OPTIMIZING THE USE OF ARSENIC-HYPERACCUMULATING FERNS FOR TREATMENT OF ARSENIC-CONTAMINATED WATER

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Water supplies in many regions of the world are contaminated by the carcinogenic metalloid arsenic (As). Though the U.S. Environmental Protection Agency (EPA) recently reduced the Maximum Contaminant Level (MCL) from 50 μg/L to 10 μg/L, water in some developing countries exceed this standard. Therefore, highly efficient, sustainable technologies that require low maintenance and have low capital costs must be considered.

Several *Pteris* fern species were recently discovered to be effective at removing As from soil and water. This study was designed to develop an understanding of the key operating parameters that would form the basis for an actual treatment process in regions where As in water exceeds the 50 μg/L limit.

This study determined that aeration facilitates greater removal efficiency of As by *Pteris* ferns in water. Following 72 hours of exposure, *Pteris* ferns demonstrated 96 ± 2% removal efficiency with aeration and 84 ± 7% without. However, the benefits of aeration can be compensated by extending the contact time to eliminate the energy cost associated with aeration.

A bench-scale experiment with 11 *Pteris* ferns in a batch reactor filled showed that the removal of As occurs much faster and to a greater extent when compared to individual plants. Individual plants were not always able to reduce the As concentration from 300 ppb to less than 50 ppb in a 4-day period. However, the batch reactor filled with multiple plants was capable of removing As concentration from 275 ± 25 ppb to less than 50 ppb in a 3-day period, even after four cycles of repeated exposure.

Because As can be adsorbed to hydrous ferrous oxides, co-precipitation and settling can aid As removal. When *Pteris* ferns were exposed to 250 ppb As in the presence of Fe (II) = 2.7 mg/L, they removed the As content to less than 50 ppb in a 24-hr period. In the absence of

Pteris ferns, the As content could not be reduced below 100 ppb in a 4-day period. Therefore, the presence of iron can significantly enhance the removal efficiency of *Pteris* ferns.

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INTRODUCTION

Safe drinking water is the world's greatest human health need and is rapidly becoming one of its scarcest resources. Therefore, innovative and sustainable means of providing safe water is a critical issue demanding the attention of the global science and engineering community. These needs are most prevalent in the developing world where an estimated global population of 1.1 Billion lacks access to improved water sources, which represents 17% of the world [World Health Organization, 2002]. Unsafe water supplies are most commonly compromised by bacterial contamination related to poor sanitation; however, chemical contaminants are becoming a more recognized problem as well.

One such example arose in the 1970s and 1980s when the Bangladeshi government partnered with international aid agencies to clean up the bacteria-contaminated surface waters that were causing children to die of diarrhea. To remedy this problem, 10 million tubewells were dug throughout the nation. At the same time, the British Geological Survey (BGS) conducted extensive tests of these tubewell water supplies, declaring them safe, neglecting to measure arsenic levels despite the advice of the World Health Organization (WHO). As early as 1983, dermatologist Kshitish C. Saha of the School of Tropical Medicine identified patients having lesions and linked them to arsenic poisoning. This finding was later confirmed when the Department of Public Health Engineering in Bangladesh discovered arsenic in the tubewell water supply and found that at least 35 million people have potentially been drinking fatal amounts of arsenic from their water supply. The WHO later confirmed that more than 95% of the 120 million Bangladesh population drink well water and more than 50 million people have been affected by As contamination.

The effects of arsenic toxicity include skin lesions, infections, neural problems, organ failure, and cancer. While the death toll from arsenic toxicity rises, scientists, engineers, and development specialists are trying to find the most cost effective ways for accurately measuring

the presence of arsenic in water and removing the arsenic from the water supply [Chowdury, 2004]. Ground waters that are well known to have high As levels can also be found in Argentina, Chile, Mexico, China, Hungary, West Bengal (India) and Vietnam [Smedley and Kinniburgh, 2002].

1.0 LITERATURE REVIEW

The most widely recognized methods for removing arsenic from contaminated water supplies include coagulation and co-precipitation with ferric chloride and alum or granular media filtration using various coated materials to accumulate arsenic. The unfortunate fate of the above mentioned materials is that they end up in chemical sludges produced by coagulation, filtration and activated alumina systems. Aside from the hazardous waste handling issues associated with spent media, the affordability of this approach impacts the feasibility of its practical use in the developing world. Table 1.1 lists the best available technologies for arsenic removal in drinking water.

Table 1.1 Best Available Technologies for Arsenic Removal [USEPA, 2000]

Factors	Ion Exchange	Activated Alumina	Reverse Osmosis	Enhanced Lime Softening	Enhanced (conventional) coagulation filtration	Oxidation Filtration	Phytofiltration
Removal Efficiency	95%	95%	>95%	90%	95% (w/ FeCl3) <90% (w/ Alum)	50-90%	>95%
Total Water Loss	1-2%	1-2%	15-75%	0%	0%	1-2%	3-5% at high flow rates
Pre-oxidation required	Yes	Yes	Likely	Yes	Yes	Yes	No
Optimal pH	6.5-9	5.5-8.3	N/A	10.5-11	5.5-8.5	5.5-8.5	4.0-10.0
Operation skill required	High	Low	Medium	High	High	Medium	Low
Waste generated[a]	1,2,3	1,3	4	3,5	3,5	3,5	6
Other considerations[b]	1,2,3,4	1,2,3	5	2	1,2	None	6
Centralized Cost	Medium	Medium	High	Low	Low	Medium	Low

[a] Waste Generated Code: 1 = spent media, 2 = spent brine, 3 = backwash water, 4 = reject water, 5 = sludge, 6 = fern biomass.

[b] Other Considerations Code: 1 = possible pre pH adjustment, 2 = possible post pH adjustment, 3 = pre filtration required, 4 = potentially hazardous brine waste, 5 = high water loss, 6 = possible post filtration and disinfection

Table 1.1 shows that phytofiltration is an effective and affordable alternative for metal remediation with high removal efficiency, a broad range of application, and minimal waste generation. By using plants to remediate water, both capital and operation and maintenance costs are relatively low, while providing an aesthetically appealing alternative to chemical based methods. The EPA projects that the long term costs of phytofiltration as a method of arsenic removal is more affordable than a conventional activated alumina system, as compared in Table 1.2.

Table 1.2 Cost Comparison of As removal by activated alumina to phytofiltration, assuming design flow of 600,000 LPD [USEPA, 2000]

Cost Parameter	Activated Alumina	Phytofiltration
Capital	$92,700	$119,500
Annual O & M	$34,300	$15, 300
Annual Waste Disposal	$1,200	$100
Year 1 costs	$128,200	$134,900
Years 1-3 costs	$199,200	$165,700
Years 1-5 costs	$270,200	$196,500
Years 1-10 costs	$447,700	$273,500

Previous attempts at phytoremediation for arsenic removal from groundwater made use of poplar trees. However, over 90% of the trees died because of arsenic toxicity [Schnoor, 1997]. A more favorable option would be a plant species that is able to survive in arsenic conditions, remove arsenic from aqueous solution, and be suitable for future reuse. These characteristics have been identified within the last few years. Promising findings have been reported about "the first known arsenic hyperaccumulator, *Pteris vittata*, commonly known as Chinese Brake fern" [Tu et al., 2004]. *Pteris cretica* cv. Mayii, also known as the Cretan brake or Table Fern, has received attention with similar notoriety [Huang et al., 2004].

The perennial nature of these ferns allows them to be reused once new fronds are allowed to grow after mature fronds are removed. However, as stated by Huang et al [2004), "…technical details still need to be finalized prior to field application of the arsenic phytofiltration technology for drinking water."

As of May 2005, no engineering or technical evaluation and review had been conducted evaluating the implementation of an actual design to make use of the plant's natural remediative qualities for a practical water treatment system. The scope of this project was to conduct an engineering analysis for the potential use of arsenic hyperaccumulating ferns as a water treatment system design component. The most practical use for such a system would be in the

developing world for small-scale applications in communities that rely on untreated water or point-of-use water treatment practice.

1.1 DISCOVERING THE FERNS

At an abandoned wood preservation site in Central Florida, a superb arsenic hyperaccumulating plant has emerged from the chromated copper arsenate soils in which it dwelled. During the course of an exploratory research project, soil and plant samples from a contaminated site were analyzed using graphite furnace absorption spectroscopy (GF-AAS). Amongst the 14 plant species analyzed, the *Pteris vittata* (Chinese Brake fern) species, was discovered to contain significantly higher concentrations of arsenic [Ma et al., 2001]. At the contaminated site, the Brake fern contained 1,440 - 7,520 mg/kg of arsenic while, in the uncontaminated site, the Brake fern contained 12 - 64 mg/kg of arsenic. This level is still higher than plants growing in uncontaminated soils, usually containing less than 3.6 mg/kg [Ma et al., 2001].

Pteris vittata is tolerant of soil conditions containing up to 1500 mg/kg and effectively translocate arsenic from the roots to its fronds [Ma et al., 2001]. When a Brake fern was planted in soil spiked with 1500 mg/kg of arsenic, the arsenic concentration in the fronds rose from 29 to 15,860 mg/kg in only two weeks. When placed in soil containing 6 mg/kg, another Brake fern accumulated 755 mg/kg in the same two-week period. The arsenic concentration in the roots was significantly lower than in the fronds. While root arsenic concentrations never exceeded 303 mg/kg, the frond concentration reached 7,230 mg/kg [Ma et al., 2001].

1.2 PHYTOREMEDIATION

Phytoremediation describes the use of plants to remove unwanted substances from water or soil and this can be accomplished in a variety of ways. In some cases, a plant plays host to symbiotic bacteria that facilitate the conversion of a contaminant to its less toxic form. In other cases, a contaminant is sequestered by adsorption to its root surface, translocated and volatized for release into the air or stored in its upper biomass. The method of phytoremediation in the *Pteris vittata* species is phytoextraction, where the plant extracts a pollutant from the surrounding medium and accumulates it in a harvestable portion of the plant. Phytoextraction is one of the phytoremediative methods with more long-term remediative potential due to its ability to sequester a contaminant, thus effectively removing it from the environment.

1.3 *PTERIS VITTATA* (CHINESE BRAKE FERN)

The Brake fern is mesophytic; it adapts to a moderately moist environment. Though considered invasive by the United States Department of Agriculture, it is known to grow in southern California and in the southeast of the United States. The brake fern is versatile, and resilient, preferring sunny alkaline environments. Some of the features that uniquely set this fern apart as an effective phytoremediator are its ability to grow rapidly, large quantities of biomass, easy propagation, and its perennial nature. The other features it possesses are common amongst metal/metalloid hyperaccumulating plants: an efficient root uptake system, an efficient root to shoot translocation, and much-enhanced tolerance to As inside plant cells [Wang et al., 2002].

Pteris Vittata is not the only known As hyperaccumulator [Zhao et al., 2002]. By conducting experiments with a variety of fern species, it has been concluded that several other *Pteris* plant species are capable hyperaccumulators: *Pteris cretica, Pteris longifolia, Pteris umbrosa, Pteris multifada*, and *Pteris oshimensis* [Zhao et al., 2002; Wang et al., 2006]. One fern that is not of the *Pteris* species that has also demonstrated arsenic hyperaccumulating

capacity is *Pitoyrogramma calomelanos*, also known as the Silverback Fern [Visoottiviseth et al., 2002]. As was the case for the Brake fern, the other species were identified by taking a survey of plant species found at sites contaminated with arsenic compounds. To classify the aforementioned ferns as hyperaccumulators, species must exhibit a bioconcentration factor (BCF), which represents the ratio of arsenic in the plant to arsenic in the surrounding soil, above 10. In addition, they should have a high frond to root concentration factor, also known as a translocation factor (TF) [Zhao et al., 2002]. BCF and TF have been used to qualify *Pteris vittata* over the others as the most effective As hyperaccumulator [Tu and Ma, 2002; Fayiga et al., 2005].

It has been suggested that *Pteris* ferns developed their ability to tolerate high levels of arsenic by its evolution around subaerial hot springs that may have contained high arsenic levels. Therefore, these early terrestrial plants learned to survive in their surroundings by accumulating large quantities of arsenic [Meharg and Hartley-Whitaker, 2002]. The theory of adapting to arsenic rich environments was also asserted by Rice et al. [1995].

1.4 ARSENIC REMOVAL BY *PTERIS VITTATA*

The mechanism of As hyperaccumulation is of great interest because, to most plants, inorganic arsenic species are very toxic. As hyperaccumulation is a trait inherent to *Pteris vittata* because of the similarities in hyperaccumulative behavior noted when comparing ferns from As-contaminated soils or from uncontaminated soils [Wang et al., 2002]. Being a nonessential element for plants, arsenic can prove to be fatal at high concentrations due to interference with metabolic processes for most plants. At low concentrations, arsenic appears to be taken up along with other nutrients, because greater concentrations of arsenic are observed in young fronds. This same pattern of localization is correlated with nutrient uptake because nutrients are preferentially directed to actively growing parts of the plants [Mengel and Kirkby, 1987]. Kabata-Pendias and Pendias [1991] reported a linear relationship between arsenic content of vegetation and soil

arsenic concentrations, suggesting that arsenic is passively removed with water flow. However, a different mechanism may describe the method of uptake at higher soil concentrations [Tu and Ma, 2002].

The level of As concentration that *Pteris vittata* is exposed to has an effect on its growth. For As soil concentrations between 50 and 100 mg/kg, the biomass of the *Pteris vittata* significantly increased. When soil As concentrations exceeding 500 mg/kg, plant biomass was significantly decreased. This indicates that a soil As concentration above 500 mg/kg has a toxic effect versus the enhancing effect noted at lower concentrations. No significant difference was noted between adding arsenic in the form of arsenite or arsenate. This may be due to the conversion of arsenite to arsenate in soil by oxidation [Smith et al., 1998].

It is important to note that while arsenic is a non-essential element for plants, phosphorus (P) is necessary for normal plant growth. P deficiency may severely increase the As uptake but this condition is short-lived as some amount of P is necessary for plant survival and to alleviate the effects of arsenate toxicity, especially at high levels of As. P improves overall plant health and growth as it is a constituent of plant macromolecular structures. A molar ratio of P/As of 12 is recommended to protect plants against arsenate toxicity [Walsh and Keeney, 1975]. In cases where the addition of high levels of arsenate enhanced biomass growth, adding phosphate produced the same result. The addition of phosphate in varying rates did not affect P concentration in the fern which suggests that while *Pteris vittata* is a verified As hyperaccumulator, it is not a P hyperaccumulator. For normal fern growth in soil, the P/As molar ratio is recommended to be at least 1.0 [Tu and Ma, 2002].

