Fern #5, 0.0077 mg for Fern #7, and 0.003 for Fern #12. For the 8 week timepoint, Total As was 0.0098 mg, 0.025 mg, and 0.05 mg for Ferns #2, #9, and #10 respectively.

Biomass for the 4 week timepoint was measured to be 0.59 g, 0.65 g, and 0.79 g for Ferns #5, #7, and #12 respectively. For 8 week, it was 0.69, 0.44, and 0.39 for Ferns #2, #9, and #10. Lastly, for the 16 week timepoint we have biomasses of 0.37 g, 0.52 g, and 0.78 g for Ferns #1, #3, and #11 respectively.

Table 4. Pre-Transplant frond data. As data by Brookside Labs.

Timepoint (wk)	Sample ID	Frond age	As (mg/kg)	Biomass (g)	Total As (mg)
4	FERN #5	Mature	4.140127	0.589	0.002439
4	FERN #7	Mature	11.83168	0.647	0.007655
4	FERN #12	Mature	3.954635	0.789	0.00312
8	FERN #2	Mature	14.32673	0.687	0.009842
8	FERN #9	Mature	57.78177	0.438	0.025308
8	FERN #10	Mature	126.25	0.398	0.050248
16	FERN #1	Mature	172.7178	0.374	0.064596
16	FERN #3	Mature	120.0746	0.523	0.062799
16	FERN #11	Mature	245.634	0.775	0.190366

There is nearly an order of magnitude increase in Pre-Transplant As for both concentration (Figure 1) and total As (Figure 2) at every consecutive time point. The averaged values for each bar are 6.64 mg/kg at 4 weeks, 66.12 mg/kg at 8 weeks, and 179.46 mg/kg at 16 weeks for Figure 1, showing an increasing trend over time. The 16 week timepoint by far shows the most bioaccumulation, more than doubling in the 8 weeks between the 8 week As concentration of and 16 week timepoint.

Total As depicted in Figure 2 shows a similarly positive increasing trend, with averaged Total As values of 0.0044 mg, 0.0285 mg, and 0.1059 mg for 4 week, 8 week, and 16 week timepoints respectively. Total As at 16 weeks is nearly four times the total As at 8 weeks.

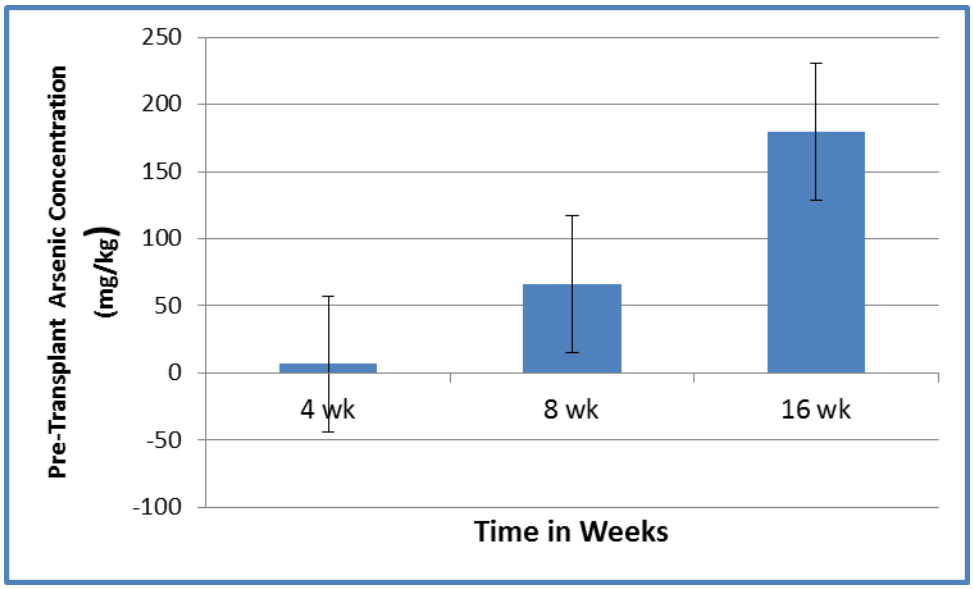

Figure 1. Pre-Transplant As concentration by biomass over Time in weeks.

