The Life and Times of *Pteris vittata*: Investigating Frond Life Stage and Harvest Techniques to Optimize Phytoremediation with an Arsenic Accumulating Fern

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ABSTRACT

Arsenic contamination in the environment is a global environmental health issue. On site (*in-situ*) remediation is an inexpensive, environmentally friendly, and minimally destructive option for cleaning up arsenic-contaminated soil and water. *Pteris vittata* has been widely studied since its discovery as a hyperaccumulator of arsenic, but little is known about the best way to harvest the fern under field conditions. I conducted two greenhouse studies in clean soil, one to observe life stage transition timing and another to determine the effects of different harvest techniques. Based on data from 40 fronds on eight different ferns, I identified the key life stage transition and optimum harvest time as when a frond transitions from mature, spore-releasing to early senescence (vital to prevent leaching of arsenic back into the soil). The cumulative time up to this transition ranged from 18.0 to 41.0 weeks, with an average of 28.7 ± 6.7 weeks. To determine effects of harvest techniques, I created harvest treatments comprised of three variables: harvest interval (12 weeks vs. 26 weeks), frond selection type (all fronds vs. mature and senescing fronds), and harvest height (2 cm vs. 15 cm). This design required eight treatments, with each treatment replicated six times for a total of 48 ferns. I observed a statistically significant difference in biomass (which can be correlated with pollutant removal) in 26-week time interval and mature and senescing frond selection treatments. These treatments should be followed when planning a harvest regime to encourage biomass production, increase arsenic uptake, and reduce arsenic leaching back into the soil.

KEYWORDS

Chinese Brake Fern, hyperaccumulator, bioremediation, phytoextraction, life cycle, harvesting

INTRODUCTION

Arsenic contamination in the environment is a global environmental health issue. The World Health Organization (WHO) has identified arsenic as one of its ten chemicals of major public health concern (WHO 2012). The source of arsenic contamination can either be naturally occurring (from soil parent material) or anthropogenic (typically from pesticides, wood preservatives, or mining) (Environmental Protection Agency (EPA) 2015, Barringer and Reilly 2013). The scale of the problem is global; arsenic contamination can be found in rural areas, industrial sites, and urban centers (Garelick and Jones 2008, Uddin and Huda 2011). Experts have estimated that more than 100 million people worldwide are exposed to arsenic from drinking water alone (Uddin and Huda 2011). In Bangladesh half the population (nearly 80 million) is at risk from drinking arsenic-contaminated water, resulting in over 9,000 deaths in 2010 (International Programme on Chemical Safety (ICPS) 2010). Routes of human exposure to arsenic include consumption of contaminated drinking water, ingestion/inhalation of contaminated soil, or consumption of food grown in contaminated soil or irrigated with contaminated water (American Cancer Society 2014, Groundwater Ambient Monitoring and Assessment (GAMA) Program 2010). Long-term exposure to arsenic can cause skin lesions, developmental effects, neurotoxicity, diabetes, cardiovascular disease, and cancers of the skin, lungs, bladder and kidney (WHO 2012). Due to the widespread nature of the problem, remediation methods that are affordable and sustainable are urgently needed.

In-situ remediation is an inexpensive, environmentally friendly, and minimally destructive option for cleaning up arsenic-contaminated soil and water (Lombi and Hamon 2004). Treating contamination on site minimizes disturbance to local ecosystems and reduces further environmental impacts associated with transportation and disposal of hazardous waste (EPA 2001). Soil is also a finite and non-renewable resource; once destroyed or disposed of (by other remediation methods) it will never be regenerated. Phytoremediation is a term for in-situ remediation techniques that use plants for treating polluted soils or water. This method is less destructive than conventional methods, and can possibly lead to improved soil conditions (Meuser 2013). *Pteris vittata* (Chinese Brake or Ladder Brake Fern) in particular is a promising choice for phytoremediation. Specifically, remediation using *P. vittata* is phytoextraction: the direct uptake of a pollutant and translocation to aboveground biomass (Lombi and Hamon 2004). *Pteris vittata* is hardy and is a hyperaccumulator (a plant that actively takes up exceedingly large amounts of one or more heavy metals from the soil, Rascio and Navari-Izzo 2011) of arsenic. It can concentrate up to 22,000-mg/kg arsenic in its fronds, an amount that would be toxic to most other plants (Ma et al. 2001, Jones 1987). *Pteris vittata* can be grown perennially in contaminated soil and harvested for its arsenic-containing fronds, which can then be disposed of as hazardous waste or recycled for arsenic recovery (EPA 2012). Phytoremediation of arsenic is a potentially useful remediation method because it is accessible to people of all socioeconomic backgrounds and requires little infrastructure or equipment (as opposed to a major undertaking like soil excavation). However, some knowledge gaps regarding the practical application of the fern have limited its use. Little is known about the best way to harvest the fern under field conditions, yet the way the fern is harvested can have a tremendous impact on the amount of arsenic removed over time and the ability of the fern to regenerate for subsequent harvests.