To understand the mechanism of As uptake noted *Pteris vittata*, experiments with varying ratios of phosphate and arsenic were conducted. Because As and phophorus (P) are both Group V_A elements with similar electron configurations and chemical properties, arsenate and phosphate often compete for sorption sites in soil and compete for uptake by plants. Arsenate can temporarily replace phosphate in ATP synthesis or in phosphorolysis reactions, but it will ultimately interfere with phosphate metabolism causing toxicity in the plant because arsenate is incapable of fulfilling the role that phosphate plays in energy transfer [Tu and Ma, 2002]. When adding *Pteris vittata* to soils with increasing phosphate concentration, As concentrations in both the roots and shoots decreased significantly. This effect was greater for root As concentrations than in the shoots. In a treatment that contained 83 μM of arsenate, increasing phosphate from

20 to 100 μM decreased the root and shoot As concentrations by 76% and 46% respectively. Similarly, increasing arsenate concentration in the solution significantly decreased the concentration of phosphate in the roots but little effect on the concentration in the shoots was observed.

It has been observed that phosphorus (P) deficiency increases the capacity of plant roots to uptake phosphate. Similarly, when *Pteris vittata* is nutritionally deprived of P for an 8-day period, the net arsenate influx increased 2.5-fold. In the absence of P, additional P transporter molecules are synthesized in the plasma membranes of root cells [Wang et al., 2002]. This suggests that the same transporters that allow for phosphate to be removed in greater capacity following P deficiency, also facilitate the removal of arsenate with greater efficiency. Arsenite influx was only a tenth of the rate of arsenate influx and was not affected by the presence or absence of P or its transporter molecules. Therefore, the mode of arsenite removal is significantly different from the mode of arsenate removal [Wang et al., 2002].

1.5 ROOT EXUDATES

Root exudates contain key constituents that make metals and metalloids more soluble for root absorption. Root exudates are metabolites that are released to the root surface or rhizosphere for the enhancement of nutrient uptake by acidification, complexation, chelation, reduction and/or oxidation [Marschner, 1995]. They are composed of mucilage and ectoenzymes, having both high molecular weights (HMW) or low molecular weights (LMW). Mucilages are primarily made of polysaccharides and polyuronic acid. Ectoenzymes are primarily made of organic acids, sugars, phenols, and amino acids [Tu et al., 2003]. Root exudates generally vary among plants and depend on the plant's biological composition and the surrounding environment in which it dwells.

To understand the role of root exudates in arsenic hyperaccumulation by *Pteris vittata* fern, a study was conducted to compare the nature of root exudates in the Chinese Brake Fern

and a non-hyperaccumulating fern species, the Boston Fern (*Nephrolepis exaltata L.*). Most arsenic found in aerobic soils is in the form of arsenate and is bound to clay minerals, Fe and Mn-oxides/hydroxides, and organic substances [Matschullat, 2000]. In both the Chinese Brake Fern and Boston Fern, phytic acid and oxalic acid are the two main LMW organic acids found in their root exudates. Phytic acid ($C_6H_{18}O_{24}P_6$) is the principal storage form of phosphorus in many plant tissues and is also known to be a strong chelator of important minerals like calcium, magnesium, iron, and zinc. Oxalic acid ($C_2H_2O_4 \cdot 2H_20$) is one of the strongest organic acids, well-known as a reducing agent enabling mineral mobilization [Jones, 1998]. High Performance Liquid Chromotography (HPLC) analysis showed that citric acid, ascorbic acid, succinic acid, and fumaric acid are also present in small quantities [Tu et al., 2003].

Both ferns had similar exudation of phytic acid in the absence of As. However, under As stress, the Chinese Brake Fern exuded 1.5-2 times more phytic acid than the Boston Fern. In the absence and presence of As, the Chinese Brake Fern exuded 3-5 times more oxalic acid than the Boston Fern. Once the root exudates were isolated, it was noted that they were able to dissolve significant amounts of As from Al-As, Fe-As, and chromated copper arsenate (CCA) contaminated soils. Root exudates from Chinese Brake fern dissolved 3-4 times more As from Al-As, 4-6 times more As from Fe-As, and 6-18 times more As from CCA soil than root exudates from the Boston Fern. It is evident that the Chinese Brake Fern has a greater ability to solubilize As than the Boston Fern [Tu et al., 2003]. Phytic acid alone, when at the same concentrations as oxalic acid, was able to mobilize 0.7 - 4.6 times more As from Al-As, 13.6 - 32.8 times more As from Fe-As, and 4.4 - 5.6 times more As from CCA-contaminated soil. This is most likely due to the greater acidity and stronger complexation capacity of phytic acid over oxalic acid [Tu et al., 2003].

1.6 ARSENIC SPECIATION

Ma et al. [2001] reported that 47-80% of As in ferns accumulated in *Pteris vittata* was inorganic arsenite. Wang et al. [2002] demonstrated that more than 85% of the As extracted from *Pteris*

vittata fronds was in the form of arsenite and Lombi et al. [2002] reported that 75% of arsenic in fronds occurred as arsenite. Lombi used et al. [2002] used X-ray absorption near edge spectroscopy analyses (XANES), to determine the physiological speciation of arsenic and its location within the plant. He suggested that using water and methanol to extract the frond samples and then determining the As species using high-performance liquid chromatography-inductively coupled mass spectrometry (HPLC-ICPMS) could affect the speciation of As. Therefore, physiological determination is much more reliable. Another consideration in understanding the speciation of As is the timeliness with which As analysis is conducted. Carbonell et al. [1998] suggested that one can only be confident that a given arsenic species is stable for only 4 days before it is highly likely that oxidation or reduction has occurred.

X-ray absorption spectrometry (XAS) and XAS imaging studies were more recently used by Pickering et al. [2006] to further understand how arsenic is transported through the plant. He reported that the roots predominantly contain arsenate (90 ± 4%) while the leaves predominantly contain arsenite (95 ± 1%) [Pickering et al., 2006]. By directly visualizing arsenic forms in intact fern tissues, *Pteris vittata* transports untransformed arsenate to the frond and it is then primarily stored within leaf tissues as arsenite [Pickering et al., 2006]. Arsenate is the dominant species of As in aerobic soils while arsenite is predominant in anaerobic conditions. Arsenite can be oxidized in soil to arsenate, removed by *Pteris* ferns as arsenate and then converted to As (III) for storage in the plant upper biomass.

Another interesting finding regarding speciation is that mature *Pteris vittata* fronds may re-oxidize arsenite to arsenate. In an unpublished work by C. Tu et al., *Pteris vittata was* grown in soil spiked with high concentrations of sodium arsenate. In young fronds they found that almost all of the As in young ferns was found as As (III) while mature to older ferns contained 62 – 73 % of As (III). This result suggests that re-oxidation of As (III) occurs as fronds mature.

Lombi et al. [2002] used another innovative method to determine how arsenic is localized in the ferns. Using energy dispersive X-ray microanalysis (EDXA), it has been determined that As is mainly localized inside of the cells and not in the cell walls. Vacuoles are the major intracellular constituent in mature leaf cells, which means that As may be stored in the vacuoles [Lombi et al., 2002]. A strategy such as this may allow for low As concentrations in the cytoplasm. If arsenic complexes with phytochelatins (PC) as a method of storage as suggested by Meharg and Hartley-Whitaker [2002], it is possible that As-PC complexes may be stored in

vacuoles where its acidic conditions are favorable for their stability. As-PC complexes can easily dissociate in alkaline pH conditions to yield both arsenite and arsenate.

Other forms of arsenic found in soil include monomethyl arsenic acid (MMA) and dimethyl arsenic acid (DMA) [Tu and Ma, 2002]. It was found that *Pteris Vittata* ferns took up more MMA than As (III) in its fronds when exposed to 1 and 10 mg As/L, as MMA or As (III). *Pteris Cretica* took up more As (III) than MMA at 1 mg As/L and more MMA than As (III) at 10 mg As/L, as MMA or As (III) [Fayiga et al., 2005]. This preference of MMA over As (III) may be explained by the assertion that As (III) is generally more toxic to plants than MMA [Sachs and Micheals, 1971].

The species of As accumulated by *Pteris vittata* has also been examined in hydroponic experiments. Fayiga et al. [2005] analyzed *Pteris vittata* fronds following exposure to 5 mg/L of As (V) or 20 mg/L of As (III) and found no As (III) present in either condition. When exposed to As (III), As (III) was only detectable in the roots after 1 day of exposure. After 15 days of exposure, comparable percentages of As (III) were found in the fronds for ferns treated with 5 mg/L As (V) and 20 mg/L As (III). The same is true with the As (III) detected in the roots of *Pteris vittata* ferns. The presence of As (III) in the roots of the *Pteris vittata* ferns actually decreased from the first day of exposure to the 15 days of exposure analysis, possibly due to the conversion of As (III) to As (V). The chart below further illustrates that As (V) is the dominant form of As in the roots while As (III) is the dominant form in the fronds [Fayiga et al., 2005].

Table 1.3 Percentage of As (III) in the fronds and roots for *P. vittata* and *N. exaltata* after As exposure [Fayiga et al., 2005]

Treatment	1 day		15 days	
	Fronds	Roots	Fronds	Roots
	5 mg/L As (V)			
P. vittata	BD*	BD	83.7**	26.9
N. exaltata	BD	BD	12.5	BD
	20 mg/L As (III)			
P. vittata	BD	40.8	83	26.8
N. exaltata	BD	31.7	24.4	10.3

* BD – below detection; ** Mean of four replicates

1.7 GROUNDWATER COMPOSITION IN BANGLADESH

There are several theories explaining the mobilization of As in Bangladeshi groundwater. At the onset of discovering the presence of toxic levels of As in the groundwater supply, anthropogenic sources such as waste disposal, fertilizer, pesticide, and insecticide use, were implicated as possible culprits [NRECA, 1997]. Early research determined that the source of arsenic was geogenic and released by natural processes. Others postulated that the oxidation of pyrite or arsenopyrite allowed for the mobilization of As once the water table was drawn sufficiently low by groundwater pumping [Mandal et al., 1996; Mallik and Rajagopal, 1996]. The reductive dissolution of iron oxyhydroxides was later verified to be the case in alluvial aquifers of the Ganges-Brahmaputra-Meghna (GBM) region [Bhattacharya et al., 1997, 2001; Ahmed et al., 1998; Nickson et al., 2000; Routh et al., 2000; McArthur et al., 2001; Dowling et al., 2002; Anawar et al., 2003]. Accharya et al. reported in 2000 that intensive groundwater extraction by pumping and the use of phosphate fertilizers precipitated As mobilization. Another theory suggests that large scale irrigation pumping drove biogeochemical processes to release arsenic at low depths where dissolved arsenic is at its peak [Harvey et al., 2002].

A survey of tubewell samples throughout Bangladesh determined that total As concentrations ranged from 2.5 – 846 μg/L. The dominant species was noted to be As (III), representing 67-99% of the groundwater As content in most wells, while As (V) was the prominent form of As in some wells. Maximum As concentrations were reported at depths between 20 m and 50 m, while samples shallower than 10 m or deeper than 150 m were relatively free of As. Deeper well pumping has been a used as a short-term solution for avoiding As-contaminated water in select regions [Ahmed et al., 2003].

Groundwater obtained from a tubewell in Kushtia Sadar, Bangladesh is representative of the groundwater used by approximately 400,000 people in the region. This region has reported signs of arsenical keratosis or skin lesions caused by arsenic toxicity. When continuously monitoring the tubewell for arsenite and arsenate, it was found that about 40% of the groundwater was contaminated with greater than 50 μg/L of total As [Hussam et al., 2003]. Despite the causes of As mobilization, it is apparent that Bangladesh is in severe need of an effective remediation method as soon as possible.

1.8 ARSENIC REMOVAL BY HYDROUS FERROUS OXIDES (HFO)

Arsenic removal by hydrous ferrous oxides (HFO) was evaluated as another possible remediation method. A survey of tubewells throughout Bangladesh revealed that total iron (Fe) concentration range from 0.4 – 15.7 mg/L [Ahmed et al., 2003]. In Kushtia Sadar, the groundwater has sufficiently high iron content (6.77 - 7.17 mg/L) that when left open to the air, a brownish precipitate is formed, which removes some inorganic species. Locally, this attenuated groundwater is known as "bashi pani" from a phrase "pani bashi kore khaben" that means "drink water after a while". In this process, hydrous ferric oxide (HFO) is precipitated and trace cations and anions are co-precipitated from the groundwater [Hussam et al., 2003]. Table 1.4 shows the results of the attenuation process.

Table 1.4 Composition of metals for fresh groundwater and naturally attenuated groundwater (NAGW) [Hussam et al., 2003]

Elements	Fresh Groundwater (FGW) mg/L	NAGW (Bashi Pani) mg/L
Aluminum, Al	<0.015-0.022	<0.015-0.022
Antimony, Sb	<0.013-0.017	<0.013
Arsenic, As	0.114-1.160	0.064-0.400
Barium, Ba	0.161-0.170	0.003-0.082
Beryllium, Be	<0.001	<0.001
Cadmium, Cd	<0.001	<0.001
Calcium, Ca	111-117	17.7-87.4
Chromium, Cr	<0.002	<0.002
Cobalt, Co	<0.002	<0.002
Copper, Cu	0.004-0.009	0.002
Iron, Fe	6.77-7.19	<0.005
Lead, Pb	<0.004-0.005	<0.004
Magnesium, Mg	21.4-23.1	19.31-20.9
Manganese, Mn	0.69-0.74	<0.001-0.01
Molybdenum, Mo	0.001-0.003	0.002
Nickel, Ni	<0.002	<0.002
Potassium, K	1.88-2.45	1.76-2.09
Selenium, Se	<0.012	<0.012

Table 1.4 (continued)

Silver, Ag	<0.002	<0.002
Sodium, Na	18.2-20.4	17.8-19.3
Strontium, Sr	0.280-0.297	0.190-0.272
Thalium, Tl	<0.067	<0.067
Tin, Sn	<0.002—0.01	0.007-0.01
Vanadium, V	<0.001	<0.001
Zinc, Zn	0.012-0.021	<0.007
pH	6.9	7.7
Ionic Strength (M)	9.66×10^{-3}	9.66×10^{-3}
Temperature (^{o}C)	27	29
Ek (mV) vs. NHE[a]	127-250	340-440
O2 (aq), mg/L[a]	0.8-1.0	1.0-2.4

When HFO is formed, large quantities of arsenite, arsenate, and other ions can be easily adsorbed to it, though arsenite removal by adsorption is much lower than arsenate [Mamtaz and Bache, 2000]. This process can be exploited in groundwater supplies with sufficiently high Fe content, where 50-75% of arsenic content can be removed in a 24 hr. period when total Fe content ranges from 5.8 to 6.7 mg/L and total As content ranges from 0.16 - 1.2 mg/L [Hussam, 2003]**.** A significant amount of iron is removed as it settles out of solution through adsorption and co-precipitation.