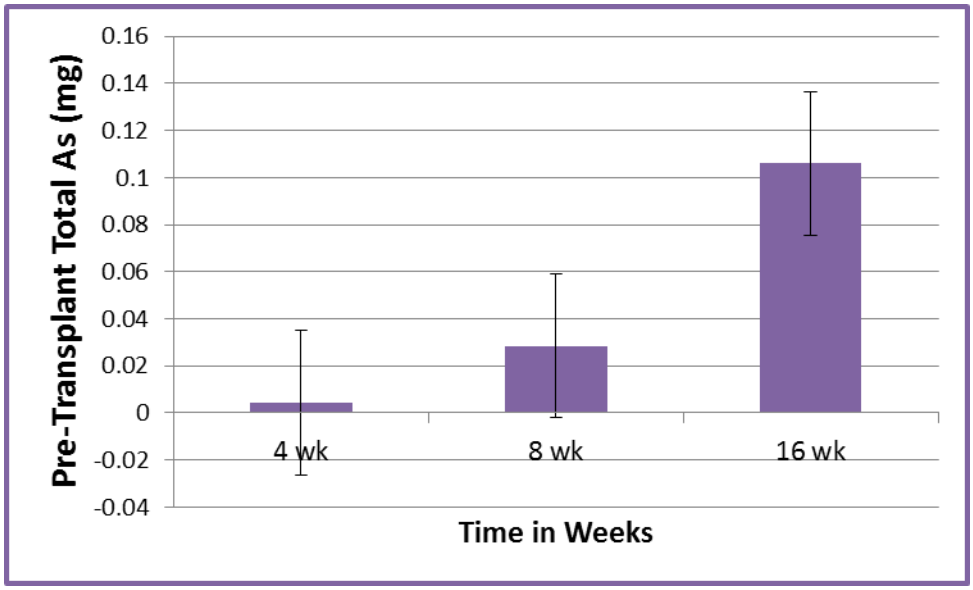

Figure 2. Averaged Pre-Transplant Total Arsenic over Time

The biomass of Pre-Transplant mature fronds at 4 weeks was 0.589 g, 0.647 g, and 0.789 g for Ferns #5, #7, and #12. At 8 weeks, it was 0.687 g, 0.438 g, and 0.398 g for Ferns #2, #9, and #10. At 16 weeks biomass for Ferns #1, #3, and #11 were 0.374 g, 0.523 g, and 0.775 g respectively. This averaged to 0.675 g, 0.508 g, and 0.557 g for 4, 8, and 16 week timepoints (Figure 3). Since these fronds were already mature prior to the experiment, little change occurred and differences were not deemed significant.

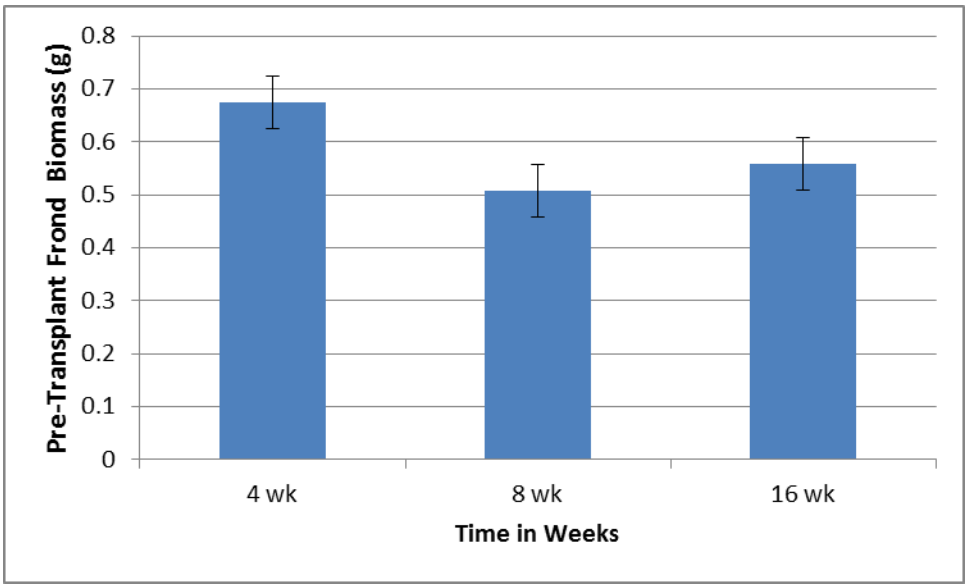

Figure 3. Pre-Transplant Frond Biomass over time in weeks.