Pteris vittata has been widely studied in hydroponics and greenhouse pot experiments since it was discovered as a hyperaccumulator of arsenic in 1999 (Chen 2002, Ma et al. 2001). In these studies the growing media vary between soil and water; most common harvest intervals are six months and the most common harvest height is 15 cm above the rhizome (a modified underground stem from which the fronds are produced, Jones 1987). Lessl and Ma (2013) stated that a six-month interval was used to coincide with peak frond maturity. On the other hand, Natarajan et al. (2011) suggested letting fronds grow until they were at their maximum arsenic accumulation capacity to minimize disposal costs, as frond harvesting was not found to impact arsenic uptake (Natarajan et al. 2009). A good understanding of harvest intervals (and subsequent biomass retrieval and regrowth) is important because numerous harvests will be necessary to remove enough arsenic from the soil or water (Gonzaga et al. 2008, Salido et al. 2003). The harvest height of 15 cm is based on an experiment performed by Stamps and Rock (2003) in which they investigated effects of harvesting *P. vittata* at heights of 5 cm, 10 cm and 15 cm. They found that increasing height yielded increases in biomass recovery, although the results were never published.

A group of UC Berkeley researchers (led by Sarick Matzen and Céline Pallud) are currently conducting field-scale phytoremediation research using *P. vittata* at the arsenic-contaminated Santa Fe Right of Way (SFROW) field site in Berkeley, CA. Preliminary results at the site show that harvesting fronds by removing only mature and senescing (the growth phase in a plant or plant part (as a leaf) from full maturity to death, Agnes 1999) fronds at 15 cm, while leaving partially emerged fiddleheads and young fronds was not a good strategy because the emerging/young fronds were delicate and were damaged during the harvesting process under field conditions. This careful frond selection and harvesting regime was also labor-intensive and time-consuming. Ultimately, frost and freeze damage caused plants to die back (rendering careful harvesting ineffectual). Further studies are needed to determine optimal harvesting heights and frond selection, as current laboratory practices are not appropriate for field scale harvesting. Further studies are also needed to determine the best harvest intervals to ensure they coincide with the maturation of the *P. vittata* frond in order to avoid senescence, which will allow the arsenic to leach back into the soil.

My research question is: What harvest method will optimize phytoremediation with *P. vittata*? My sub questions are (1) when do *P. vittata* fronds transition from mature to senescing life stages? (2) At what height should fronds be harvested? (3) How should fronds be selected? And (4) at what time interval should subsequent harvesting occur? Based on preliminary results at the SFROW field site, I hypothesized that the optimum harvest method for maximizing biomass and pollutant removal is to harvest all fronds, just above the rhizome, at a longer time interval. I have tested my hypothesis by determining average lengths of time associated with different life stages in the life cycle of *P. vittata* fronds, from fiddlehead through senescence, and determining the effect of harvest interval, age/type of fronds harvested per plant, and height at which fronds are cut, on biomass retrieval over subsequent harvests.

METHODS

I conducted two experiments to test my hypothesis, running over the course of a year (April 2014 to April 2015). Experiment 1 focused on observing the life stages of *P. vittata* and Experiment 2 focused on quantifying the effects of different harvest methods.

Planting and soil sampling

All *P. vittata* ferns were previously subdivided from ferns purchased from Edenspace Systems Corporation (Manhattan, KS) approximately two years prior to the beginning of the experiments. For the life stage observations, I chose ferns that had at least two emerging fiddleheads at approximately 2 cm tall. For the harvesting experiment I chose ferns that were similar in growth (5 – 7 fronds each). I transplanted all ferns into 3-gallon pots with soil dug up from the nearby-uncontaminated Oxford Tract (1.5 acres of open field planting space used by UC Berkeley for research). I used fourteen wheelbarrows full of soil, took a sample from each wheelbarrow (for a total of fourteen samples of soil), and combined them into one composite sample. I sieved the soil to 4 mm, took six subsamples, and shipped three of the subsamples to the Soil and Plant Tissue Testing Laboratory at the University of Massachusetts for nutrient analysis. The other three subsamples were shipped to Brookside Laboratories in New Bremen, Ohio for arsenic analysis.

Growing conditions

After I transplanted the ferns, I distributed them among four benches in the lathe house of the Oxford Tract Greenhouse in Berkeley, CA and assigned randomized IDs to harvest experiment ferns. A lathe house is an open-air room next to the greenhouse, intended to allow seedlings to harden off before being transplanted to a field while being shaded and protected from some wind (Jones 1987). I chose this for the location of my experiment to examine fern performance under semi-controlled outdoor conditions. Berkeley has a Mediterranean climate characterized by hot, dry summers and mild, wet winters, with average temperatures ranging from 5.6 – 23.8 °C (state average is 14.8 °C, National Oceanic and Atmospheric Administration (NOAA) 2015). The greenhouse staff watered the ferns as needed and fertilized the ferns once with 14-14-14 (N-P-K ratio) Osmocote slow release fertilizer, (1 tbsp./ pot) in April 2014. The greenhouse staff sprayed the ferns with DiPel Bt three times (April 24, 2014, July 24, 2014, and March 26, 2015) at the label rate (Bt, *Bacillus thuringiensis* is a soil-dwelling bacterium used as a biological pesticide, Valent BioSciences Corporation 2014). I recorded the light in micromoles at each of the four benches in the lathe house every month using a LI-189 light meter from LI-COR, Inc.