Removal of As by Fe alone can be achieved if the Fe/As ratio is ≥ 10. If the water to be treated meets this criterion, it can be added to a plastic container, manually shaken for 1 minute and allowed 3 days for particle settling. If the treated water is discharged at a rate not exceeding 0.5 L/min, there is reasonable expectation that the Bangladesh Water Quality Standard of 0.5 μg/L of arsenic will be met. This application is recommended for iron concentration between 1.0 – 20.0 mg/L and arsenic concentration between 0.1 – 0.5 mg/L [Mamtaz and Bache, 2000]. In the event that this Fe/As ratio cannot be met, other removal alternatives must be utilized.

1.9 OPTIMIZING ARSENIC REMOVAL BY *PTERIS* FERNS

Simulations of real-world application using *Pteris* ferns to remove arsenic will help to optimize the functionality of this application. In some regions of the United States, there are municipalities that are not in compliance with the 2006 EPA Standard of 10 μg/L of As for public water supply. A substation located in South Florida that has been contaminated by the legal application of arsenic-containing herbicides, has an average total As concentration of 46 μg/L and total P concentration of 20 μg/L. When *Pteris vittata* ferns were added to the contaminated groundwater supply, a single fern was capable of depleting the As concentration in 600 mL of water to less than 10 μg/L. Young ferns (three months age) were much more efficient at removing As than older ferns (twelve months age). However, an advantage that older ferns have over younger ferns is their significantly larger biomass. This study also concluded that adding P in excess of the groundwater content significantly inhibited As uptake [Tu et al., 2004].

In Albuquerque, New Mexico, a pilot-scale demonstration was carried out at the Parks and Recreation Department where As levels in drinking water range from 6.6 to 14 μg/L. A modular, continuous flow phytofiltration system was developed, consisting of ten treatment tanks linked in series. For a 3 month period, at various ambient temperatures, daylight exposure lengths, and relative humidity levels, the system consistently produced an effluent with less than 2 μg/L of total As. It has been concluded that arsenic uptake by *Pteris* ferns occurs continuously throughout the day and night and is not affected by day length. Variations in humidity and light intensity also had little effect on arsenic uptake [Elless et al., 2005].

Broader water quality effects were noted such as slightly lower turbidity in the effluent and slightly greater dissolved oxygen content possibly due to aeration throughout the system, while pH and mineral content remained constant. An increase in heterotrophic plate count (HPC), which measures microorganisms that require organic carbon for growth, was noted in the effluent. This count includes bacteria, yeast and molds; however, no other organisms like protozoa, nematodes, amoeba, ciliates, etc. were present.

2.0 MATERIALS AND METHODS

2.1 PLANT PREPARATION

All *Pteris cretica cv. Mayii* and *Nephrolypis Exaltata* ferns were acquired from Milestone Agriculture™ in 4" pots and transplanted from soil to aqueous nutrient solution contained in Ball® Quart Regular Mason Jars coated with aluminum foil. The mason jars were covered to provide the dark environment, similar to soil, that plant roots most readily thrive in. All nutrient solutions were prepared using Peter's Professional® All Purpose Plant Food, Water Soluble 20-20-20 (Nitrogen-Phosphate-Potash) with Micronutrients Solution diluted at 1:200 ratio with Pittsburgh tap water. The ferns were suspended in the mason jars by supporting the base of each fern with 1 ½" thick Styrofoam™.

Figure 2.1 *Pteris Cretica cv. Mayii* Plants Received in Potted Soil

Figure 2.2 *Pteris Cretica cv. Mayii* Plants Transferred to Hydroponic System

2.2 PLANT CARE

Ferns were placed under grow lights for 16-hr day lighting with a measured luminance of 1200 FC using an Amprobe® Digital Light Meter (DLM2). Humidity was supplied to the plant environment using a Bionaire™ Warm Mist Humidifier (CP-0470) and measured routinely using Testo® Humidity/Temperature Probe (610) to ensure that Relative Humidity was at 60 – 70 % and that the temperature was maintained at 22 – 25 ^{o}C.

Nutrient solution was supplied to plants every other day to replenish water volume losses due to transpiration and uptake by plants. The fronds were misted daily to prevent drying and to raise the relative humidity level. A plastic canopy covered the plants to simulate a greenhouse environment and prevent humidity losses.

The nutrient solution for each plant was changed on a weekly basis to ensure that all necessary nutrients were readily available for plant uptake. All nutrient solutions were aerated by lab-supplied air and distributed to each mason jar via Fisher Scientific™ Flexible Clear Plastic Tubing with corresponding T-tube connectors.

As the Pteris fronds age, they become brown and dried, which was observed in all plants independent of experimentation. Therefore, weekly pruning was employed to remove unsightly and aging fronds and foster new frond growth.

2.3 SOLUTION PREPARATION

Experimental stock solutions of arsenic were prepared using distilled deionized water produced in an environmental lab using a Barnstead™ Nanopure system, acidified with 2 % Fisher Scientific™ Trace Metal Grade Nitric Acid to pH 3, and stored in 4 ^{o}C cold room. Arsenate stock solution was prepared by dissolving 0.625 g Sodium Hydrogen Arsenate (Na_2HAsO_4-$7H_2O$) in a 100-mL volumetric flask to prepare 1.5 g As/L solution. Arsenite stock solution was prepared by adding 40 mL of 0.1 N Sodium Arsenite ($NaAsO_2$) solution in a 100-mL volumetric flask to prepare 1.5 g As/L solution. Iron stock solution was prepared by dissolving 0.0996 g $FeSO_4 \cdot 7H_2O$ in 100-mL volumetric flask to prepare a 200 mg Fe/L solution. When Fe stock solutions were diluted for experimentation, a carbonate buffer was added to supply 0.005 M $NaHCO_3$ content to maintain a pH level between 6.5 and 7.5 which is suitable for normal plant growth.

Spex CertiPrep™ plasma grade arsenic standard solution of 1000 ppm was used to prepare standard solutions for As analysis and Fisher Scientific™ certified reference iron standard solution of 1000 ppm was used to prepare standard solutions for Fe analysis. The As standard solution was used to calibrate the Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) and the Fe standard solution was used to calibrate the Flame Atomic Absorption Spectrophotometer (AAS). All solutions were diluted using DI water.

2.4 CLEANING GLASSWARE

All glassware, pipettes, beakers and test tubes in contact with As were soaked overnight in a polyethylene basin containing 10 % v/v Fisher Scientific™ Nitric Acid diluted in DI water. After soaking the glassware, they were triple-rinsed with Pittsburgh tap water and then triple-rinsed with DI water.

2.5 ARSENIC ANALYSIS

Total arsenic content was determined using Perkin Elmer™ model 4100ZL Zeeman GF-AAS, shown in Figure 2.3, equipped with a graphite tube atomizer and programmable sample dispenser. Argon gas of ultra high, 5.0 grade purity was used to purge the volatilized matrix materials and protect the heated graphite tube from air oxidation. Arsenic hollow-cathode lamp was used at a wavelength of 193.7 nm with a slit width of 0.7 nm. The heating program for the GF-AAS used during As analysis is listed in the Table 2.1.

Table 2.1 Heating Program for As Analysis by GF-AAS

Step	Temperature (^{o}C)	Ramp Time (sec)	Hold Time (sec)	Internal flow (mL/min)
1	110	7	18	250
2	140	5	10	250
3	200	5	10	250
4	900	15	20	250
5	2200	0	5	0
6	2450	1	3	250

Figure 2.3 Graphite Furnace Atomic Absorption Spectrophotometer (Perkin Elmer™ 4100 ZL)

Samples and calibration standard solutions were prepared according to approved Environmental Protection Agency (EPA) method (U.S.EPA National Exposure Research Laboratory – NERL Method No. 206.2). GF-AAS was calibrated for As analysis in the linear range of 10 μg/L to 100 μg/L. All samples were analyzed in triplicate by GF-AAS and were re-analyzed if the relative standard deviation between each of the three samples was greater than 10%.

2.6 IRON ANALYSIS

Total iron analysis was performed using Perkin Elmer™ model 1100B Flame-AAS, shown in Figure 2.4, following the Method 3111B of the Standard Methods. Samples were analyzed by direct aspiration into air-acetylene flame and absorbance was measured at 248.3 nm for iron. Flow rates were adjusted to 2.5 L/min for acetylene and 8 L/min for air as specified by the manufacturer to give maximum sensitivity for Fe measurement. Operating current of the hollow-cathode lamp was 20 mA.

Figure 2.4 Flame Atomic Absorption Spectrophotometer (Perkin Elmer™ 1100B)

Samples and standards were acidified using Fisher Scientific™ Trace Metal Grade Nitric Acid at 2 % v/v to dissolve any precipitated iron particles and retain the solubility of Fe in solution. Flame-AAS was calibrated for Fe analysis in the linear range from 1 mg/L to 5 mg/L.

2.7 SAMPLE HANDLING

Samples were extracted from the experimental solution contained in jars at various time intervals using a 5-mL glass syringe and passed through a 0.45 μm Millipore™ Membrane Filter (Table 2.2) supported by a Pall™ 25 mm-diameter Easy Pressure Syringe Filter Holder. Filtration is necessary to remove particulates that may affect As analysis by GF-AAS. Samples were then transferred to a test tube and acidified with concentrated Nitric Acid to provide 2 % v/v acid content and refrigerated. Acidification allows for the metals to remain dissolved in solution without precipitation. Samples were diluted to attain the concentration range between 10 μg/L and 100 μg/L and, following the protocol for As analysis by GF-AAS, concentrated 1 % v/v Nitric Acid was added again. To minimize analysis interference by other sample constituents, 5 % Nickel Nitrate (prepared by diluting 24.780 g of $Ni(NO_3)_2 \cdot 6H_2O$ in 100 mL of DI water) was added to provide 2% v/v matrix modifier content.

Table 2.2 Filter Specifications for MF-Millipore™ Membrane Filters

Content	Mixed Cellulose Ester
Pore Size (μm)	0.45
Thickness (μm)	150
Diameter (mm)	25

Table 2.2 (continued)

Water Flow Rate (mL/min/cm^2)	60
Air Flow Rate (L/min/cm^2)	4
Porosity (%)	79

3.0 RESULTS AND DISCUSSION

The goal of this research effort was to understand how the innate properties of the *Pteris* fern could be utilized for the real-world application of a sustainable, low-maintenance arsenic (As) removal system. In doing so, a variety of conditions were explored to determine the most effective use of the plants and how they can be best applied. More specifically, the following operating conditions were examined:

- The impact of As speciation (adding As as arsenite or arsenate)
- The impact of aeration on As removal
- The impact of iron on As removal
- As removal by *Pteris* ferns in tap water and deionized water
- As removal following repeated exposure of ferns
- As removal by individual and multiple ferns in a single reactor

3.1 IMPACT OF SPECIATION ON ARSENIC UPTAKE

The rate of total arsenic removal was compared when As is added as arsenite or arsenate. Wang et al. [2002] reported that arsenate influx by *Pteris* ferns is significantly greater than arsenite influx and was verified after conducting experiments in the absence of phosphorus (P). Similarly, arsenate, the form of arsenic that most similarly resembles phosphate, is removed a rate 2.5 times greater following P starvation than in the case of sufficient P concentration. When *Pteris* ferns are exposed to As in the form of arsenite, the rate of removal is not affected by

absent or sufficient P concentration which illustrates that the method of arsenite removal is significantly different than that of arsenate removal [Wang et al., 2002].

To evaluate the effect of speciation on As removal, single *Pteris* ferns were placed in a mason jar containing 800 mL of aerated deionized water with As added from a stock arsenite solution. The aqueous As content of the experimental solution was sampled and observed to exponentially decrease over time as shown in Figure 3.1. Though As was added as arsenite at the onset of these experiments, no experiment tested if or to what extent arsenite converted to arsenate during the course of the experiment.

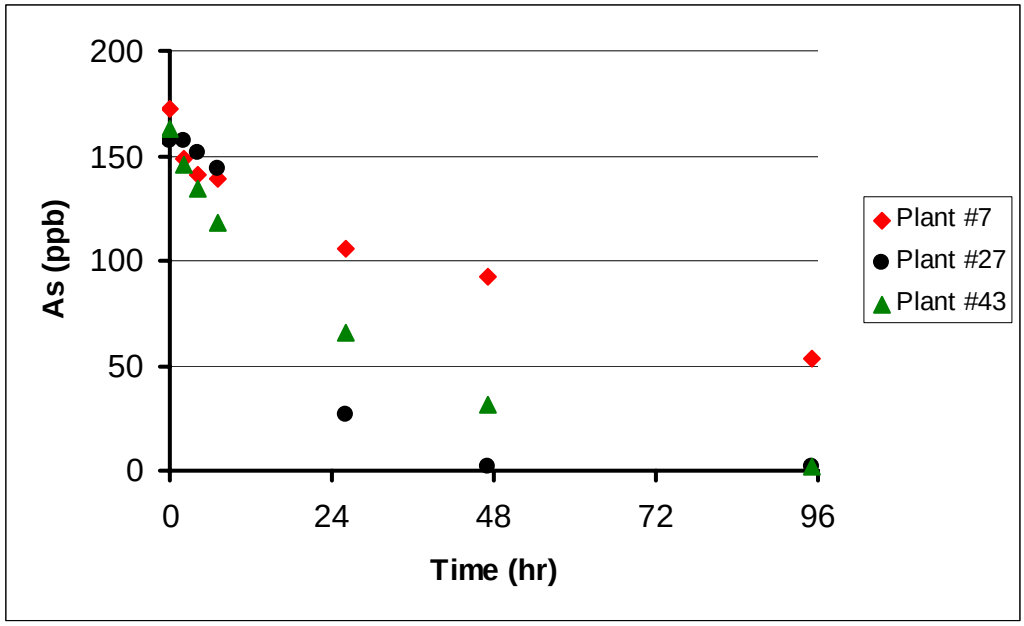

Figure 3.1 Arsenite Removal by *Pteris* Ferns in Aerated Deionized Water

To further develop the results presented in Figure 3.1, a first-order depletion kinetics model can be applied to the data to develop a rate constant with which data analysis can be made. The model is described as:

$$dC/dt = -kC$$

$$(1/C)\, dC = (-k)\, dt$$

$$\ln C - \ln C_o = -kt + \text{constant}$$

$$\ln (C/C_o) = -kt + \text{constant}$$

Where C is concentration, C_o is initial concentration, k is first order kinetics rate constant, and t is time. The data fit to the first-order depletion model is depicted in Figure 3.2, with the model parameters presented in Table 3.1.