Post-Transplant frond data is listed in Table 5. 4 week data is not available due to low biomass at the 4 week timepoint that prevented sampling.

8 week timepoint As concentration in biomass was 712.71 mg/kg, 909.24 mg/kg, and 1620.31 mg/kg for Ferns #4, #6, and #8 respectively. The 8 week temporal replicate for the 12 week timepoint had concentrations of 1733.62 mg/kg, 1262.92 mg/kg, and 4841.35 mg/kg, again for Ferns #4, #6, and #8. The last 8 week temporal replicate at 16 weeks had concentrations of 2764.29 mg/kg, 1814.1 mg/kg, and 4307.5 mg/kg. The 12 week timepoint had concentrations of 831.61 mg/kg, 2107.9 mg/kg, and 1819.61 mg/kg for Ferns #1, #3, and #11. The 12 week temporal replicate at 16 weeks had concentrations of 1808.8 mg/kg, 1437.1 mg/kg, and 3746 mg/kg. Lastly, the 16 week timepoint had concentrations of 543.81 mg/kg, 2446.7 mg/kg, and 2195.2 mg/kg for Ferns 5#, #7, and #12 respectively.

Post-Transplant Total As at the 8 week timepoint was calculated to be 0.14 mg, 0.074, and 0.21 for Ferns #4, #6, and #8 respectively. The 8 week temporal replicate at 12 weeks had total As of 0.36 mg, 0.31, and 1.07 mg. The last temporal replicate had total As of 0.478 mg, 0.235 mg, and 0.788 mg. At the 12 week timepoint, total As was 0.225 mg, 0.563 mg, and 0.575 mg for Ferns #1, #3, and #11. The 12 week temporal replicate at 16 weeks for total As was 0.204 mg, 0.206 mg, and 0.577 mg again for Ferns #1, #3, and #11. At the 16 week timepoint, total As

was 0.195 mg, 1.211 mg, and 0.834 mg for Ferns #5, #7, and #12. The value of 4841.35 mg/kg for Fern #8 in the 8 week temporal replicate at 12 weeks is the highest concentration of As by biomass for all samples. The value of 1.211 mg for Fern #7 at 16 weeks is the highest amount of accumulated total As across all samples. Both values are from the Post-Transplant fronds.

Biomass for Post-Transplant fronds was 0.201 g, 0.081 g, and 0.132 g for Ferns #4, #6, and #8 at the 8 week timepoint. The 8 week temporal replicate at 12 weeks had biomass of 0.208 mg, 0.249 g, and 0.222 g. The last 8 week temporal replicate had biomass of 0.173 g, 0.13 g, and 0.183 g. At the 12 week timepoint, biomass was 0.271 g, 0.267 g, and 0.316 g for Ferns #1, #3, and #11. The 12 week temporal replicate at 16 weeks for biomass was 0.113 g, 0.143 g, and 0.154 g again for Ferns #1, #3, and #11. At the final 16 week timepoint, biomass was 0.358 g, 0.495 g, and 0.38 g for Ferns #5, #7, and #12. Biomass values are significantly smaller than in Pre-Transplant due to the fronds not having reached maturity.

Table 5. Post-Transplant frond data. As data by Brookside Labs.