Experiment 1: life stages

To observe the life stages of *P. vittata*, I collected data on frond height (continuous) and frond life stage (categorical) of multiple fronds on eight ferns. I tagged each initial fiddlehead with an ID (two per fern). Fiddleheads that sprouted afterwards were tagged until I reached a total of 40 fronds. I performed weekly observations on the selected fronds, recording the length of the frond and the life stage. To obtain the length, I measured from the base of the stipe to the end of the rachis using a tape measure and recorded the length in cm.

I defined and recorded five life stages based on relevance to field harvesting: fiddlehead, young frond, mature frond pre spore-releasing, mature frond spore releasing (indicated by the presence of spores on a loop of clear packing tape placed on 2 different pinnae), and 5 pinnae senescing (Appendix A: Definitions and pictures). This final stage and the life cycle of the frond as a whole ended when all the pinnae on the frond had senesced.

I chose to mark the key transition between maturity and senescence at 5 pinnae senesced, because I observed that fronds mature from the bottom up. Pinnae at the bottom of the fern mature, release spores, and senesce first, and pinnae at the top of the fern mature, release spores, and senesce last. This pinnae-by-pinnae growth indicated that I could not pinpoint the exact time between the mature and senescing stages, the ideal harvesting point I was looking for. By the time the fronds had fully senesced, many individual pinnae had broken off, which would allow arsenic to leach back into the soil. To account for the possibility of atypical pinnae breakage resulting from my handling, I defined the last stage based on when 5 pinnae had senesced.

Experiment 2: harvest methods

Variables

To observe the effects of subsequent harvests using different harvest methods, I created harvest treatments comprised of three variables: harvest interval (12 weeks vs. 26 weeks), frond selection type (all fronds vs. mature and senescing fronds), and harvest height (2 cm vs. 15 cm). This gave me eight treatments total, with each treatment replicated six times for a total of 48 ferns (Table 1).

Table 1. Harvest Treatments. Three variables with two factors each gave me eight unique treatments. Each treatment was replicated six times.

Harvest height	Frond selection	Time interval
2 cm	All fronds	12 weeks
2 cm	All fronds	26 weeks
15 cm	All fronds	12 weeks
15 cm	All fronds	26 weeks
2 cm	Mature and senescing fronds	12 weeks
2 cm	Mature and senescing fronds	26 weeks
15 cm	Mature and senescing fronds	12 weeks
15 cm	Mature and senescing fronds	26 weeks

Harvest

Before each harvest I counted the fronds on each fern and recorded their life stage. To determine where I would cut the fronds, I marked 2 cm and 15 cm on my garden shears using a measuring tape and Sharpie permanent marker. For the "all" frond treatments, I gathered all fronds on the fern in one hand and cut at the treatment height with the other hand. I put all of the biomass in a paper bag (Uline) marked with the fern ID, harvest ID, and date.

For the "mature and senescing" treatments, I looked at each frond's life stage and cut each mature and senescing frond individually. I made separate sample bags for the mature fronds and the senescing fronds. After each harvest, I dried the biomass in the greenhouse drying room for at least one week at 100 °F. After the biomass dried, I took the ferns to the Pallud lab at UC Berkeley. I labeled 1-gallon Ziploc bags with the fern ID, harvest ID, and date. I placed a 1000 mL plastic beaker on a balance (Denver Instrument model P603) and tared it, weighed the Ziploc bags and recorded the weight in grams. I transferred each biomass sample into a Ziploc bag and weighed the biomass. I subtracted the bag weight from this weight to get the net biomass weight.

Data Analysis

All results were expressed as an average of 8 treatments with 6 replicates each in a completely randomized design. Treatment effects were determined by an analysis of variance (ANOVA) run on a linear regression model with categorical variables (harvest interval, cut height, and frond selection) corrected for error introduced by location within the greenhouse. I used the linear model in R to run the regression. Duncan's New Multiple Range test was used for post hoc comparisons to separate treatment differences. P-values less than 0.05 were accepted as statistically significant.

RESULTS

Soil Analysis

All macro- and micronutrients tested within normal, optimum, or above optimum range and there was no contamination of heavy metals in the soil (Table 2). The averaged soil pH was 5.8, which is normal for agricultural soils like the Oxford Tract but a little low for *P. vittata* growing preferences (Jones 1987).

Table 2. Soil analysis results. Soil analysis was performed at the University of Massachusetts, except for arsenic analysis, which was performed at Brookside Laboratories, Ohio.

			Optimum/ Normal	Sample 1	Sample 2	Sample 3	Mean	Standard Deviation (P)
Soil pH (1:1, H2O)			-	5.7	5.7	6.0	5.8	0.1
Modified Morgan extractable (ppm)	*Macronutrients*	**P**	4 - 14	32.4	32.8	15.4	26.9	8.11
		K	100 - 160	253	254	168	225	40.3
		Ca	1000 - 1500	3329	3312	2279	2973	491.0
		Mg	50 - 120	646	662	410	573	115
		S	>10	34.4	34.2	23.7	30.8	5.00
	Micronutrients	**B**	0.1 - 0.5	0.3	0.3	0.3	0.3	0.0
		Mn	1.1 - 6.3	30.3	31.7	14.3	25.4	7.89
		Zn	1.0 - 7.6	13.9	14.1	8.0	12.0	2.83
		Cu	0.3 - 0.6	0.5	0.5	0.3	0.4	0.09
		Fe	2.7 - 9.4	4.9	5.3	6.1	5.4	0.50
	Heavy metals	**Al**	<75	15	15	27	19	5.7
		Pb	<22	7.9	8.2	5.1	7.1	1.4
		As*	-	6.46	6.95	6.26	6.56	0.290
Cation Exchange Capacity, meq/100g			-	30.9	30.5	21.5	27.6	4.34
Exch. Acidity, meq/100g			-	8.3	7.9	6.4	7.5	0.82
Ca Base Saturation, %			50 - 80	54	54	53	54	0.47
Mg Base Saturation, %			10 - 30	17	18	16	17	0.82
K Base Saturation, %			2.0 - 7.0	2	2	2	2	0
Scoop Density, g/cc			-	1.10	1.03	1.25	1.13	0.0918