Table 3.1 First Order Depletion Kinetics Model Parameters for Arsenite Removal by *Pteris* Ferns in Aerated Deionized Water

Plant #	k Value	Constant	R^2 Value
7	0.0113	-0.1139	0.9773
27	0.0572	-0.0124	0.8388
43	0.0474	0.1041	0.9751
AVERAGE	0.0386	-0.0074	0.9304
ST. DEV	0.0242	0.1091	0.0793
RSD (%)	62.57	----------	---------

By comparing the k-values observed above for arsenite depletion, it is apparent that the k values vary significantly with an average value of 0.0386 ± 0.0242 hr^{-1} and a relative standard deviation of almost 63%.

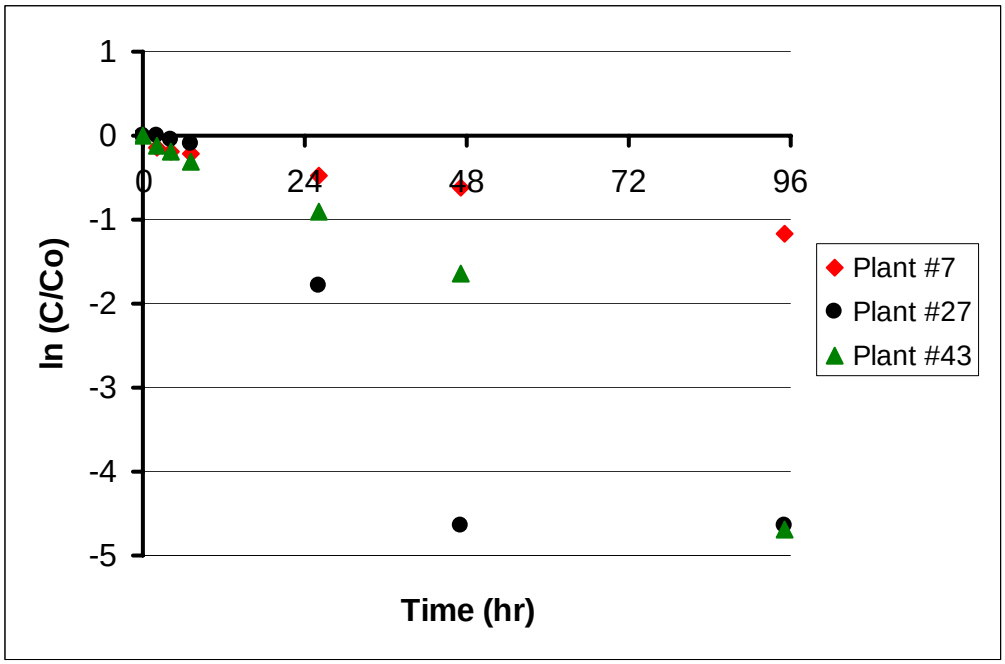

Figure 3.2 Arsenite Removal by *Pteris* Ferns in Aerated Deionized Water fit to First Order Depletion Kinetics Model

It is possible that the variability in the case of arsenite removal is related to the complexity of arsenite removal mechanisms noted in the *Pteris* fern species. Arsenite influx is significantly slower than arsenate influx into the *Pteris* fern and the supply of aeration was inconsistent amongst the three ferns, the rate of oxidation from arsenite to arsenate could have varied which would thus affect total arsenic removal [Wang et al., 2002].

Similar to the experiments conducted with As added as arsenite, experiments were conducted with the addition of As from an arsenate stock solution. The As content over time is presented Figure 3.3. This data is converted to the first-order kinetic model in Figure 3.4 with the parameters tabulated in Table 3.2.

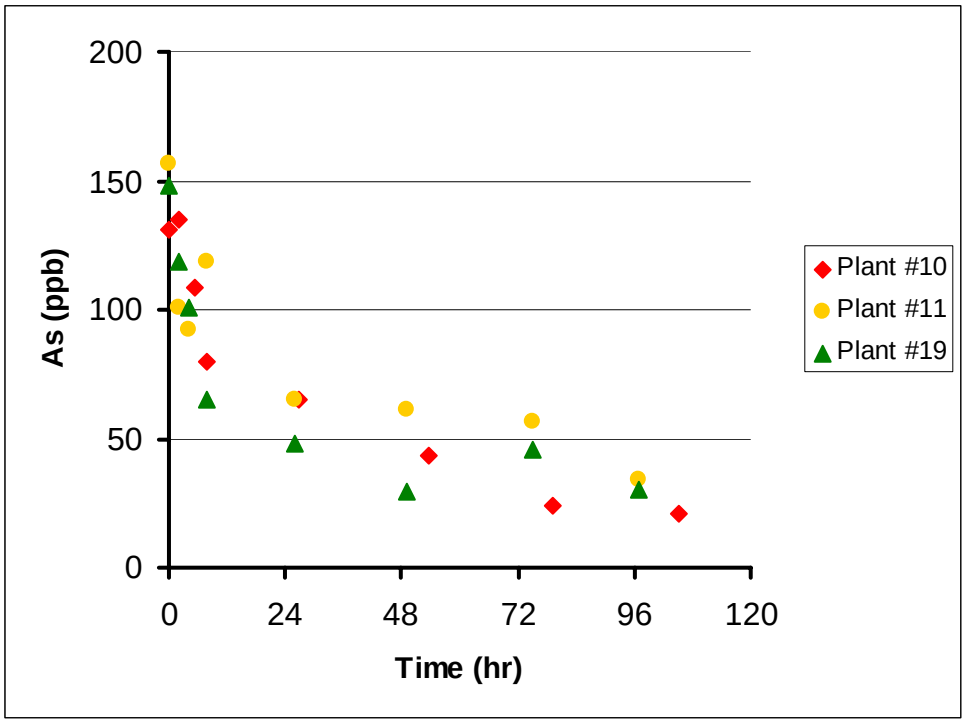

Figure 3.3 Arsenate Removal by *Pteris* Ferns in Aerated Deionized Water

Table 3.2 First Order Depletion Kinetics Model Parameters for Arsenate Removal by Pteris Ferns in Aerated Deionized Water

Plant #	k Value	Constant	R^2 Value
11	0.0124	-0.2799	0.8436
19	0.0128	-0.4841	0.6535
10	0.0172	-0.1764	0.9592
AVERAGE	0.0141	-0.3135	0.8188
ST. DEV	0.0027	0.1566	0.1544
RSD (%)	18.84	----------	----------

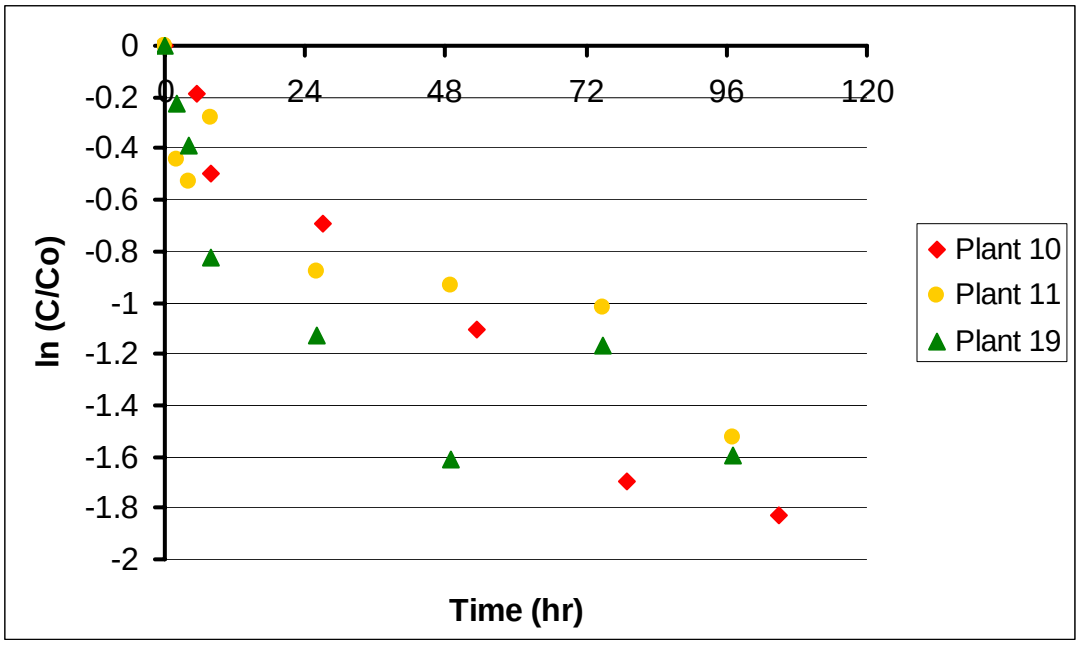

Figure 3.4 Arsenate Removal by *Pteris* Ferns in Aerated Deionized Water fit to First Order Depletion Kinetics Model

In the case of arsenate removal, the average k-value is 0.0141 ± 0.0027 hr^{-1} and a relative standard deviation of approximately 19 %. The variability of arsenate removal in *Pteris* ferns in the case of these three independent ferns is significantly lower than the variability of arsenite removal observed. This may be due to the more rapid influx of arsenate than arsenite by *Pteris* ferns due to arsenate's similarity to phosphate [Ma et al., 2001; Wang et al., 2002; Lombi et al., 2002]. Because the total As content was added as arsenate, the form more readily removed by Pteris ferns, the As removal could have been facilitated. When adding As as arsenite, the interim step of converting arsenite to arsenate may be the step that slows the overall rate of removal.

3.2 IMPACT OF AERATION ON ARSENIC UPTAKE

If As removal by *Pteris* ferns is effective without aeration, considerable energy savings can be achieved because mechanical aeration would not be necessary. To evaluate the need for aeration, three single Pteris ferns were placed in a mason jar containing 800 mL of aerated deionized water with As content of nearly 300 ppb comprised from both arsenite and arsenate stock solutions in approximately equal concentration (target concentration of 150 ppb was prepared for each species). One set of experimental solutions received lab-supplied air via plastic tubing while the other set remained without aeration. As content was monitored and can be described by exponential decrease over time as illustrated below in Figure 3.5.

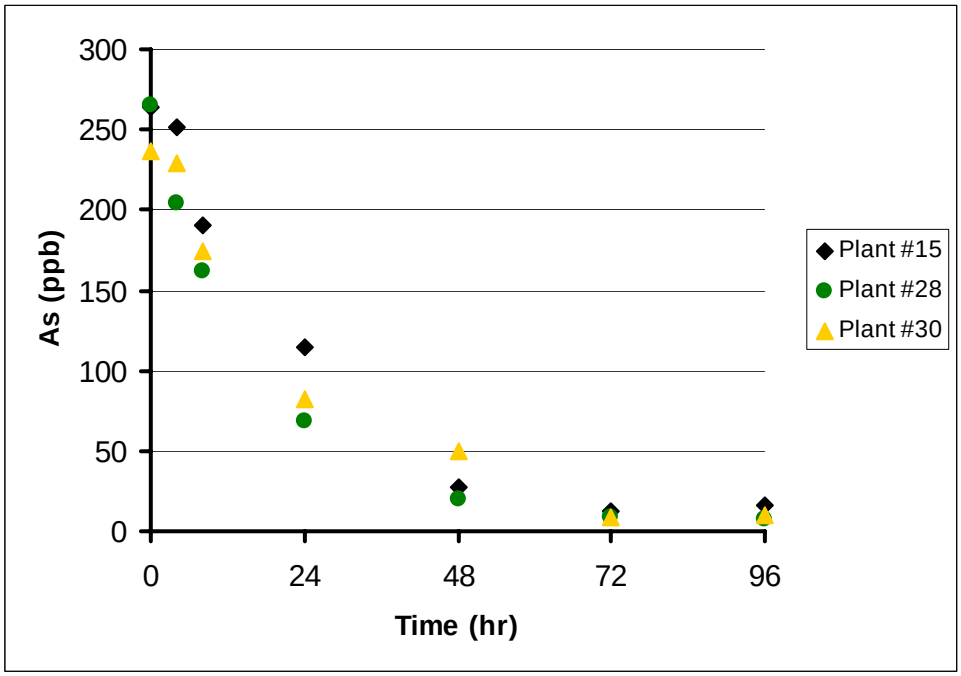

Figure 3.5 Arsenic Removal by *Pteris* Ferns in Aerated Deionized Water

Table 3.3 First Order Depletion Kinetics Model Parameters for Arsenic Removal by *Pteris* Ferns in Aerated Deionized Water

Plant #	k Value	Constant	R^2 Value
15	0.0344	-0.0953	0.9198
30	0.0372	-0.0165	0.9400
28	0.0405	-0.2303	0.9577
AVERAGE	0.0374	-0.1140	0.9392
ST. DEV	0.0031	0.1081	0.0190
RSD (%)	8.1715	----------	----------

Table 3.3 shows the average k value for total As removal in aerated deionized water as 0.0374 ± 0.0031 hr^{-1} and a relative standard deviation around 8 %. These values were derived from the graph depicted in Figure 3.6.