Sample ID	Frond age	As (mg/kg)	Biomass (g)	Total As (mg)
FERN #4	8 wk	712.7118	0.201	0.143255
FERN #6	8 wk	909.2391	0.081	0.073648
FERN #8	8 wk	1620.312	0.132	0.213881
FERN #1	12 wk	831.6091	0.271	0.225366
FERN #3	12 wk	2107.931	0.267	0.562818
FERN #11	12 wk	1819.607	0.316	0.574996
FERN #4	8 wk	1733.615	0.208	0.360592
FERN #6	8 wk	1262.921	0.249	0.314467
FERN #8	8 wk	4841.345	0.222	1.074779
FERN #5	16 wk	543.8053	0.358	0.194682
FERN #7	16 wk	2446.666	0.495	1.2111
FERN #12	16 wk	2195.192	0.38	0.834173
FERN #1	12 wk	1808.75	0.113	0.204389
FERN #3	12 wk	1437.096	0.143	0.205505
FERN #11	12 wk	3745.999	0.154	0.576884
FERN $4	8 wk	2764.285	0.173	0.478221
FERN #6	8 wk	1814.141	0.13	0.235838
FERN #8	8 wk	4307.534	0.183	0.788279

Post-Transplant fronds show a slight decrease in arsenic concentration by biomass over time (Figure 4). Averaged values are 2218.5 mg/kg As concentration for the 8 week timepoint, 1958.5 mg/kg for 12 week, and 1728.55 mg/kg for 16 week.

However, averaged Total arsenic shows an increase over time, with the highest at 16 weeks. The averaged values are 0.409 mg, 0.392 mg, and 0.747 mg for 8 week, 12 week, and 16 week timepoints. The total As nearly doubles from the 12 week to 16 week time point. The highest amount of averaged Post-Transplant Total arsenic, 0.746 mg at 16 weeks, is about seven times larger than the Pre-Transplant maximum Total arsenic of 0.106 mg at 16 weeks.

Averaged Biomass also increased rapidly over the 16 weeks, from 0.18g at 8 weeks, 0.211 g at 12 weeks, and 0.411g at 16 weeks (Figure 6). This is expected since plants grow over time.

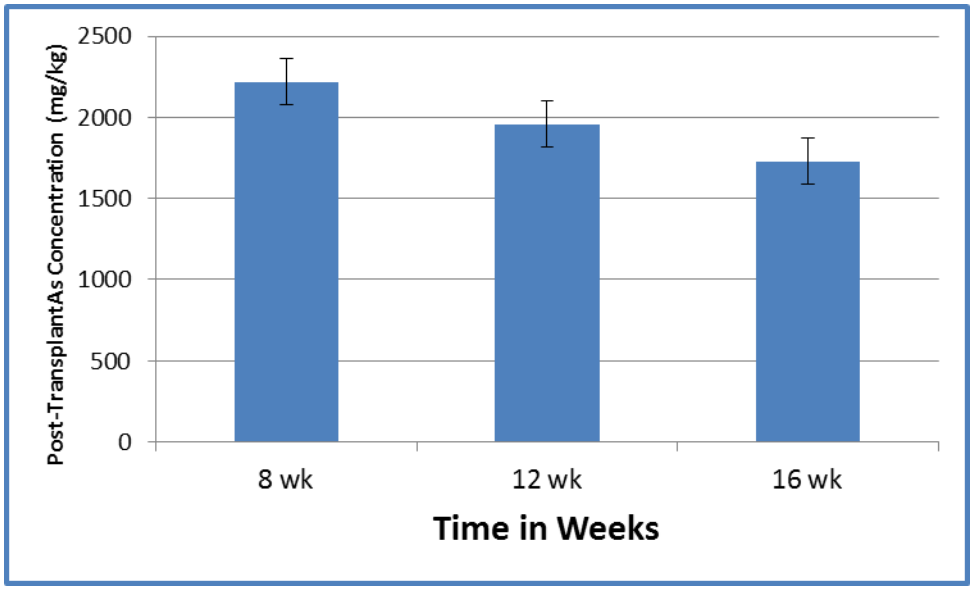

Figure 4. Post-Transplant As concentration by biomass over time.