*Method 6010B

Life Stage

Each frond grew and matured independently of the other fronds or of the fern as a whole. The fronds started in the fiddlehead stage and grew for 6.1 ± 2.0 weeks (Table 3). After the fiddleheads unfurled, the young fronds grew for 4.7 ± 0.8 weeks. It is in this stage that most fronds reached their maximum height, 46.0 ± 12.5 cm. Fronds spent 3.7 ± 1.2 weeks in the pre spore-releasing stage and 14.2 ± 5.8 weeks in the spore-releasing stage. I did not observe whether spores were actually released during this whole stage, but considered the fronds in this stage until they had at least 5 pinnae senesce. Fronds spent 11.9 ± 5.8 weeks in the 5 pinnae senesced stage, after which their life stages were complete (all pinnae senesced). The cumulative time it took fronds to reach 5 pinnae senesced ranged from 18.0 to 41.0 weeks, with an average of 28.7 ± 6.7 weeks (Figure 1). The averaged total time of all observed life stages was 40.5 ± 6.7 weeks.

Table 3. Time spent in each life stage. Values shown are the minimum, maximum, average values, and standard deviation for the 40 fronds reported in weeks. N=40 fronds on 8 ferns.

Life Stage	Min	Max	Avg	Std Dev (P)
Fiddle head	3.0	11.0	6.1	2.0
Young Frond	3.0	6.0	4.7	0.8
Mature Frond, Pre Spore Releasing	2.0	7.0	3.7	1.2
Mature Frond, Spore Releasing	5.0	24.0	14.2	5.8
5 Pinnae Senesced	0.0	26.0	11.9	5.8
Cumulative time to 5 Pinnae Senesced	18.0	41.0	28.7	6.7
Total in weeks	19.0	51.0	40.5	6.7

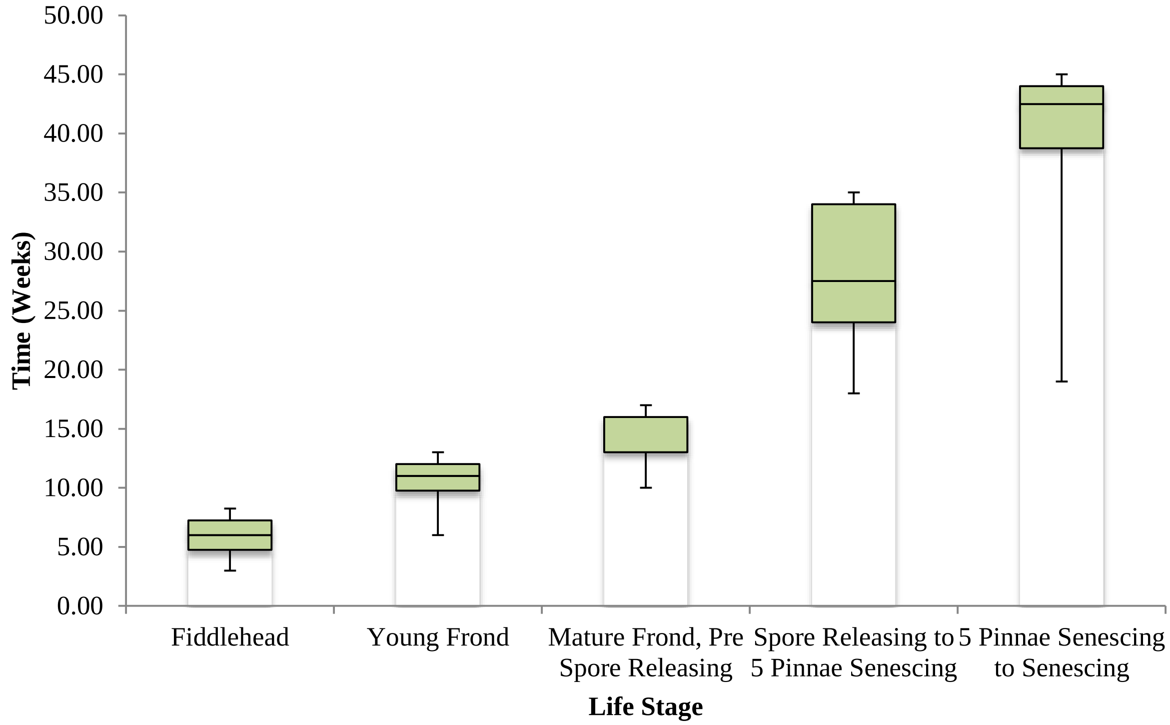

Figure 1. Time spent in each life stage. The chart reflects the cumulative time spent in each life stage for the 40 fronds reported in weeks. N=40 fronds on 8 ferns.