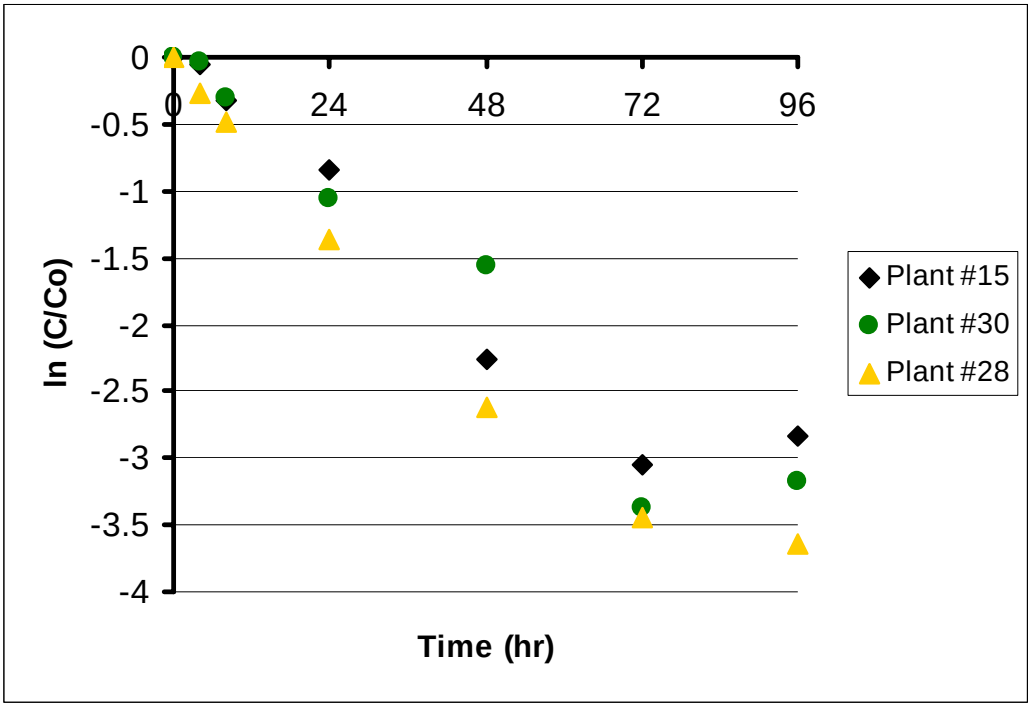

Figure 3.6 Arsenic Removal by *Pteris* Ferns in Aerated Deionized Water fit to First Order Depletion Kinetics Model

In Figure 3.7, the As content over time for a similar experiment conducted without aeration is reported below and is shown to follow a fairly linear pattern of decrease.

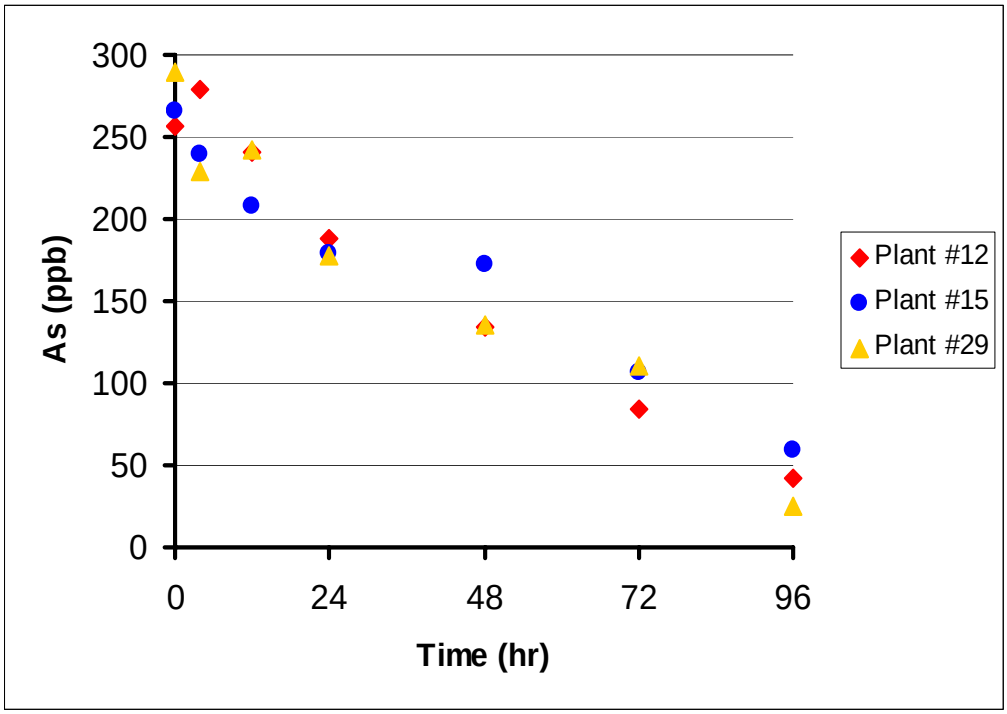

Figure 3.7 Arsenic Removal by *Pteris* Ferns in Non-Aerated Deionized Water

By converting the data presented in Figure 3.7 to fit a first-order kinetic model, the rate constants can be derived and compared as presented in Table 3.4. The average k value for total As removal without aeration is 0.0179 ± 0.0037 hr^{-1} and a relative standard deviation of nearly 21 %.

Table 3.4 First Order Depletion Kinetics Model for Arsenic Removal by *Pteris* Ferns in Non-Aerated Deionized Water

Plant #	k Value	Constant	R^2 Value
12	0.0187	0.1335	0.9790
15	0.0139	0.0037	0.9374
19	0.0212	0.0487	0.8609
AVERAGE	0.0179	0.0620	0.9258
ST. DEV	0.0037	0.0659	0.0599
RSD (%)	20.68	----------	----------

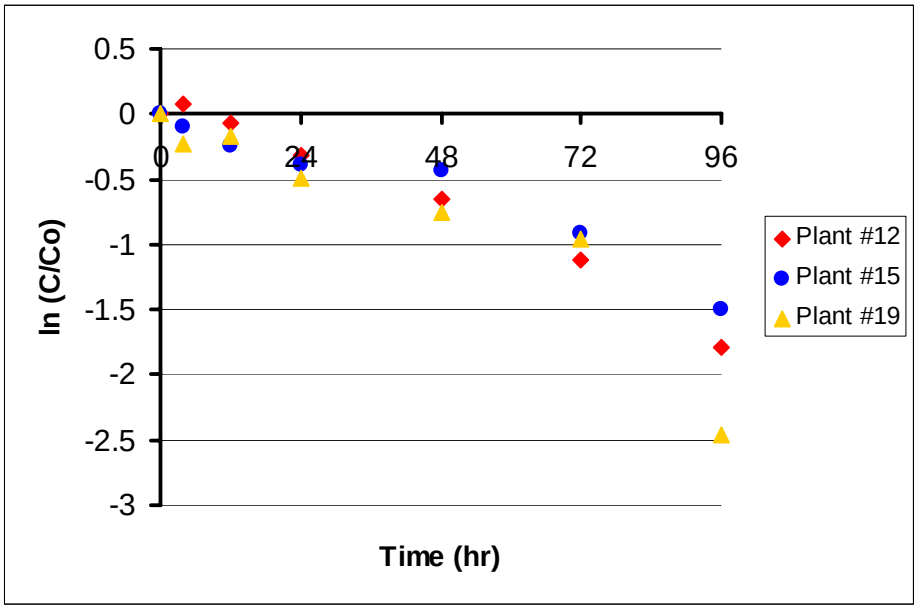

Figure 3.8 Arsenic Removal by Pteris Ferns in Non-Aerated Deionized Water fit to First Order Depletion Kinetic Model

The rate of As removal by *Pteris* ferns can be compared to determine the positive effect of aeration on removal efficiency. The rate constant for As uptake in the presence of aerated DI water is 0.0374 ± 0.0031 hr^{-1} with a relative standard deviation around 8 %. The rate constant for As uptake in DI water that is not aerated is 0.0179 ± 0.0037 hr^{-1} with a relative standard deviation of almost 21 %. For the aerated experiments the rate constant is more than twice the rate constant in the experiments without aeration. Also, there is less variability between the three experiments conducted under aerated conditions than observed without aeration. The cause of this finding may be due to the more rapid oxidation of the arsenite content to arsenate due to aeration or the effect that high dissolved oxygen levels have on overall plant health. Without aeration, Pteris ferns may need a longer period of exposure to the As-contaminated solution to achieve the removal efficiency observed in aerated conditions.

3.3 THE IMPACT OF BACKGROUND IONS ON ARSENIC UPTAKE

To apply phytofiltration by *Pteris* ferns as a method of As removal, it is important to know how the presence of competing anions that are present in natural water supplies would effect As removal. To evaluate this, experiments were conducted to compare the removal of arsenite and arsenate in solutions prepared with Pittsburgh tap water and deionized (DI) distilled water.

Single ferns were placed in a mason jar containing an experimental solution prepared with arsenite and arsenate stock solution, in equal concentration, in aerated Pittsburgh tap water. In 2005, the Pittsburgh Water and Sewer Authority (PWSA) reported the average Pittsburgh tap water constituents as presented in Table 3.5. Conducted in triplicate, this condition was contrasted by ferns placed in experimental solutions prepared with DI water. The results depicted in Figure 3.9 show the depleted As content in the experimental solution in which each plant follows exponential decrease in varying degrees.

Table 3.5 Pittsburgh Tap Water Content

Contaminant (Unit of Measure)	Violation (Y/N)	Level Detected	Action Limit
Lead (ppb)	N	90^{th} Percentile = 9.5	15
Copper (ppm)	N	90^{th} Percentile = 0.099	1.3
Barium (ppm)	N	0.041	2
Chromium (ppb)	N	2.9	100
Flouride (ppm)	N	1.1	2
Nitrate (ppm)	N	0.2	10
Nickel (ppb)	N	10	100

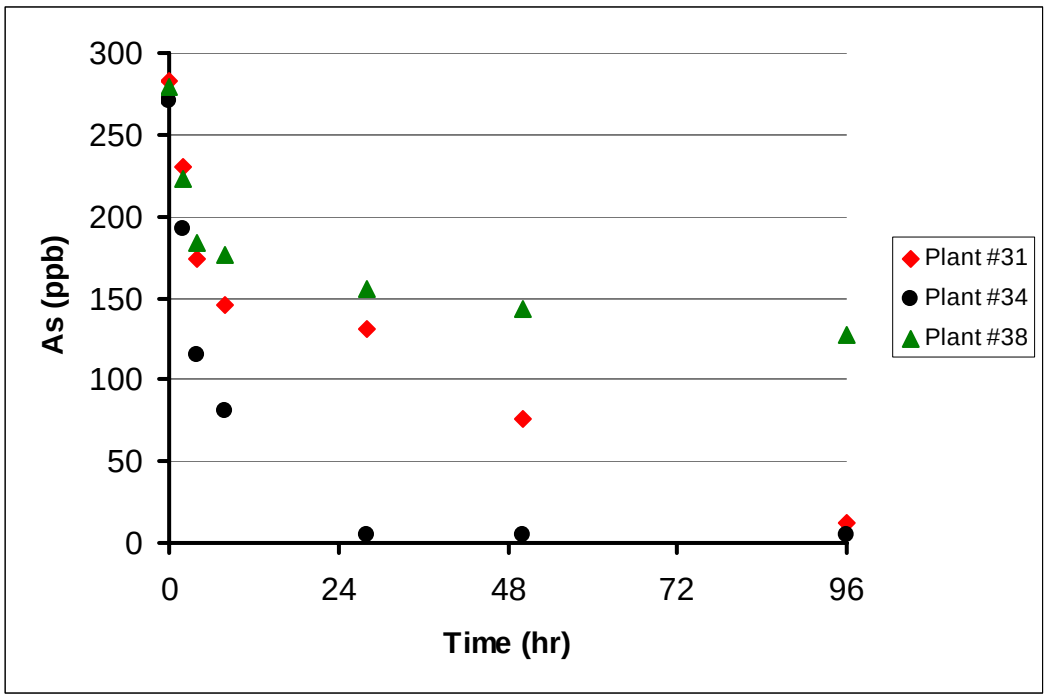

Figure 3.9 Arsenic Removal by *Pteris* Ferns in Aerated Tap Water

Table 3.6 First Order Depletion Kinetics Model for Arsenic Removal by *Pteris* Ferns in Aerated Tap Water

Plant #	k Value	Constant	R^2 Value
31	0.0289	-0.1584	0.9552
34	0.0441	-0.9155	0.6706
48	0.0061	-0.2836	0.6636
AVERAGE	0.0264	-0.4525	0.7631
ST. DEV	0.0191	0.4058	0.1664
RSD (%)	72.54	----------	---------

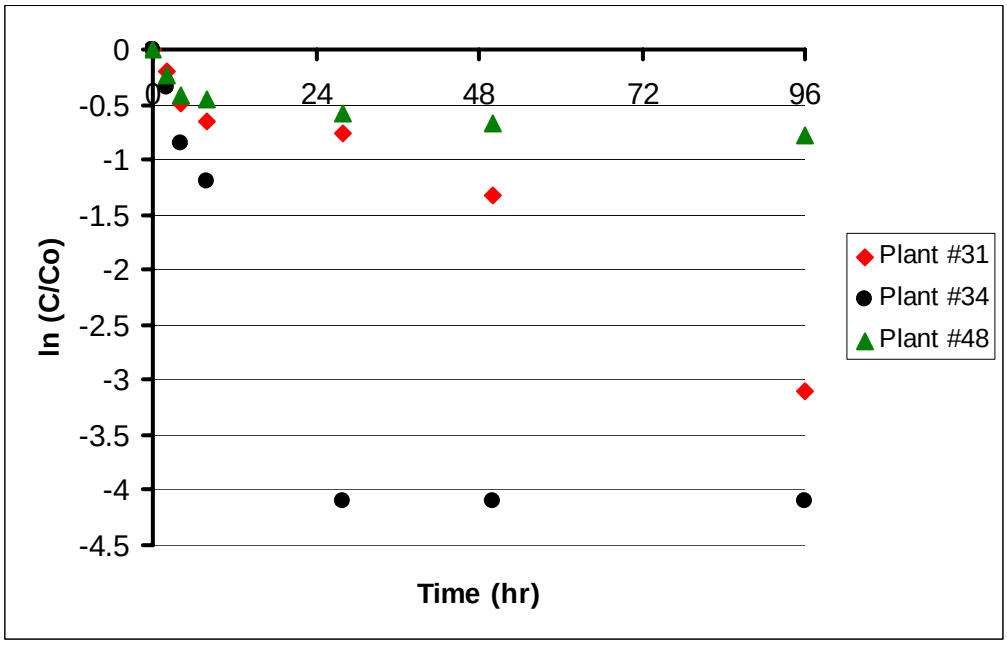

Figure 3.10 Arsenic Removal by *Pteris* Ferns in Aerated Tap Water fit to First Order Depletion Kinetics Model

In the experiments conducted with solution made with tap water, there is a significant scatter between the derived k-values with an average value of 0.0264 ± 0.0191 hr^{-1} and a relative standard deviation of 73%, as shown in Table 3.6. As was stated earlier, the k value for experiments conducted in aerated deionized water was averaged as 0.0374 ± 0.0031 hr^{-1} with a relative standard deviation around 8 %, as shown in Table 3.3. The *Pteris* ferns are more effective at removing As when the ions present in Pittsburgh tap water are unable to compete for uptake. It is not clear at this time why the performance of the ferns in aerated tap water produced such varied rates of As removal.