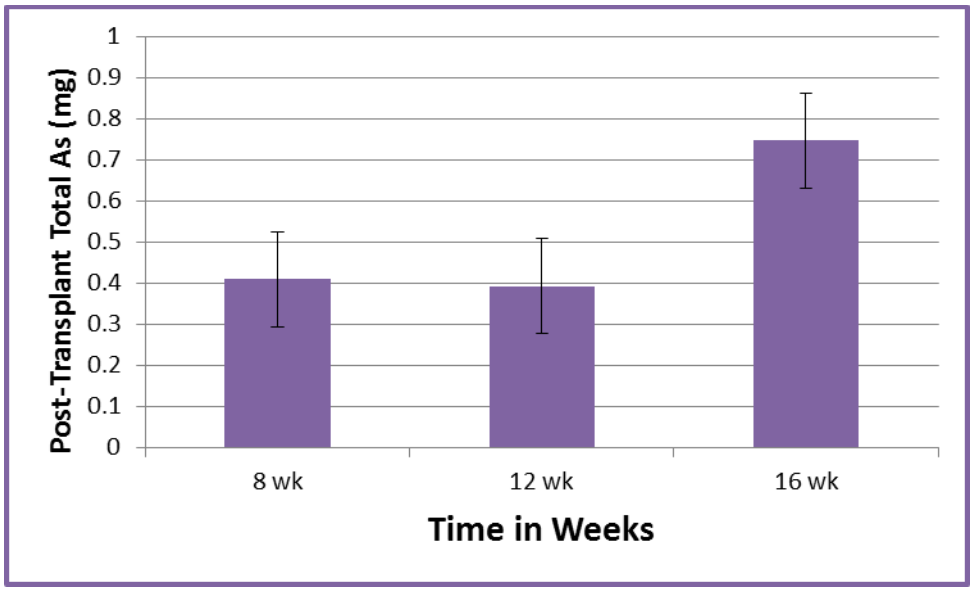

Figure 5. Post-Transplant frond Total Arsenic concentration over time in weeks.

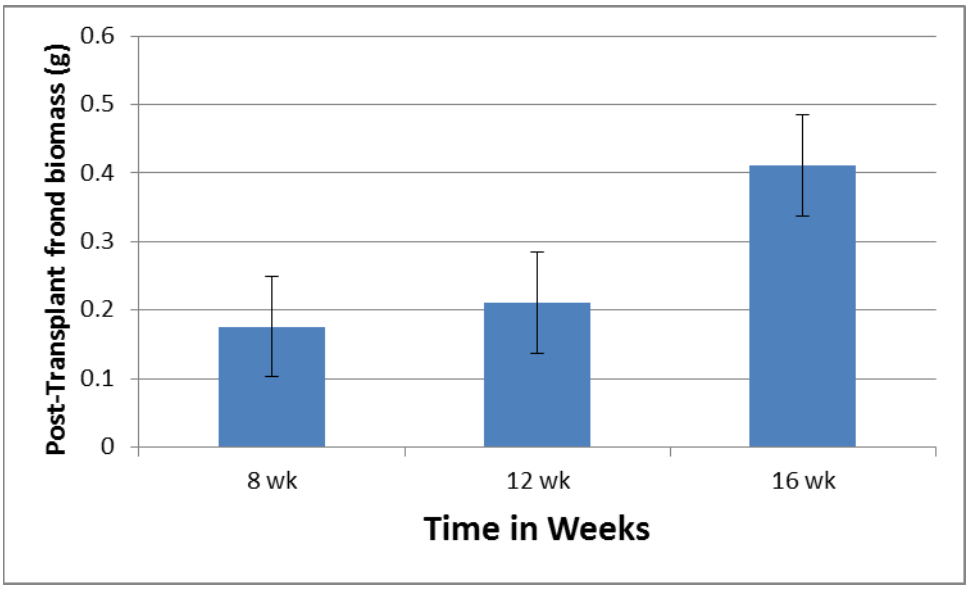

Figure 6. Post-Transplant frond biomass over time.

DISCUSSION

Relationship between arsenic uptake and time

The statistically significant increase in arsenic concentration within sampled ferns over time suggest that with increasing age within our time frame, *P. vittata* is more effective at accumulating arsenic. This correlates with previous findings (Gonzaga et al. 2007), since our time frame is within the youngest stages of frond growth, where metabolic activity and amounts of glutathione, an important antioxidant for detoxifying metals, are highest (Scott et al. 1993). Total arsenic accumulated for both Pre-Transplant and Post-Transplant fronds showed positively increasing and statistically significant trends.