Harvest

For Height, there was not a significant difference in biomass harvested between the 15 cm and 2 cm treatments (Figure 2). For Frond Selection, significantly more biomass was harvested from the "mature and senescing" treatments than the "all" treatments (Figure 3). For Harvest Interval, significantly more biomass was harvested from the 26-week treatments than the 12-week treatments (Figure 4). Additionally, there was a statistically significant difference in the biomass harvested according to the physical location in the lathe house (different locations received different amounts of natural sunlight). Overall, the 15cm/mature and senescing/26 week treatment provided the most biomass ("15cm/MS/26weeks", Figure 5). However, there were not any significant differences based on interactions between the treatments. Significance was determined by p-values less than 0.05.

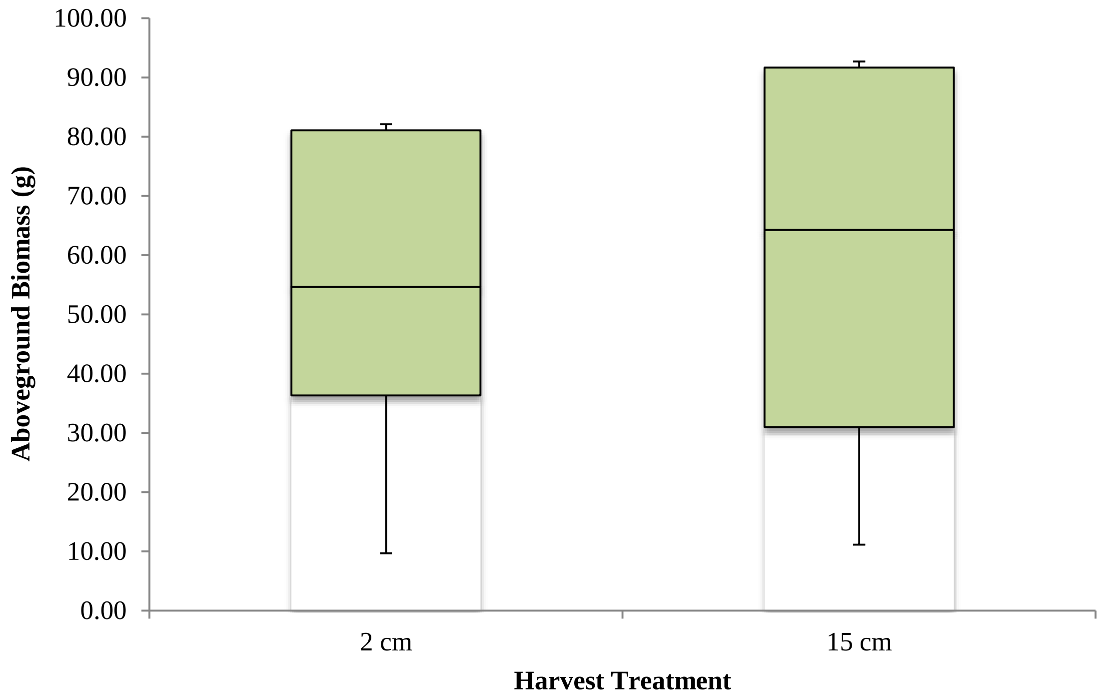

Figure 2. Frond cut height. Total biomass for all harvests, separated by cut height (2 cm vs. 15 cm). There was no statistically significant difference in the biomass harvested from either treatment.