3.4 IMPACT OF ARSENIC REMOVAL IN PRESENCE OF IRON

Iron is common constituent of groundwater and has an effect on As removal. Groundwater iron content in Bangladesh can vary between 0.4 - 15.7 mg/L [Ahmed et al., 2003]. Iron alone is capable of removing arsenic by the mechanisms of adsorption and co-precipitation if the Fe/As ratio is sufficiently high. Roughly 85 – 88 % As removal efficiency has been noted where the ratio of Fe/As ≥ 10 [Mamtaz and Bache, 2000]. With such high iron content, paired with high As content noted throughout Bangladesh, it is important to know what added effect would As hyperaccumulation by *Pteris* ferns have on the rate of As removal. To examine this, *Pteris* ferns were placed in a mason jar containing approximately 250 ppb As, as arsenate and arsenite in equal proportion, with 2.7 mg/L Fe (II) prepared in DI water. The Fe content was added in the form of ferrous sulfate and buffered with 0.005M $NaHCO_3$ to maintain the pH range between 6.5 and 7.5. A parallel experiment was conducted using *Nephrolypsis exaltata* (Boston Ferns), a known non-hyperaccumulator of As [Ma et al., 2001]. By comparing the rates of As removal in the presence of iron with an As hyperaccumulator and non-hyperaccumulator, the effect contributed by *Pteris* fern can be noted.

The As content was monitored throughout the experiment and is reported in Figure 3.11 and Figure 3.12. Figure 3.11 compares the As removal in the presence of iron with and without the non-accumulating Boston fern. Figure 3.12 compares the As removal in the presence of iron with the Boston fern versus the *Pteris* fern.

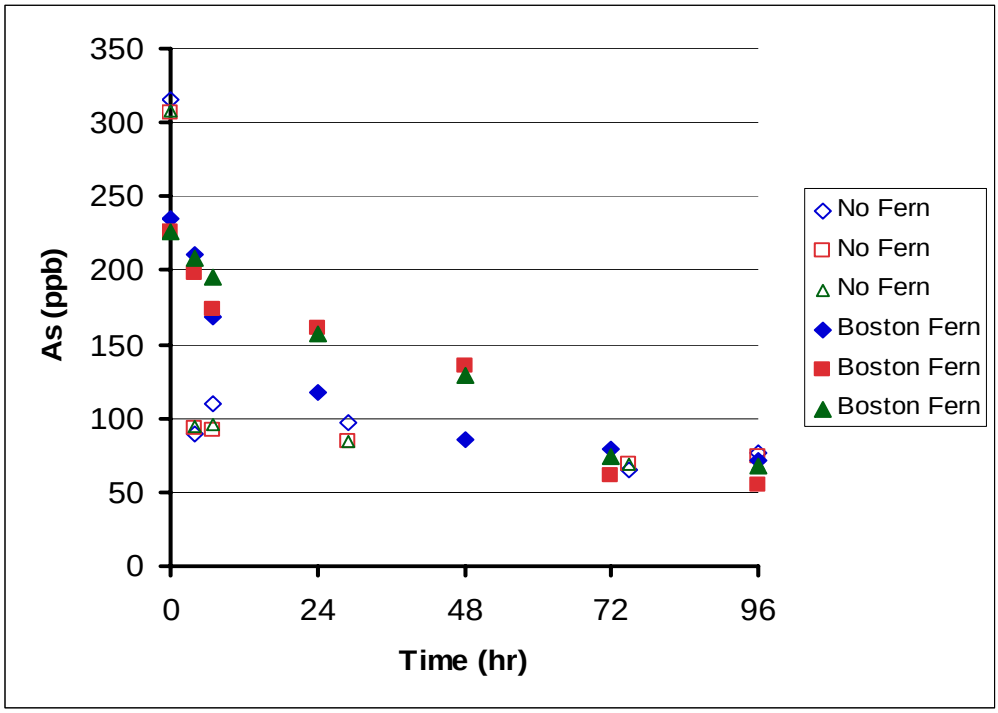

Figure 3.11 Comparing Arsenate Removal with and without Boston Ferns in Presence of Fe (II) = 2.7 mg/L

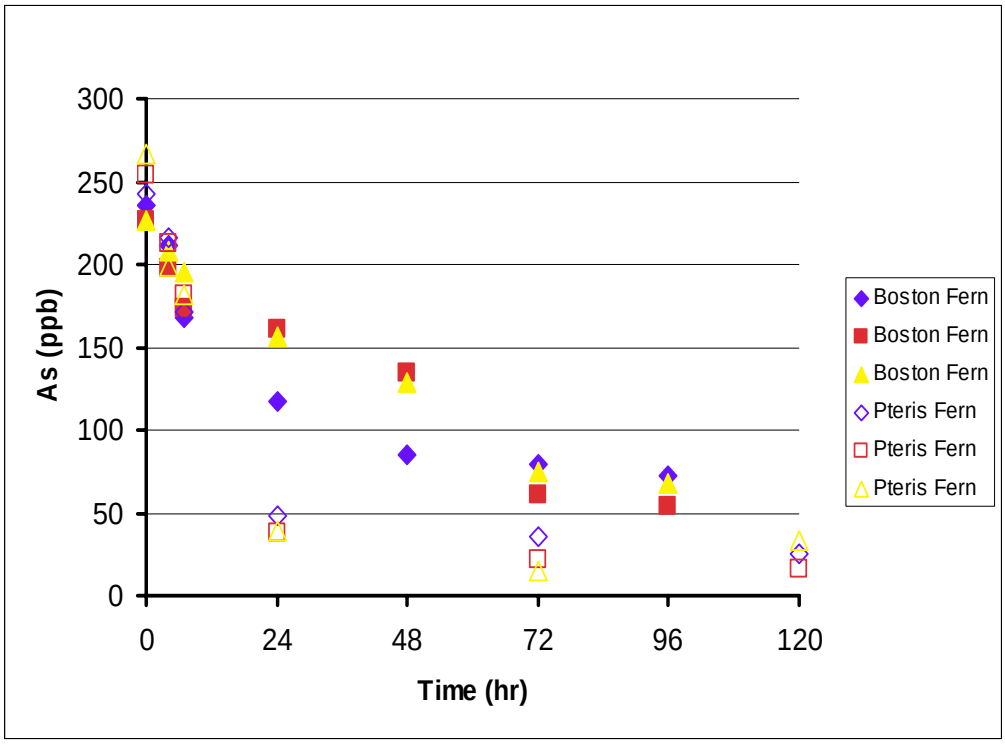

Figure 3.12 Comparing Arsenate Removal by Boston Ferns and Pteris Ferns in Presence of Fe (II) = 2.7 mg/L

The trend shown in Figure 3.11 indicates that the non-accumulating Boston fern species does little to enhance the effect of As removal by iron. When iron is present as ferrous or ferric, HFO form and adsorb As to their surface. The dominant mechanism of As removal in the presence of Boston ferns is HFO adsorption and co-precipitation. The trend shown in Figure 3.12 indicates that the removal efficiency of the *Pteris* fern in the presence of iron is significantly greater, demonstrating that As can be removed from about 250 ppb As to less than 50 ppb in a 24-hr period when the iron content is at least 2.7 mg/L. This condition provides a Fe/As ratio of 10.8. These experiments can be further compared by their rate constants as noted in Table 3.7 and Table 3.8. Figure 3.13 and Figure 3.14 depict the data fit to a first-order kinetic model.

Table 3.7 First Order Depletion Kinetics Model Parameters for Arsenate Removal by Boston Ferns in Deionized Water with Fe (II) 2.7 mg/L

Plant	k Value	Constant	R^2 Value
B2	0.0122	0.1964	0.8654
B3	0.0148	0.0407	0.9396
B6	0.0130	0.0324	0.9739
AVERAGE	0.01333	0.08983	0.92630
ST. DEV	0.00133	0.09238	0.05546
RSD (%)	9.99	---------	--------

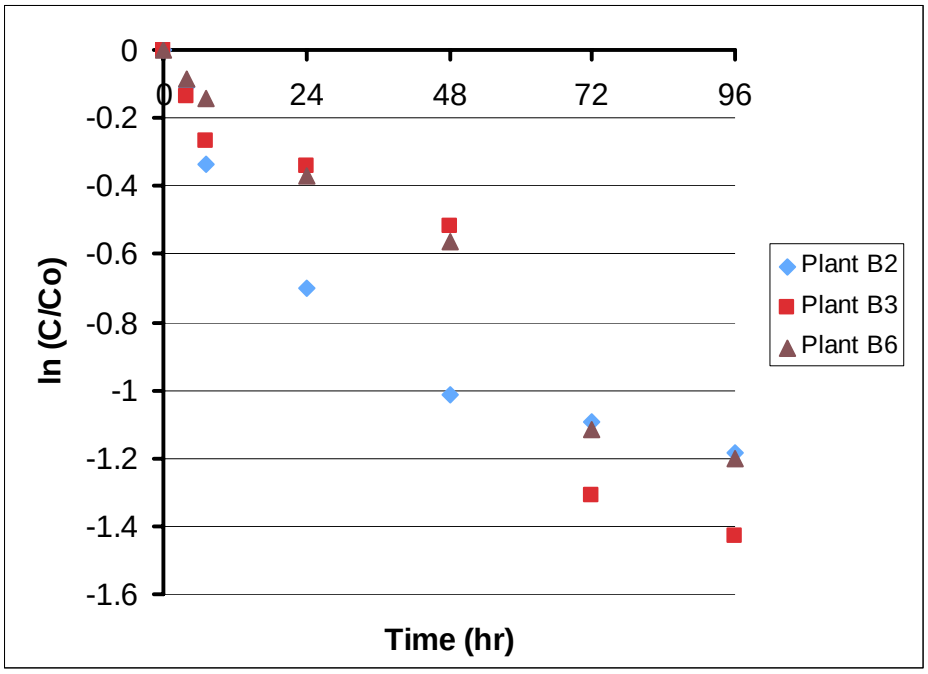

Figure 3.13 Arsenate Removal by Boston Ferns in Deionized Water with Fe (II) = 2.7 mg/L Fit to First Order Depletion Kinetics Model

Table 3.7 shows that in the case of non-hyperaccumulating Boston Ferns in the presence of Fe(II) = 2.7 mg/L, the average k value is 0.01333 ± 0.00133 hr^{-1} and a relative standard deviation of 10%. These values were derived from the graph presented in Figure 3.13. The rate constant for As removal for *Pteris* ferns in the presence of Fe(II) = 2.7 mg/L is 0.0202 ± 0.0026 hr^{-1} with a relative standard deviation of 13% as listed in Table 3.8. These values are derived from the graph presented in Figure 3.14. The effect of As removal by *Pteris* ferns and HFO co-precipitation can be described as additive or synergistic by comparing the following rate constants. Without iron, As removal by *Pteris* ferns in non-aerated deionized water with an initial As concentration of 300 ppb shows a first order rate constant of 0.009479 ± 0.00721 hr^{-1} (Table 3.11). The first order rate constant of As removal by iron alone in the presence of the non-accumulating Boston fern is 0.01333 ± 0.00133 hr^{-1} (Table 3.7). Adding these rate constants produce a combined rate constant of 0.02281 ± 0.00854 hr^{-1}. To compare this to the actual rate constant (0.0202 ± 0.0026 hr^{-1}) observed for As removal by *Pteris* ferns in the presence of iron, it appears that the combined effect of the *Pteris* ferns and iron co-precipitation is additive.

Table 3.8 First Order Depletion Kinetics Model Parameters for Arsenate Removal by *Pteris* Ferns in Deionized Water with Fe (II) = 2.7 mg/L

Plant #	k Value	Constant	R^2 Value
20	0.0184	-0.3459	0.7881
28	0.0232	-0.3936	0.8084
7	0.019	-0.542	0.5988
AVERAGE	0.0202	-0.4272	0.7318
ST. DEV	0.0026	0.1023	0.1156
RSD (%)	12.95	--------	--------

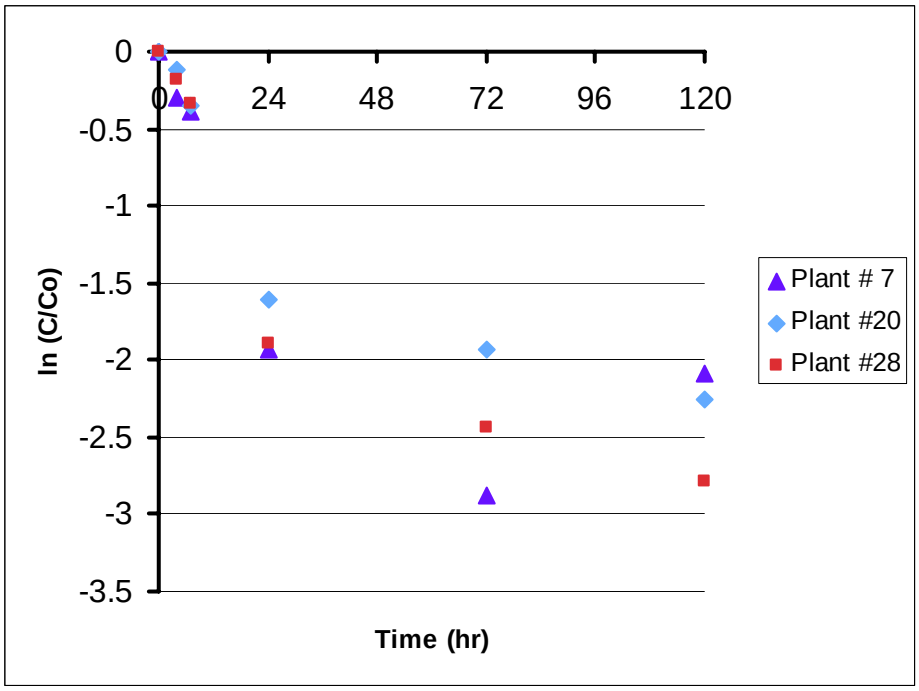

Figure 3.14 Arsenate Removal by *Pteris* Ferns in Deionized Water with Fe (II) = 2.7 mg/L Fit to First Order Depletion Kinetic Model

The k-value in the case of the *Pteris* Ferns in the presence of iron is more than 1.5 times greater than in the case of Boston ferns in the presence of iron. This indicates that the added effect of As hyperaccumulation by *Pteris* ferns enhances the effect of As removal when compared by the effect of hydrous ferrous oxide precipitation alone.

3.5 IMPACT OF REPEATED EXPOSURE ON ARSENATE UPTAKE

To test the ability of *Pteris* ferns to be used as an effective method of As remediation, it must be verified that they can handle and continue to remove As despite repeated exposure to As. To accomplish this goal, single *Pteris* ferns were exposed to an aerated experimental solution made from arsenate stock solution, diluted using DI water. Each fern experienced a period of exposure

to 150 ppb As(V) lasting for 96 hours, followed by 48 hours in a nutrient solution, and another 48 hours in 0.1mM $CaCl_2$ solution before being reintroduced to As. This regimen was repeated five times, in series, for the same set of three ferns. Their removal efficiency is tabulated in Table 3.10 and plotted in Figure 3.16.

