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Phytoremediation of arsenic-contaminated groundwater using arsenic hyperaccumulator *Pteris vittata* L.: Effects of frond harvesting regimes and arsenic levels in refill water

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ABSTRACT

A large-scale hydroponic system to phytoremediate arsenic-contaminated groundwater using *Pteris vittata* (Chinese brake fern) was successfully tested in a field. In this 30-wk study, three frond-harvesting regimes (all, mature, and senescing fronds) and two water-refilling schemes to compensate for evapotranspiration (high-As water of 140–180 μg/L and low-As water of <7 μg/L) were investigated. Two experiments (*Cycle 1* and *Cycle 2*) were conducted using the same plants in 24 tanks with each containing 600 L of arsenic-contaminated groundwater and 32 ferns. During *Cycle 1* and with initial As of 140 μg/L, As in tanks refilled with low-As water was reduced to <10 μg/L in 8 wks compared to <10 μg/L in 17 wks in tanks refilled with high-As water. During *Cycle 2* and with initial As of 180 μg/L, the remediation time was reduced by 2–5 wks, indicating that more established ferns were more efficient. In areas where clean water is limiting, refilling high-As water coupled with harvesting senescing fronds is recommended for more effective As phytoremediation.

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1. Introduction

Arsenic (As), a carcinogenic metalloid, is ubiquitous in the environment. Human activities including mining, smelting, and As-based pesticides are major sources of arsenic contamination in the environment [1,2]. Chronic exposure to arsenic via drinking contaminated water (>50 μg/L) leads to cancers, birth defects, and other diseases [3].

In the past, industrial sites such as electrical power substations across the United States used As-based herbicides for many years to control weeds. This practice has resulted in arsenic contamination in soil and groundwater. Since many of these sites are located in densely populated residential areas, many people may be at risk of arsenic exposure. Conventional technologies such as membrane separation, ion exchange and nanotechnology to clean As-contaminated water have significant limitations in terms of both cost and substrate disposal issues [4,5].

Phytoremediation uses plants to remove contaminants from the environment. Many bench scale and greenhouse studies were conducted using arsenic hyperaccumulator *Pteris vittata* L. [6]. These studies showed that this fern has potential as a biofilter for As-contaminated groundwater [7–10]. However, cost-effective cultivation practices to grow the ferns have not been well defined because this fern is of little commercial importance. Few studies determined its nutritional requirements and biomass harvesting methods [11–15]. Developing environmentally safe cultivation practices at field scale are also limited. Hence, cost effectiveness, environmental safety, and practical applicability have been of concerns for effective use of phytoremediation in cleaning up arsenic-contaminated sites.

For cost-effective commercial applications of As-phytofiltration using *P. vittata*, several operational constraints have not been addressed: (1) labor cost for harvesting the aboveground As-rich biomass and hazardous biomass handling, (2) additional care while air-drying, storing and transporting As-rich biomass because of the potential problems of human exposure and arsenic leaching from the fronds [16,17], and (3) disposal costs for harvested biomass at waste management facilities. Typically, arsenic concentrations exceeding 73 mg/kg are considered as hazardous and the tipping fee ranges from $75 to 100/ton [Personal communication to EPA].

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Fig. 1. Photos of a field-scale arsenic phytofiltration setup in hydroponic tanks: (a) 600 L capacity hydroponic tank with aeration system, arrows indicate aeration nozzle locations and direction of air flow, (b) Styrofoam float holding 32 net pots with fern plugs, (c) overall arrangement of hydroponic tanks under rainout shelter with fully grown ferns, and (d) fully established ferns with extensive root system.

Hence, the question arises—what is the best method to harvest the As-rich fronds for cost-effective As-phytofiltration while sustaining good plant growth? A suitable harvest method is necessary for cost-effective As-phytofiltration and to make this technology viable for practical application.

An environmental concern that has not been addressed when using this phytofiltration method is secondary contamination from fertilizer application, particularly nitrogen and phosphorus. A recent study suggested application of 200 mg/kg NH_4^+-N as the preferable fertilizer for *P. vittata* to maximize As-removal [11]; however the study failed to monitor nutrients leaching from the pots. Such leaching could result in secondary environmental contamination.