The slight decrease in arsenic concentration by biomass seen in Figure 3 for Post-Transplant fronds may at first suggest new fronds are not as efficient at arsenic uptake over time; however, visualizing the Total arsenic in Figure 4 shows that the increase is in fact very large and positively increasing. The factor of increasing biomass over time may be causing a dilution effect on the data that depicts arsenic concentrations as falling due to rapidly increasing biomass in Post-Transplant fronds. Because biomass is increasing, it appears that less mg of arsenic is accumulated per kg biomass. This observed dilution effect correlates with past findings on *P. vittata* that show evidence for arsenic uptake promoting biomass growth, possibly as a form of defense against the contaminant (Han et al. 2016).

Difference in As concentration between Pre-Transplant and Post-Transplant fronds

In fact, for both arsenic concentration and Total arsenic, Post-Transplant fronds accumulated nearly an order of magnitude higher arsenic at every time interval. Pre-Transplant fronds still showed increasing trends in accumulation, but not nearly as high as the Post-Transplant fronds that emerged after arsenic exposure. This may be due to the rapid metabolic activity and growth of the new fronds, and the slowed growth and metabolic activity of mature fronds nearing senescence (Gonzaga et al, 2007). It is practical to know what fronds to harvest to maximize arsenic removal, and, in experimental work, it is important to know how harvesting a

pre-transplant frond biases early experimental results compared to selecting a post-transplant frond.

Limitations

My study was limited by the short time frame of 4 months for the greenhouse observational experiment and sampling; a majority of phytoextraction projects are done over a long time period of months or years (Kopittke et al. 2010). Therefore it might be not as reflective of long-term phytoremediation conditions of interest and only be representative of a short time span. My study was also inhibited by slow growth of fronds that led to low biomass, which we accommodated by combining fronds from outside the random sample to make the 1 gram analysis requirement. This may have influenced the data and affected the statistical significance of the results and introduced bias. My sample size was also a bit small at 12 ferns; more would have increased the statistical power of my results and given more data points to track accumulation over time. The greenhouse conditions of my study are also very ideal and again not reflective of actual phytoremediation projects done on site, where weather, local environment and disruptions from humans and animals may occur.

Future Directions

Long-term controlled greenhouse or field studies on *P. vittata*'s ability to extract arsenic over time will be beneficial to reflect the long-term conditions of phytoremediation projects to clean contaminated sites. 16 weeks showed maximum accumulation in my project, so future studies should grow ferns beyond that to find any points of inflection in which arsenic accumulation stabilizes or drops to better identify optimal accumulation time. Identifying specific time frames where senescence begins is also an important next step due to the risk of arsenic accumulation being lost as the fern ages (Gonzaga et al. 2007). We also did not analyze soil after the study for changes from the initial arsenic concentration; examining changes post-experiment in soil may give useful insight of the effectiveness of accumulation for phytoremediation. A field site version of my experiment will also give more insight to the "real-

world" conditions and factors that inhibit phytoremediation projects and give light to ways to improve phytoextraction in the face of outside factors.

Conclusions

Younger *P. vittata* ferns may be the best to use for quick and effective phytoremediation of arsenic contaminated sites, as evidenced from the Post-Transplant frond arsenic results. Mature, Pre-Transplant fronds were also shown to accumulate increasing amounts of arsenic over time and can still contribute to phytoextraction of arsenic, just at lower relative amounts to younger fronds. Combinations of mature and young fronds may be most optimal for remediation if exclusively young fern plants are not as available. The findings of this study should be useful in assisting planners to identify the best, youngest ferns and time frames to target remediation efforts for maximum contaminant extraction. In the bigger picture, *P. vittata* may be a viable option in sustainably remediating arsenic contamination of urban soils that can then be repurposed for urban agriculture or other community projects without worry. Larger public health issues of arsenic contamination in water and soils can consider *P. vittata* as a non-invasive, cost-effective tool.

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