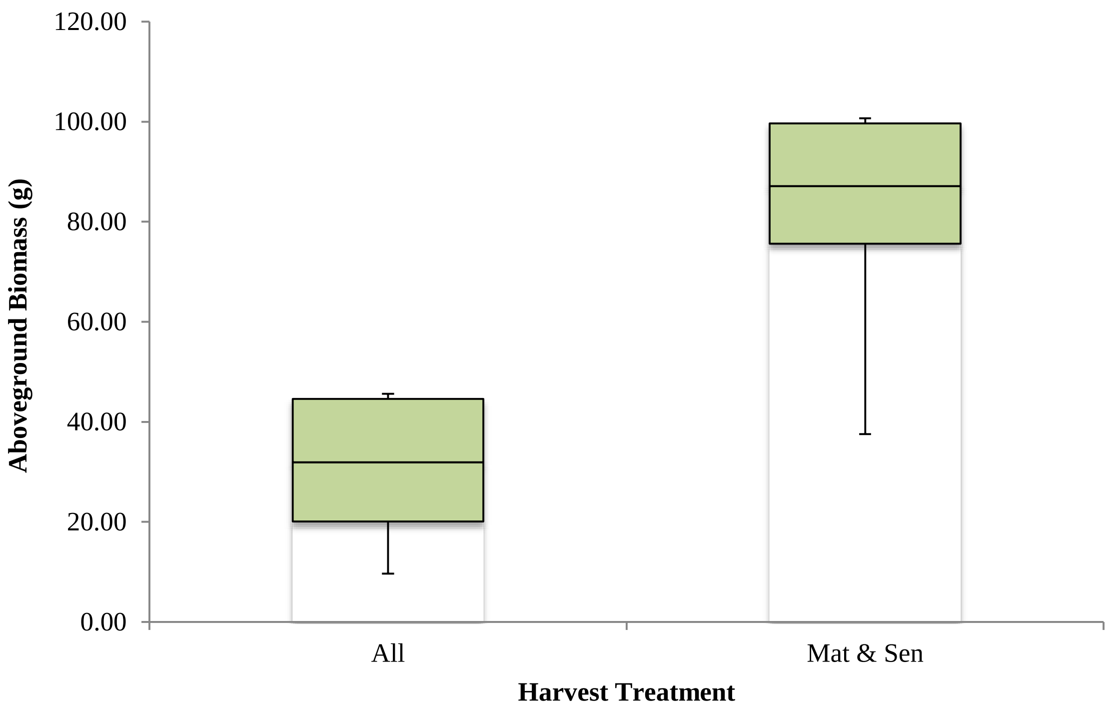

Figure 3. Frond selection. Total biomass for all harvests, separated by frond selection (all vs. mature and senescing). The Mat & Sen treatments produced significantly more biomass that the All treatments (p = 0).

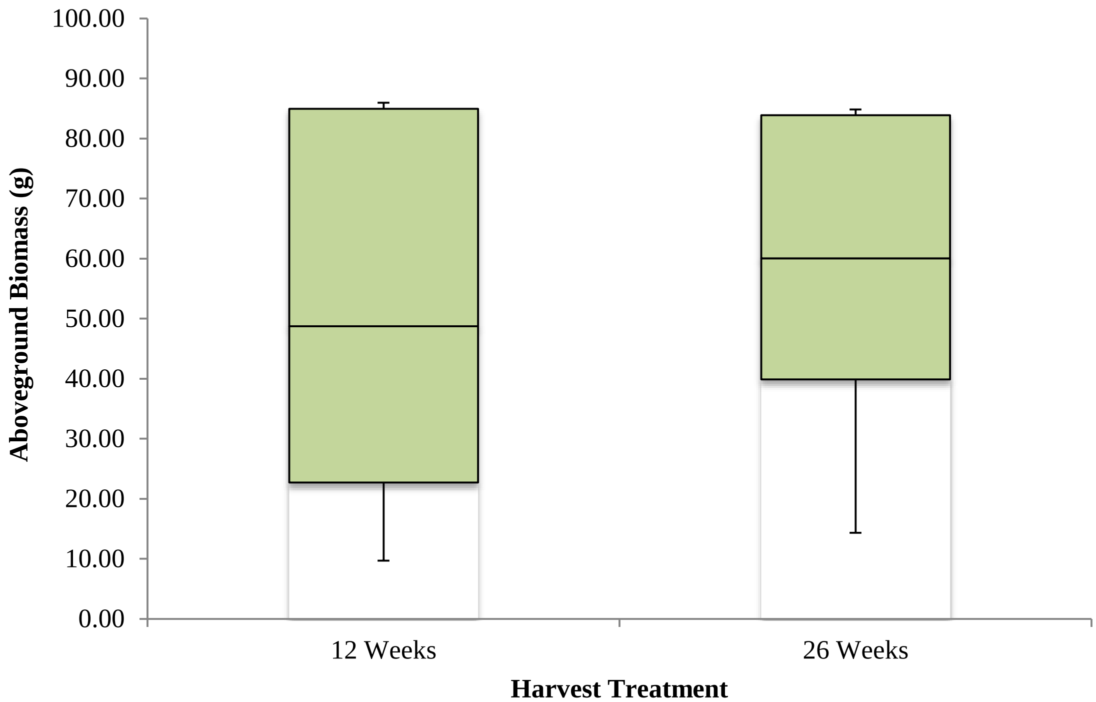

Figure 4. Frond harvest interval. Total biomass for all harvests, separated by harvest interval (12 weeks vs. 26 weeks). The 26-week treatments produced significantly more biomass than the 12-week treatments (p = 0.001).