In a batch system as used in this study, it is critical to track water volumes to calculate arsenic mass balance over the long term. Hence water was refilled to replace evapotranspiration (*ET*) loss. Preliminary field studies indicated that ~10% of the water in 600-L hydroponic tanks holding 32 ferns was lost through *ET* in 2 wks. In most bench scale or greenhouse experiments, the amount of water lost through *ET* (<1 L) was replaced with water or nutrient solution. This may not be applicable in a large-scale operation on a contaminated site where clean water may be limited. A pilot study using dynamic As-phytofiltration where 10 hydroponic tanks (8 ferns/45 L) were connected in a series and continuously filled with slightly As-contaminated water (~14 μg/L) has shown promising results [15]. However, the long-term effects on the ferns and As decontamination when using water contaminated with high arsenic concentrations is unknown. Hence, two water refill methods, one with high-As water (140 and 180 μg/L) and another with low-As water (As <7 μg/L) was compared in this study.

To address the concerns for practical application of *P. vittata* in phytofiltration, a large-scale field demonstration on phytofiltration of As-contaminated groundwater was conducted for the first time. The objectives were to: (1) demonstrate the feasibility of using *P. vittata* to effectively remediate arsenic-contaminated groundwater without producing secondary contamination from using fertilizers; (2) study the effects of frond harvesting regimes and arsenic levels in refill water on phytofiltration by *P. vittata*, and (3) test the reusability of *P. vittata* ferns for long-term As phytofiltration.

2. Materials and methods

2.1. Rainout shelter and hydroponic tanks

A rainout shelter (14.6 m × 25.6 m) covering an area of approximately 375 m^2 was installed to accommodate 25 hydroponic tanks of 600 L capacity each at an electrical power substation in Florida. Its roof was covered with two layers of 30% light exclusion shade cloth (gray ChromatiNet, Polysac Plastic Industries, Negev, Israel) with a 6 mil clear greenhouse poly cover in the middle layer. Side and end walls were covered with one layer of the 30% light exclusion cloth to allow for air exchange (Fig. 1a).

Hydroponic tanks were built on a leveled sand base covered with woven polypropylene ground cloth (Fig. 1b). They were all equipped with an aeration system consisting of PVC pipe and eight brass nozzles with 1.6 mm diameter orifices. The tanks were built with 5 cm × 25 cm non-arsenic containing pressure-treated (copper oxide and quaternary compounds, ACQ) lumber. The hydroponic tanks, with inside dimensions of approximately 1.2 m × 2.4 m × 0.24 m, were lined with 10-mil thick black

polyethylene sheet. All tanks were placed at least 3–4.3 m inside the rainout shelter to prevent rain water from getting into the tanks.

2.2. Two experiment cycles using P. vittata

This field experiment was conducted in two cycles (*Cycle 1* and *Cycle 2*) using *P. vittata*. *Cycle 1*—plants germinated from spores at the Mid-Florida Research and Education Center of University of Florida were used. Thirty-two plants at the 6–8 fronds stage (~10 months after germination), growing in rockwool plugs (horticultural rock wool, AgroDynamics, Coppell, TX) were planted in each tank (one fern/ft^2) (Fig. 1c). Each plug was placed in an 8-cm net pot suspended through a hole in 5-cm thick, 2.4 m × 2 m extruded closed-cell polystyrene (Styrofoam®, Dow Chemical, Midland, MI), which was floating in a hydroponic tank filled with 600 L of As-contaminated groundwater (~140 μg/L). Initially, each tank was filled with 600 L of groundwater using a flow meter (M150, Elster Metering Ltd., Luton, Bedfordshire, UK) to measure water volume. The water level was then marked so that the tanks could be refilled biweekly to the original level to compensate *ET* loss. All tanks were amended with weak fertilizer (0.25 strength Hoagland's solution #2) with nitrogen and phosphorus concentrations reduced further to 21 and 1.2 mg/L, respectively.

Cycle 2 was initiated after discharging the remediated water and refilling the tanks with 568 L of fresh As-contaminated groundwater. The volume of water in the tanks was reduced in this cycle to accommodate the extensive root system of the now established ferns (Fig. 1d). Also, it should be noted that As concentration in the water pumped from the same well during *Cycle 2* was higher than *Cycle 1*, i.e., 180 vs. 140 μg/L.

Throughout the experiment, one tank filled with As-contaminated water at 140 μg/L with a Styrofoam float and Rockwool-filled net pots with no ferns was maintained as a control, and water lost through evaporation was replaced biweekly with low-As water.