Table 3.9 First Order Depletion Kinetics Model Parameters for Arsenate Removal by *Pteris* Ferns in Deionized Water with Fe (II) = 2.7 mg/L

Trial #	**1**	**2**	**3**	**4**	**5**
Plant #	% Removal	% Removal	% Removal	% Removal	% Removal
10	67	35.2	52.6	44.3	82.2
11	63.9	45	58.3	39.1	67.9
19	68.9	65.5	78.3	79	73.4
AVERAGE	66.6	48.6	63.1	54.1	74.5
ST. DEV	2.52	15.46	13.50	21.69	7.21
RSD (%)	3.79	31.84	21.40	40.07	9.68

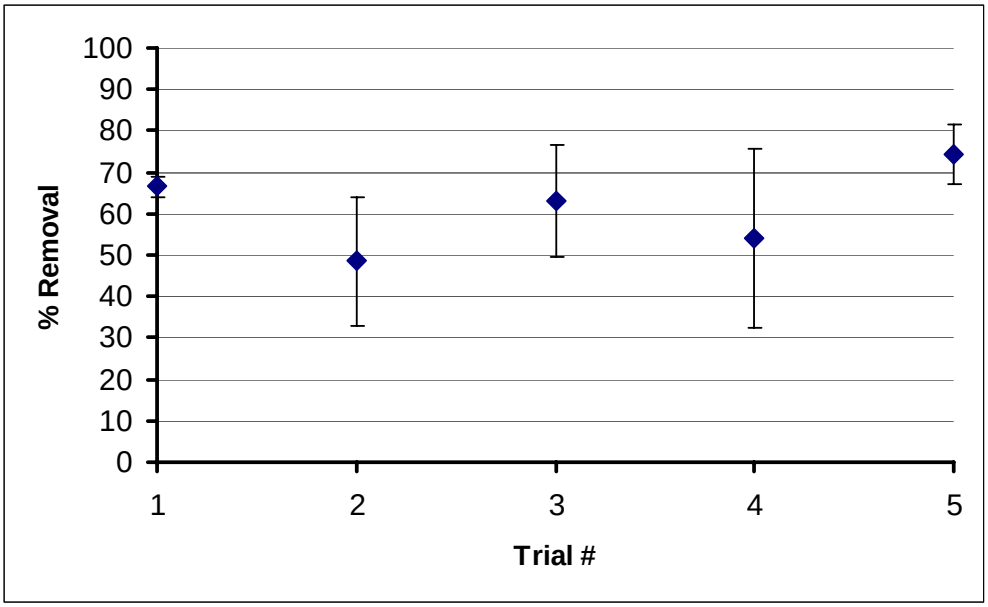

Figure 3.15 Arsenate Removal Efficiency by *Pteris* Ferns in Aerated Deionized Water

There is no clear pattern or trend of removal noted in Figure 3.16. With a 96-hr rest period between trials of As exposure, the *Pteris* ferns were able to function as effective hyperaccumulators to varying degrees. The causes of the variability noted in the observations presented in Figure 3.16 are unknown at this point and in need of further exploration.

3.6 IMPACT OF INITIAL ARSENIC CONCENTRATION ON ARSENATE REMOVAL

When applied in the field, *Pteris* ferns may encounter varied As concentrations. Knowing that the mechanism of arsenite removal is not well-known and that arsenite is readily oxidized to arsenate prior to uptake by *Pteris* ferns, the subsequent experiments focused on adding As as arsenate alone. Individual ferns were placed in mason jars containing experimental solutions prepared using the arsenate stock solution, diluted with DI water, to provide different initial

concentrations. Three ferns for each level of As concentration were selected for experimentation. The experimental solutions were not aerated due to energy efficiency considerations for future application. The average rate of As removal for three plants at each initial As concentration is shown in Figure 3.16.

To better understand the impact of initial arsenate concentration on total As removal, statistical software was recommended for analysis. To determine the extent to which the first-order kinetic model actually represents our data, statistical analysis can quantify the fit of the model with a confidence interval. Using Statistical Analysis System (SAS) software, these analyses were conducted with the assistance of a faculty member, Dr. Sati Mazumdar, and graduate student, Rick Blakesley, in the area of biostatistics. The data analyzed by the SAS software program is depicted in Figure 3.16.

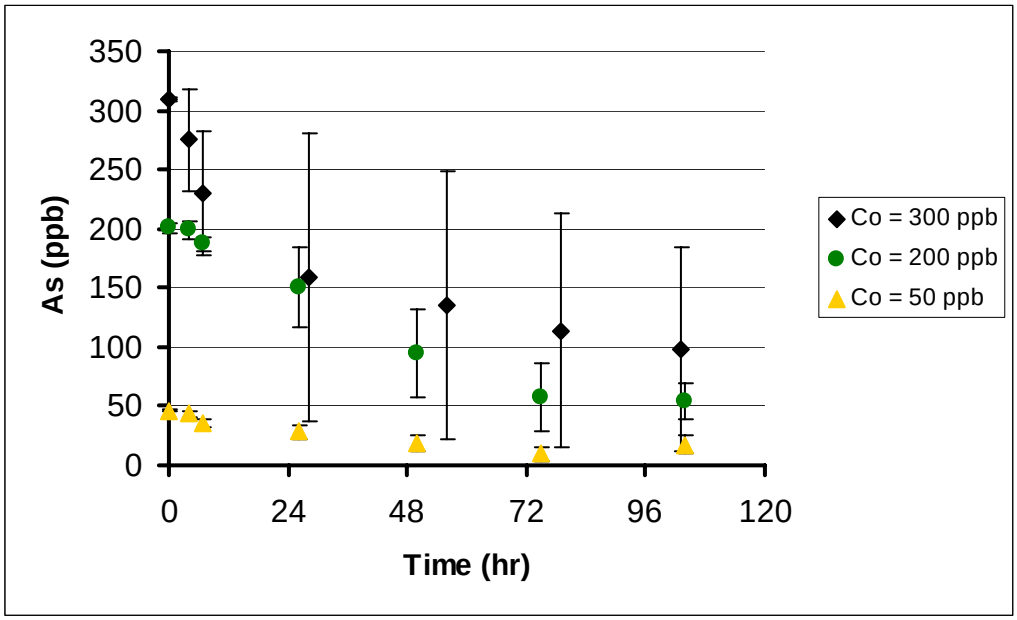

Figure 3.16 Arsenate Removal by *Pteris* Ferns in Aerated Deionized Water at Different Initial Concentrations

The statistical analysis of the experiments conducted at different initial concentrations indicated that the initial concentration has a significant effect on the rate of As removal. The null

hypothesis was that for each experiment the rate constant would equal zero and this hypothesis was clearly rejected. The results also concluded that the length of exposure time has a significant effect on As concentration in the solution. All analyses were conducted with a significance value, described as α, of 0.05. The confidence interval of the results produced are [100 - (1 - α)] %, which is 95% in this analysis. The rate constant values for experiments conducted at different initial concentrations are depicted in Table 3.11.

Table 3.10 First Order Depletion Kinetic Model Parameters for As Removal Efficiency by *Pteris* Ferns at Varied Initial Concentrations

Co (ppb)	k Value (hr^{-1})	Significance Level, α
50	0.02115 ± 0.0167	0.05
200	0.01471 ± 0.0116	0.05
300	0.009479 ± 0.00721	0.05

The use of statistical analysis offers insight to the effect of As removal at increasing initial concentrations. As displayed in Table 3.10, the experiment conducted with an initial As concentration of 300 ppb exhibits the lowest k value, indicating the slowest rate of removal, of all of the As concentration levels tested. The experiment conducted with an initial As concentration of 50 ppb exhibits the highest k value, indicating the highest rate of removal, of all of the As concentration levels tested. The average k value for experiments conducted at an initial As concentration of 200 ppb is 1.6 times greater than the rate constant for experiments conducted at an initial As concentration of 300 ppb. At an initial As concentration of 50 ppb, the rate constant is 2.2 times greater than the experiments conducted with an initial concentration of 300 ppb. Therefore, initial concentration exerts a significant effect on the rate of As removal by *Pteris* ferns. Slower removal efficiency as initial As concentration increases may indicate a reduced tolerance to As and possible toxicity as As concentration approaches 500 ppb, at which Tu and Ma [2002] report toxicity effects in *Pteris* ferns.

3.7 ARSENATE REMOVAL BY MULTIPLE *PTERIS* FERNS IN A SINGLE BATCH REACTOR

To a give a better understanding of how *Pteris* ferns can remove As in a larger population of plants, experiments were conducted with multiple ferns in a single batch reactor. The notable variability in As removal efficiency observed from plant to plant produces incredible challenges to predicting the rate of As removal or developing preliminary kinetics data. The rate of uptake for As (III) removal exhibited a significant degree of scatter when compared to that of As (V) removal. The average rate constant for total As removal in aerated DI water was 2.1 times greater than the rate constant of As removal in non-aerated DI water. The As removal rates by *Pteris* ferns in the DI water was more than 1.4 times greater than the experiments conducted in Pittsburgh tap water. Also, the experiments conducted in tap water produced results with great variability. Even with the same experimental conditions and initial As concentration as the only variable, the rate of removal varies. The irreproducibility of results makes it difficult to predict how a large population of plants will perform in the field.

11 plants were placed in a basin containing 8.8 L of solution with samples withdrawn at various locations throughout the basin as shown in Figure 3.18. By sampling throughout the basin, the total As content could be tested as consistent throughout the basin. Each fern was proportionally exposed to the equivalent fern to water volume ration of 1 fern: 800 mL observed in earlier experiments conducted with one plant in a single vessel. The group of ferns was repeatedly exposed to 250-300 ppb of As (V) every 7 days with the aqueous As content monitored daily. The average As content in the experimental solution for each exposure trial is presented in Figure 3.18.

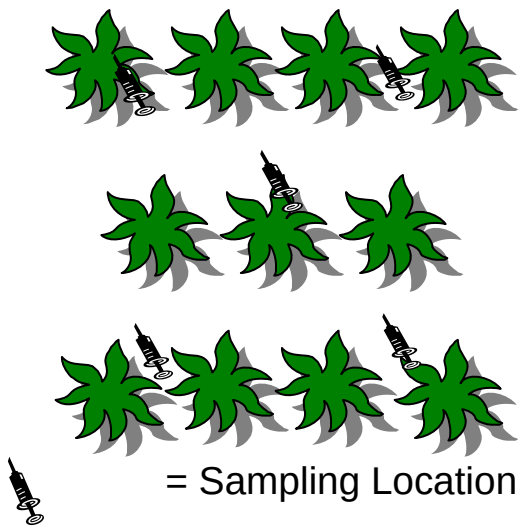

Figure 3.17 Illustration of Sampling Location in Batch Reactor

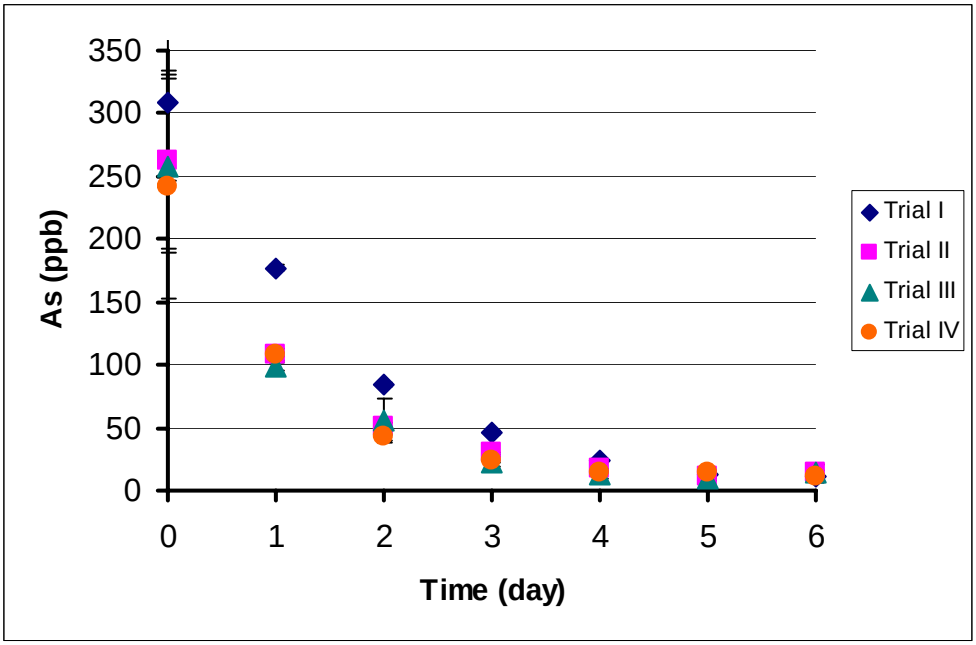

Figure 3.18 Arsenate Removal by Pteris Ferns in Deionized Water in Batch Reactor for Successive Exposure Periods

Figure 3.18 displays that in each As exposure trial, the current Bangladeshi maximum contaminant level of 50 μg/L is met after 3 days of treatment by the *Pteris* ferns. After 5 days of treatment, the current U.S. EPA maximum contamination level of 10 μg/L is met. When individual ferns were placed in a solution of 250 – 300 ppb As (V) without aeration, as shown in Figure 3.7, most of the plants were able to reduce the As content to less than 50 ppb after 4 days. The improved effect of multiple ferns in a single batch reactor may be due to the competitive effect amongst the ferns. The solution was prepared with DI water and all of the ferns are competing for As as a 'nutrient' source since no other ion is present in any significant quantity. Also, the total As removal efficiency in this experiment is an average of each individual plant's efficacy. With the results observed in the batch experiments, the possibility of developing a kinetics model for field application becomes a more realistic possibility. The results obtained were analyzed and provide the following statistical results displayed in Table 3.12.