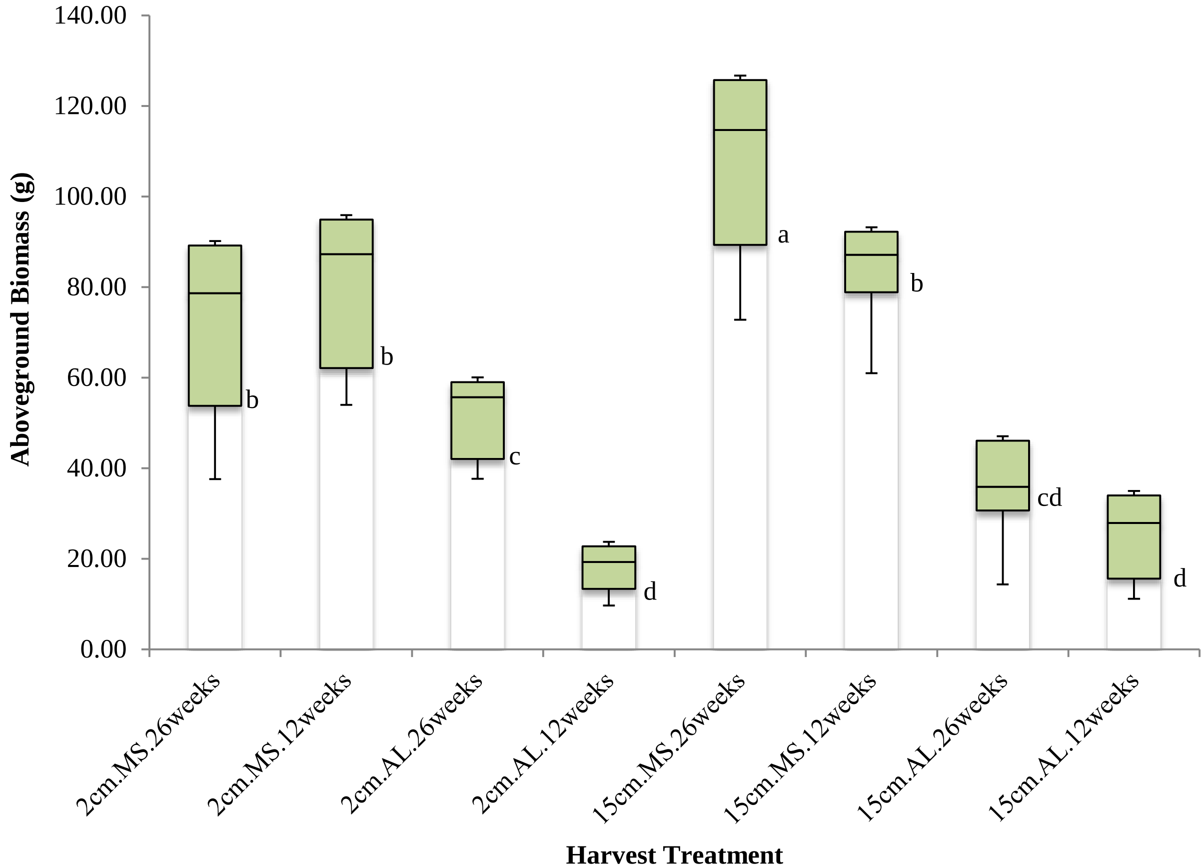

Figure 5. All treatments. Total biomass for all harvests, separated by each unique treatment. Means with the same letter are not significantly different.

Additionally, I compiled data on how the biomass retrieved from each harvest compared to the previous harvest (Table 4).

Table 4. Change in Biomass over Subsequent Harvests. Percent change in biomass retrieved from each harvest to the next.

	Change, Harvest 1 to Harvest 2	Change, Harvest 2 to Harvest 3	Change, Harvest 3 to Harvest 4
Overall Biomass	148.83%	-35.90%	-12.17%
2 cm	100.04%	-57.92%	-8.67%
15 cm	197.62%	-13.88%	-15.09%
All	-23.26%	-64.69%	-60.23%
Mat & Sen	320.92%	-7.11%	27.87%
12 Week	242.73%	n/a	n/a
26 Week	54.93%	n/a	n/a

DISCUSSION

The amount of arsenic removed from a site through phytoextraction is related to both the biomass produced by the fern and the biomass removed from the site through harvesting (Fayiga and Ma 2005, Gonzaga et al. 2008). Harvesting can affect the amount of biomass produced for subsequent harvests, which are necessary for many contaminated sites. If soil is managed and ferns are harvested carefully, arsenic removal and remediation efficiency can increase over time and subsequent harvests (Lessl and Ma 2013). If larger ferns accumulate more arsenic, it is important to identify harvest strategies that maximize biomass retrieval from *P. vittata* in order to maximize removal of arsenic from contaminated media. In this study, I separated harvest strategy into three components: time interval, cut height, and frond selection. I identified the length of time it takes fronds of *P. vittata* to get to optimum cutting age and found significant differences in biomass retrieval depending on the harvest strategy used. These results suggest a harvest strategy of selecting mature and senescing fronds, with subsequent harvests occurring at a longer time interval (28.7 ± 6.7 weeks to avoid senescence) will maximize biomass/pollutant retrieval while minimizing time and effort.

Life Stages and Timing

I observed that pinnae of ferns mature and grow from the bottom up, which agrees with the findings of Lombi et al. (2002). This bottom-up growth means that timing of harvests cannot wait until the fronds are fully senesced, because by this time pinnae may have fallen and arsenic will have leached back into the soil through the decomposing biomass (Lombi et al. 2002). I identified the point of beginning senescence to occur on average at 28.7 ± 6.7 weeks. These results suggest that ideal harvest intervals occur after 22 to 35.5 weeks, in order to maximize the removal of arsenic and avoid leaching of arsenic back into the soil. Other timing considerations should include local climate, as ferns may die back upon exposure to frost.

Harvest Height

The 15 cm harvest height treatments produced a slightly higher amount of biomass than the 2 cm harvest height treatments (1546 total g vs. 1366 total g). However, these results are not statistically significant ($p > 0.05$). Stamps and Rock (2003) visually observed that the higher cut height (15 cm) resulted in faster fern recovery. Gonzaga et al. (2008) also found that "plant biomass… was generally better" when harvesting at a higher cut height (15 cm). The cut height should also take into account where the lower pinnae occur as the lowest pinnae also contain the greatest amount of arsenic (Lombi et al. 2002), and the cut stipe and any pinnae left on it will die soon after harvest. In my harvesting I did not notice a significant amount of pinnae left on the stipe when I made cuts at 15 cm. These observations support the conclusion that a higher cut height should be used to support fern recovery and biomass, as long as the cut height does not leave too many pinnae behind (15 cm is okay).