2.3. Water refill treatments and water sampling

Water lost due to *ET* was replaced biweekly with water from two sources—a well with contaminated groundwater (~140–180 μg/L, high-As water) or a well with clean groundwater (<7 μg/L, low-As water). Every 2 wks, water was refilled to initial level for each tank. Fertilizer solution, calibrated to the amount of replacement water, was added. After allowing for overnight mixing, 10 mL of water samples were collected for arsenic analysis. Nitrogen and phosphorus concentrations were monitored monthly.

2.4. Frond harvesting regimes

The three frond harvesting regimes included (1) periodically cutting off all fronds 15 cm above rhizomes (all fronds), (2) selectively harvesting matured fronds (mature fronds), and (3) infrequently harvesting senescing fronds (senescing fronds). Overall, fronds were harvested three times during the experiment: *1st harvest* was 8 wks after initiation (all fronds and mature fronds), *2nd harvest* occurred 11 wks after the *1st* harvest or 2 wks after *Cycle 2* (mature fronds and senescing fronds) and *final harvest* at 30 wks after initiation (all fronds). At each harvest, three representative sub-samples among 32 plants per tank were harvested and bagged separately for As-analysis, and the remaining 29 plants were also harvested to obtain total frond biomass per tank.

2.5. Arsenic analysis

Biweekly water samples were analyzed for total arsenic using an inductively coupled plasma mass spectrometer (HP 4500 ICP-MS, Hewlett-Packard). Blanks, calibration and internal standards, and calibration checks were included for quality assurance/quality control. Fern tissue from each harvest was bagged separately for each tank and oven dried at 55 °C for 5 days. Dried plant samples were ground to fine powder (20 mesh) and digested with concentrated HNO_3 and H_2O (1:1 v/v), followed by 30% H_2O_2 for As determination [18]. The tissue As-concentration was determined using a graphite furnace atomic absorption spectrophotometer (SIMMA 6000; PerkinElmer, Wellesley, MA). Blanks and internal standards were included for quality assurance/quality control.

2.6. Nitrogen and phosphorus analysis

Water samples were analyzed for NO_3-N and PO_4-P. Nitrate content was determined using a combination nitrate-ion selective electrode (Accumet®Model 13-620-534, Fisher Scientific, Pittsburg, PA, USA) connected to a pH/conductivity meter (Accumet®Model 20, Fisher Scientific). Phosphate content was analyzed by a colorimetric method based on ascorbic acid reduction of ammonium phosphomolybdate complex (blue color) [19]; the absorbance was determined by a UV–visible spectrophotometer (UV160-U, Shimadzu Corporation, Columbia, MD, USA).

2.7. Experimental design and statistical analysis

This was a 3 harvest regimes × 2 water sources factorial experiment in a completely randomized block design with 4 replicates. Data were analyzed using PROC GLM and ANOVA procedures of SAS [20]; Tukey's least significant differences (LSD) test was used to compare the means.

3. Results and discussion

3.1. P. vittata reduced As in groundwater from 140 to <10 μg/L in 8 wk in Cycle 1

P. vittata was effective in removing arsenic from contaminated groundwater in all tanks with ferns. As expected, arsenic concentrations in tanks refilled with high-As water were much higher than those with low-As water (Fig. 2a). After 8 wk, arsenic dropped to <5 μg/L in low-As water treatments and 40 μg/L in high-As water treatments. It took an additional 9 wk for As concentration to drop to <5 μg/L in high-As water treatments.

All-frond and mature-frond harvest was performed during *1st harvest* (after 8 wk of growth) whereas mature-frond and senescing-frond harvest was performed during *2nd harvest* (after 11 wk of growth) (Table 1). The three harvest regimes in *Cycle 1* showed no effect on arsenic reduction rate in both water refill treatments (Fig. 2a). Similar conclusions were derived from our previous greenhouse studies, where ferns with harvest (15-cm from rhizome) and without harvest were equally effective in removing arsenic from water [10]. Earlier studies indicated that traits of *P. vittata* such as its perennial nature, high arsenic accumulation in the fronds, rhizomes as alternate tissue for As storage, and little arsenic in the roots, all made it suitable for reuse in long-term As phytofiltration [9,10,21,22]. Hence, in this study, at the end of *Cycle 1* (17 wks), remediated water from all tanks with ferns was discharged and the plants were reused for *Cycle 2*.