Table 3.11 First Order Depletion Kinetics Model Parameters for Arsenate Removal by Pteris Ferns in Batch Reactor

Batch	k Value (day^{-1})	LCL (day^{-1})*	UCL(day^{-1})**
I	0.6159	0.6296	0.6022
II	0.6093	0.6309	0.5876
III	0.6453	0.6743	0.6164
IV	0.6088	0.6311	0.5866
AVG	0.6198	0.6415	0.5982
ST DEV	0.01729	0.02189	0.01407
RSD (%)	2.78	3.41	2.35

*** LCL: Lower Confidence Limit**
**** UCL: Upper Confidence Limit**

The removal efficiency of multiple plants versus individual plants in a single batch reactor can be evaluated by comparing their respective rate constants. For multiple plants in a single batch reactor, a significant correlation is noted for the first order rate constants, despite the successive trials. The average k value in this case is 0.6198 ± 0.0173 day^{-1} or 0.02583 ± 0.000721 hr^{-1} and a relative standard deviation of 3 %. These preliminary results suggest that with a plant ratio of 1 fern for each 800 mL of solution containing 250-300 ppb As, the 50 μg As/L standard can be met following 3 days of exposure and the 10 μg As/L standard can be met following 5 days of exposure. The individual plants in a single batch reactor without aeration have a rate constant of 0.0179 ± 0.0037 $hr^{-1,}$ as shown in Table 3.4. This observation demonstrates that the rate of As removal by multiple plants in a single batch reactor is 1.4 times greater than that of individual plants. Therefore, the composite effect of multiple ferns working together is much greater than the single fern's independent ability.

4.0 SUMMARY AND CONCLUSIONS

The results of this work are useful in planning and designing a system where *Pteris* ferns can contribute to the constant provision of a drinking water supply with significantly reduced concentrations of As. The *Pteris* ferns were examined in a variety of conditions for the development of useful design parameters. The results of this work can be summarized in the following points.

- *Pteris* ferns are capable of removing As from aqueous solution regardless of its form (arsenite, arsenate or both).
- *Pteris* ferns function more efficiently in aerated solutions but the lack of aeration can be compensated by extending the contact time in non-aerated solutions.
- *Pteris* ferns function more efficiently in the absence of competing anions as found when comparing results in deionized water and in Pittsburgh tap water.
- At initial As (V) concentrations of 50 ppb, 200 ppb, and 300 ppb, the first order decay rate constants, k, are 0.02115 ± 0.0167 hr^{-1}, 0.01471 ± 0.0116, and 0.009479 ± 0.0781, respectively decreasing as initial As concentration increased.
- *Pteris* ferns can withstand repeated exposure to high concentrations of As (150 -300 ppb) for up to four trials and maintain high levels of removal efficiency.
- With experiments conducted in a batch reactor containing 11 plants, the first order decay rate constant, k, is 0.6198 ± 0.0173 day^{-1} or 0.02583 ± 0.000721 hr^{-1}. This is greater than the k values for experiments conducted with individual plants.
- In a single batch reactor containing multiple plants, *Pteris* ferns are cooperatively capable of removing As from 250-300 pbb to less than 50 ppb in a 3-day period, consistently with little variability amongst four successive exposure trials.

- In the presence of Fe (II) = 2.7 mg/L, *Pteris* ferns can remove As from 250 ppb to less than 50 ppb in a 24-hr period (Fe/As = 10.8).
- The combined effect of As removal by *Pteris* ferns and HFO co-precipitation can be described as additive.

4.1 PRELIMINARY DESIGN CONSIDERATIONS

The usefulness of this work lies in its ability to translate to real-world application. Based on the observations noted in this research effort, a preliminary design can be developed that would require further modification based on pilot studies and adaptation to the environment in which it will be applied. To begin with a modest goal of supplying 50 L/day, which can supply a family of approximately seven people with an adequate amount of potable drinking water according to the WHO's recommendation of 7.5 L/day/person, the following preliminary design considerations can be offered. The size and age of the plants used in this project can be described as approximately 8 ± 2 g in mass and 37 ± 10 mL in root volume. Plant age is not the best means of characterization because of the cyclical nature of growth patterns observed by *Pteris* ferns. Therefore, the *Pteris* fern can be described by its approximate upper biomass and root volume. The maximum capacity of the *Pteris* ferns is reported in the literature as ranging between 1,440-7,520 mg/kg (Ma et al, 2001) and the water volume treated by each plant was 0.8 L.

Our first design assumption is that with an initial As concentration of 300 ppb in the absence of iron, the ferns accumulate 232 μg ((300 μg/L – 10 μg/L) * 0.8 L = 232 μg) during each treatment period (Figure 3.19). At an initial As concentration of 300 ppb in the presence of Fe (II) = 2.7 mg/L without *Pteris* ferns, the hydrous ferrous oxide alone removes 200 ppb of As from the solution within the first 2 hours (Figure 3.12). Therefore, the *Pteris* ferns would only remove 72 μg ((100 μg/L – 10 μg/L) * 0.8 L = 72 μg) in the presence of Fe (II) = 2.7 mg/L.

4.2 DESIGN CONSIDERATIONS FOR GROUNDWATER FREE OF IRON CONTENT

To supply 50 L/day x 1 plant/0.8 L = 62.5 plants ≈ 63 plants

In a rectangular basin, arrange 4 plants by width and 16 plants by length (Figure 4.1).

Basin dimensions: 3 ft x 1 ft x 1 ft = 3 ft^3 = 0.085 m^3

Water volume: 3 ft x 1 ft x 0.7 ft = 2.1 ft^3 = .06 m^3 = 60 L

Assuming 5% of water volume is loss due to transpiration and evaporation, treated volume of water supplied is 57 L.

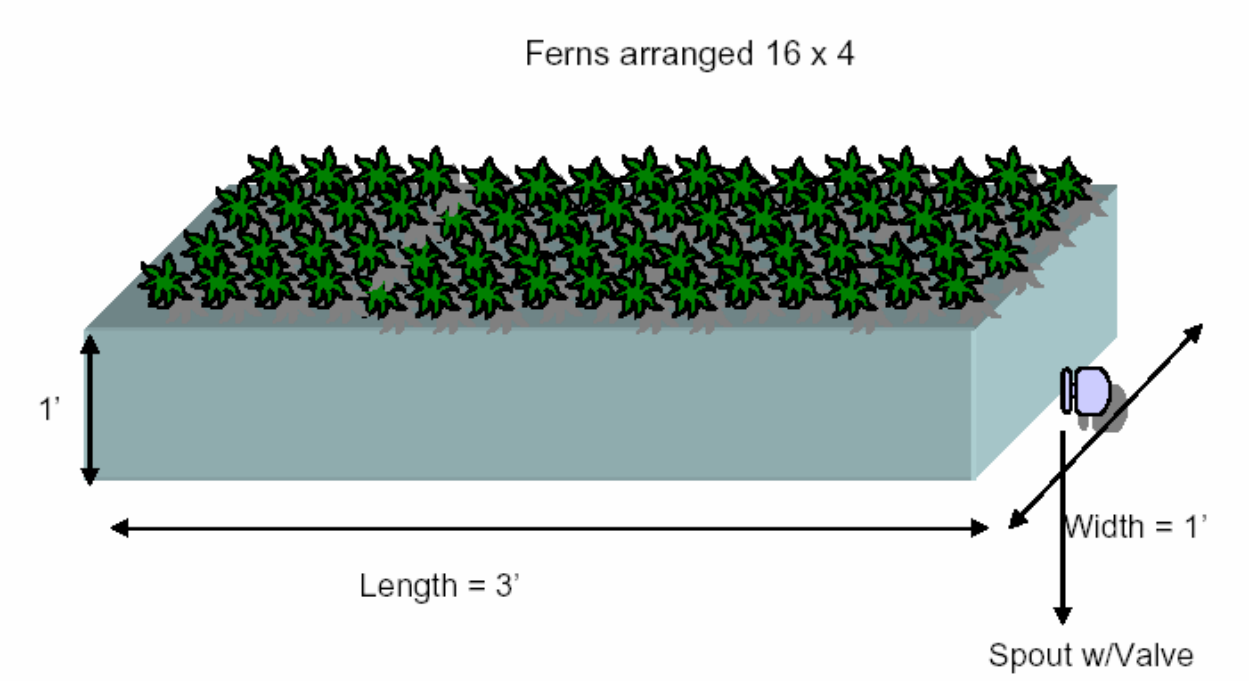

Figure 4.1 Schematic of *Pteris* Fern Batch Reactor

The number of basins to be utilized depends on the required detention time. If a detention time of five days is established by the pilot studies, six identical basins arranged with 64 plants in each basin should be provided with the ferns suspended by a meshed material that can consist of lightweight wire, plastic, etc. Assuming average amount of As accumulated by

each plant is 2000 mg/kg or 2 mg/g and the average mass of ferns in this project is 8g, this allows for a total possible accumulation of 16 mg of As.

If each treatment exposure accumulates 232 μg of As, approximately 69 treatment periods can be replicated before each plant reaches their maximum capacity. To ensure the sustainability of this effort and long-term use of the *Pteris* ferns, the plants should have a rest period. In this project, it has been noted that plants require 3-5 weeks to effectively grow new fronds. Therefore, the recommended rest period is 40 days. The extra basin included in the design is for an extra set of 64 ferns that can be rotated in and out of active treatment to provide the needed respite. An eighth or 12.5% of the ferns can be removed every 40 days or after approximately 8 treatment periods. In this case, the maximum amount of treatment periods a single fern will experience before being removed from the active treatment basin is 64 exposure periods. The ferns can be replaced with plants that have been removed from As exposure and are watered with uncontaminated water supplied by the excess treated water or by collected rainwater. This respite period will allow the ferns to grow new fronds and prepare for re-exposure to As. A total estimate of 384 *Pteris* ferns will be necessary to support this system.

The projected land space for this design entails an edifice that is approximately 14 ft x 7 ft for a total square area of 98 ft^2 (9.2 m^2). The treatment basins should be arranged on tables or stands to prevent debris from entering the water supply. It is estimated that this system can be maintained by one family member with no electrical input if sufficient lighting and humidity levels are met. The task requirements including pumping well water for the treatment basins, collecting treated water for use, pruning the ferns as necessary, disposing of the pruned ferns to a compost container for ultimate disposal as a hazardous waste, watering the ferns in respite with rainwater or treated water from the extra basin, and general maintenance and care for the plants.

If iron content of at least 2.7 mg/L is present in the water system being treated, a smaller system can be established due to the shorter exposure period time and less accumulation by *Pteris* ferns in each exposure period. Accumulating only 72 μg in each treatment period and assuming that only 2 days of contact time are needed for adequate As removal, requires only three of the basins described earlier. For plants of the size used in this project, the *Pteris* ferns in the presence of iron should be able to withstand 222 exposure trials (16 mg of total As accumulation capacity/0.072 mg accumulated in each exposure period). The third basin would be for the ferns in need of respite. Eight of the used ferns can be replaced every 40 days or after 20

exposure periods. The maximum period of time a single fern in this system would be in active treatment is 160 exposure periods. The total plants necessary for this system is 192 (64 plants/basin x 3 basins). However, due to hydrous ferrous oxide precipitate that is laden with As in the system, a disposal method must be developed for this generated waste. The treated water would need to be filtered and the retained precipitate disposed of appropriately as a hazardous waste.

If it is concluded that this is an extensive investment for a system that only supplies 50 L/day, it must be noted that these design considerations are based upon a smaller variety of the *Pteris* ferns. *Pteris* ferns of the size used in this research project may not be the most suitable for real-world application. It is possible that larger plants with greater root volume and rates of removal efficiency would be better suited for a system that requires less maintenance and care. However, further research would need to be conducted to evaluate the specific design considerations.

4.3 DESIGN CONSIDERATIONS FOR LARGER PLANTS

If the same design considerations are used for *Pteris* ferns the size of those used in a pilot-scale demonstration in Albuquerque, NM (Elless et al, 2005), the design should be updated as follows:

To supply 50 L/day x 1 plant/6 L = 8.33 plants ≈ 8 plants

In a rectangular basin, arrange 2 plants by width and 4 plants by length.

Basin dimensions: 3 ft x 1 ft x 1 ft = 3 ft^3 = 0.085 m^3

Water volume: 3 ft x 1 ft x 0.7 ft = 2.1 ft^3 = .06 m^3 = 60 L

Assuming 5 % of water volume is loss due to transpiration and evaporation, treated volume of water supplied is 57 L.

The amount of basins to be replicated depends on the necessary detention time. If a detention time of three days is observed in the pilot studies, four identical basins arranged with 8 plants in each basin should be established with the ferns suspended by a meshed material that can consist of lightweight wire, plastic, etc.

Assuming average As accumulation by each plant of 2000 mg/kg or 2 mg/g. The average mass of ferns in this project is 27 g, which allows for a total possible accumulation of 54 mg. If each treatment exposure accumulates 232 μg, approximately 232 treatment periods (54 mg of total As accumulation capacity/ 0.232 mg As accumulated in each exposure period) can be replicated before each plant reaches their maximum capacity. If 1 plant is removed every 40 day period or after 13 treatment periods, the maximum amount of trials a single plant would be exposed to As is 106 treatment periods. The extra basin in the design would contain the plants that have been removed from As exposure for rotation in and out of the system. A total amount of 56 ferns would be required to support this system (8 plants/ basin x 4 basins= 32 plants). The projected land space would also be 85 ft^2 (8 m^2). This system design for larger *Pteris* ferns can be maintained by one family member with space and labor input being the most significant constraint.

5.0 RECOMMENDATIONS FOR FUTURE WORK

Accumulation by *Pteris* ferns has the potential to be an effective, inexpensive, and easily operated method of arsenic (As) removal. The operating conditions examined in this research effort have offered insight as to how the use of *Pteris* ferns can be optimized and enhanced, which is essential for a preliminary treatment design. Experiments conducted with multiple ferns in a single batch reactor gives confidence that the field application of this treatment method can be far-reaching. As this was a new research project, the start-up challenges were many. Significant effort and energy was necessary to simply maintain the health and viability of the plants and provide an environment in which they could thrive. Many revisions of the experimental protocol were adopted and the research journey has been dynamic. Nonetheless, a foundation has been laid upon which future work can be built in hopes of attaining the ultimate goal of providing As-free drinking water to those in need.

There are many recommendations for enhancing the results and strengthening the conclusions presented in this thesis. Because the statistical analysis tools were introduced at a late stage in this project, all of the experiments were not designed in a manner necessary to produce sound statistical results. All future experiments should begin with a thorough experimental design process and training in the appropriate statistical tools, like SAS software, for data analysis.

The As removal efficiency of different fern population sizes should be compared, as well as different plant sizes and fern to water volume ratios. Pittsburgh groundwater or simulated groundwater should be used in the experiments to mimic actual groundwater conditions experienced in the field. The experiments with multiple plants in a single batch reactor should be repeated to determine what period of time *Pteris* ferns can be exposed to high concentrations of As before removal efficiency decreases.

The recommendations mentioned here could prove to impact this research effort and allow for hyperaccumulation by *Pteris* fern to be regarded as a viable alternative of arsenic remediation in the near future.

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