Harvest Frond Selection

Frond selection is a harvest factor that determines whether harvest could be done mechanically or manually. Selecting only mature and senescing fronds is a more selective treatment and can only be performed manually. This type of treatment is very time and labor intensive, requiring one to kneel around the field, check every frond and make a careful cut that does not harm the fragile young fiddleheads. I found that the treatments that selected only mature and senescing fronds produced significantly ($p < 0.05$) more biomass than the treatments that selected all fronds (2099 total g vs. 813 total g). These results agree with other studies that found that harvesting all fronds made it difficult for plants to regenerate (Gonzaga et al. 2008, Raj and Singh 2015) or hinders plant regrowth due to a lack of photosynthesis (Lessl and Ma 2013). After three harvests of selecting senescing fronds and cutting at ground level, Kertulis-Tartar et al. (2006) left fiddleheads and 1 – 2 live fronds on ferns to help facilitate survival of the ferns during the winter. Although study results indicate that the mature and senescing treatment is preferable in terms of biomass, regrowth in the ferns indicates that selecting all fronds for harvest is possible if needed. These results imply that mechanized harvest is possible for *P. vittata* with respect to frond selection. But, if a cleanup were done on a small scale that can support careful frond selection, fern biomass and regrowth would be much higher.

Harvest Time Interval

Determining the timing of harvests is an important management decision in phytoremediation because we must manage for maximizing the amount of contaminant removed with minimizing contaminant returns to the soil. By maximizing arsenic removal through careful timing of harvests, the length of time required for site remediation is therefore minimized (Kertulis-Tartar et al. 2006). We know that the ideal harvest interval is approximately 29 weeks from the life stage observations. This is further substantiated by the statistically significant differences seen in the time interval treatments of the harvest experiment, where I saw the 26-week treatment ferns produced more biomass than the 12-week ferns (1652 total g vs. 1260 total g). I also observed that harvesting too often can cause detrimental effects to the fern. The ferns in the 12-week treatment, when coupled with cutting all fronds at 2 cm, were most likely to die off completely. When the ferns did have regrowth, the fronds were stunted in size and did not seem to produce as many sori as ferns in other treatments (produced more infertile fronds). These results and observations support the conclusion that a longer time interval between harvests is preferable for *P. vittata*.

Unlike Lessl and Ma (2013), I found that harvested biomass did not always increase over subsequent harvests (Table 4). These results are not uncommon and agree with most studies in which subsequent harvesting occurred (Mandal et al. 2012).

I originally hypothesized that the optimum harvest method for maximizing biomass and pollutant removal is to harvest all fronds, just above the rhizome, at a longer time interval. I was correct about the time interval, but incorrect about the frond selection (and height does not make a big difference), and ultimately answering my research question: What harvest method will optimize phytoremediation with *P. vittata*?

Limitations and Future Directions

Although a 26-week/mature and senescing fronds only approach is promising because of its ability to maximize biomass while still encouraging growth, these *P. vittata* ferns were grown in clean, non-arsenic-contaminated media. Studies have shown differences in fern biomass growth between clean and uncontaminated media (Ma et al. found biomass increases in arsenic-contaminated soil, 2001), but we do not know if the presence of arsenic could affect the timing of life stages, or how arsenic concentrations change over the frond life stages. This study was only performed over the course of a year, but field remediation will most likely require years of harvests (Salido et al. 2003). In addition, depending on one's budget or time frame for remediation, there are other factors to consider like time and labor-intensiveness, or the number of ferns used and their spacing. Continued research and field use of *P. vittata* should be considered to address these limitations and explore further opportunities in making phytoremediation a more viable tool.

Broader Implications and Conclusion

Each phytoremediation project has unique conditions to take into account when planning a harvest regimen. Each treatment variable has a winner and loser in terms of biomass retrieval; in this study I found that the mature and senescing/26-week treatments produced a statistically significant difference in biomass retrieval. This result implies that harvesting *P. vittata* by selecting mature and senescing fronds (leaving fiddleheads and young fronds to grow) and choosing a greater harvest time interval (around 29 weeks to avoid leaching arsenic back into the soil) will produce the most biomass and subsequently remove the most arsenic, in the least amount of time. The fact that the interaction of harvest variables did not produce significant results implies that if a contaminated site cannot follow one the more stringent regimes (harvesting only older fronds), then it can be compensated for in other harvest variables like cut height or time interval without significant losses to biomass or regrowth.

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APPENDIX A: Definitions and pictures

Figure A1. Fiddleheads. New fronds, which emerge tightly coiled. Also called croziers.

Figure A2. Young fronds. Fronds that have unfurled from a fiddlehead, but does not yet have mature sori. Immature sori are small and pale green.

Figure A3. Mature fronds. Fronds that have matured sori (enlarged and darkened to a brown color). A loop of clear packing tape indicates whether the pinnae are releasing spores.

Figure A4. Senescing. The process of aging, characterized by browning and drying of the pinnae.