3.2. P. vittata reduced As in groundwater from 180 to <10 μg/L in 6 wk in Cycle 2

Cycle 2 was initiated by filling all tanks with ferns with fresh As-contaminated water containing 180 μg/L As. Biweekly water refill for *ET* loss and water sampling for As analysis were repeated as

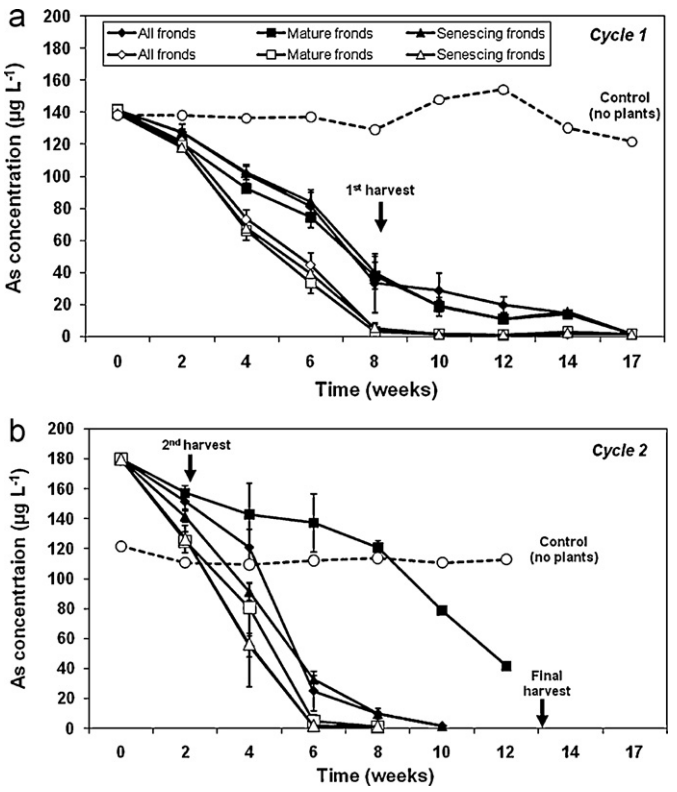

Fig. 2. Effects of arsenic levels in refill water and frond harvesting regimes on As phytofiltration using *P. vittata* in two cycles: (a) *Cycle 1* started with 140 μg/L As and lasted 17 wk, and (b) *Cycle 2* started with 180 μg/L As after harvesting ferns from *Cycle 1* and lasted 13 wk. The solid points represent high-As water (140/180 μg/L) and open points represent low-As water (<7 μg/L) refill. All fronds were harvested periodically 15 cm above rhizomes, mature fronds were harvested selectively, and senescing fronds were harvested infrequently. Water in control tanks contained 140 μg/L As and refilled with low-As water biweekly. Error bars are means ± SE.

in *Cycle 1*. During this cycle, mature and senescing fronds were harvested 2 wk after the 2nd cycle (*2nd harvest*) (Fig. 2b).

By reusing established fern plants, As concentrations declined from 180 μg/L to <5 μg/L in 6 wk (2 wk shorter than *Cycle 1*) with low-As water, whereas it took 10 wk (7 wk shorter than *Cycle 1*) with high-As water (Fig. 2b). The more established plants, with more root and frond biomass (Table 1), were more efficient in As removal [15]. Similar results were reported in our greenhouse experiments, wherein reusing ferns dropped phytofiltration time from 35 d to <1 d due to increased biomass [9]. However, in *Cycle 2* of this experiment, in the treatment with mature-frond harvest and refill with high-As water, it took >12 wk to reduce arsenic concentration to <10 μg/L.

The slower As-removal was attributed to the slower regrowth of *P. vittata*. This is because efficient As uptake by *P. vittata* depends on its extensive root and frond growth. Poor performance of the ferns in this treatment was due to multiple stresses on the ferns from frequent harvesting practice (2 harvests in 18 wks) and continuous As exposure that affected rhizome vigor and the ferns' regrowth potential [8,23].

During the two cycles, the control tank with no plants maintained a high As concentration, confirming the ferns' major role in As removal (Fig. 2). It was refilled with low-As water biweekly to compensate for *ET* loss. During both cycles, arsenic in the control tank dropped gradually, from 140 to 122 μg/L over 17 wk (~13% reduction) in *Cycle 1* and from 122 to 113 μg/L over 13 wk (~7% reduction) in *Cycle 2*. Though the level of arsenic reduction was not substantial, some arsenic was lost in the control tank. In a hydroponic study, we examined optimum P levels on arsenic removal from groundwater by *P. vittata* [24]. With no P being supplied, 100% arsenic removal is accounted by plant removal. However, the amount recovered by *P. vittata* decreased from 70 to 35% as P increased from 150 to 600 μm, an indication of microbially mediated arsenic methylation, which is currently being investigated.

3.3. Healthy ferns were more efficient in arsenic removal

For successful phytofiltration, production of plant biomass is always an important factor. For instance, low efficiency of certain aquatic plants in metal removal, such as duck weed (*Lemma minor*), water velvet (*Azolla pinnata*) and pennywort (*Hydrocotyl umbellate*), was primarily due to their low biomass production [25]. *P. vittata* has great potential for As phytoextraction from contaminated site because of its hyperaccumulation trait and high biomass production [6,9]. However, harvesting regimes to remove As-rich biomass has not been well studied. Hence, in this study, three frond harvesting regimes were compared to determine the best method for biomass removal to achieve cost efficiency and high biomass production.

Fronds harvested three times during the entire experiment (*1st*, *2nd* and *final harvest*) (Table 1). Compared to the *1st harvest*, frond biomass from mature-frond harvesting was almost doubled from *2nd harvest*. During *final harvest*, when all fronds were harvested, frond biomass in the treatment with mature-frond harvesting and high-As water refill was 0.6 kg/tank. In these ferns, the rhizome

Table 1
Effects of arsenic levels in refill water and frond harvesting regimes on biomass (g/tank dw) from three harvests of *P. vittata* grown in As-contaminated groundwater.

Treatments		Fronds				Roots
Arsenic in refill water	Frond harvest regimes	*1st harvest* (8 wk)	*2nd harvest* (11 wk)	*Final harvest* (11 wk)	Total	Total
High-As water (140–180 μg/L)	All fronds	164		1227	1421	534
	Mature fronds	220	403	598	1221	430
	Senescing fronds		112	1574	1686	638
Low-As water (<7 μg/L)	All fronds	170		1591	1790	754
	Mature fronds	241	435	1404	2080	821
	Senescing fronds		96	1507	1603	719
Significance						
Refill Water Source (RWS)		*	ns	**	**	**
Frond Harvest Regimes (FHR)		***	–	**	ns	*
RWS × FHR		ns	–	*	*	*

ns indicates not significant at $P < 0.05$.
* Significance at $P < 0.05$.
** Significance at $P < 0.01$.
*** Significance at $P < 0.001$.

Table 2
Effects of arsenic levels in refill water and frond harvesting regimes on As concentrations in the biomass (mg/kg dw) from three harvests of *P. vittata* grown in As-contaminated groundwater.

Treatments		Tissue arsenic concentration				
Arsenic in refill water	Frond harvest regimes	Fronds			Rhizomes	Roots
		1st harvest (8 wk)	*2nd harvest* (11 wk)	*Final harvest* (11 wk)	*Final harvest*	*Final harvest*
High-As water (140–180 μg/L)	All fronds	151		145	137	10.2
	Mature fronds	122	97.0	195	131	21.8
	Senescing fronds	[a]	71	140	112	6.6
Low-As water (<7 μg/L)	All fronds	156		79.4	68.6	11.0
	Mature fronds	125	42.4	82.0	58.2	17.4
	Senescing fronds		51	95.2	75.0	11.8
Significance						
Refill Water Source (RWS)		ns[b]	***	***	***	ns
Frond Harvest Regimes (FHR)		ns	–	ns	ns	*
RWS × FHR		ns	–	ns	ns	ns

[a] Blanks indicate fronds not harvested at *1st harvest*.
[b] ns indicates non significant at $P < 0.05$.
* Significance at $P < 0.05$.
*** Significance at $P < 0.001$.

and root growth were severely affected (data not shown). In comparison, with the same harvest scheme but with low-As water, the frond biomass was 1.4 kg/tank. Poor plant growth affected As-uptake ability of the ferns and delayed the arsenic phytofiltration by several weeks (Fig. 2b). Frequent frond harvest when ferns were continuously exposed to arsenic affected plant vigor, and may not be a wise choice for efficient As phytofiltration. Similar results were reported earlier in greenhouse studies [8,23].

On the other hand, the frond biomass at *final harvest* with senescing-frond harvest in both refill treatments produced similar biomass yield at 1.5–1.6 kg/tank (Table 1). Biomass of all frond harvest (1.6 kg/tank) in low-As water was slightly greater than that in high-As water (1.2 kg/tank). Among the three harvesting regimes, mature-frond harvest yielded the lowest biomass when combined with high-As water refill (1.2 kg/tank), whereas with low-As water refill the yield was 2.1 kg/tank. Regardless the arsenic in refill water, harvesting senescing-fronds produced equal biomass (1.7 kg and 1.6 kg/tank). In comparison, biomass from all-frond harvest produced higher yield with low-As water (1.8 kg/tank) than high-As water refill (1.4 kg/tank).

Our results suggest that periodic harvesting of As-rich fronds in a large scale As-phytofiltration, either all fronds or mature fronds, may add expenses such as labor costs for harvesting and storage space for harvested biomass with little benefit to phytoremediation. Elless et al. [15] suggested that ferns could be allowed to accumulate arsenic to its maximum capacity (~20,000 mg/kg) before harvest to minimize disposal cost. Alternatively, ferns could be harvested frequently to allow disposal as non-hazardous waste (biomass As below 73 mg/kg) in a landfill. Based on our results, frequent harvesting when ferns are continuously exposed to arsenic may not be a wise choice. This is because it resulted in poor fern regrowth and hampered its As-uptake capacity. Hence, it may be unnecessary to harvest fronds so long as ferns are healthy and effectively removing arsenic. However, senescing-fronds should be removed periodically, which requires less labor and also prevents unwanted dispersal of As-rich frond tissues.

3.4. Arsenic was primarily accumulated in the fronds of P. vittata

Arsenic accumulation in *P. vittata* fronds increases with growth time and frond age (young < mature < old fronds) [16,26,27]. A suitable frond harvest method to remove maximum arsenic, without negatively affecting ferns' regrowth, is essential. Fronds from different harvests contained different amounts of arsenic depending upon harvest method and water refill treatment (Table 2). Arsenic concentration in the fronds harvested after 8-wk of growth (*1st harvest*) in both refill treatments showed no difference, i.e., 150 and 155 mg/kg in all-frond harvest, and 122 and 125 mg/kg in mature-frond harvest. However, in mature-frond harvest from the *2nd harvest*, frond As concentration was two-fold higher in high-As water refill (97 mg/kg) than in low-As water refill (42 mg/kg). This could be partly due to continuous As addition and partly from high As concentration remaining in tanks (~40 μg/L) after 8-wk of growth in *Cycle 1* (Fig. 2). Moreover, As concentration in senescing-fronds from *2nd harvest* were 51–71 mg/kg, which can be treated as non-hazardous waste since it was below 73 mg/kg. Hence, if only senescing fronds were removed periodically, the biomass can be disposed of as non-hazardous material in a landfill. Also, the labor cost and total biomass for disposal may be significantly reduced this way.

During the *final harvest*, As concentrations in different parts were different. Across all treatments, As concentrations were the highest in the fronds, followed by rhizomes and the roots (Table 2). Between the two water refill treatments, ferns in the high-As water treatment had greater As accumulation than the low-As water treatment. Fronds from mature-frond harvest and high-As water treatment showed the highest arsenic concentration (195 mg/kg), which was not the case in the low-As water treatment.

Lower arsenic concentration in the fronds of low-As water treatment at *final harvest* compared to *1st harvest* may be attributed to the "dilution" effect due to substantial dry matter production (Table 1). Conversely, higher As concentrations in the fronds of mature-frond harvest in the high-As water refill than low-As water may be due to significantly less biomass accumulation (0.6 vs. 1.4 kg/tank, Table 1). Earlier studies suggested the "dilution" effect, due to high biomass that reduces arsenic toxicity, is a possible survival mechanism of *P. vittata* [16]. Similar dilution effect was observed in the roots (Table 2). Among the three harvest regimes, the roots with senescing-frond and all-frond harvest accumulated the lowest As (6–12 mg/kg), whereas the roots with mature-frond harvest in both refill treatments accumulated the highest As (17–22 mg/kg). Rhizomes accumulated almost equal arsenic as the fronds, with greater accumulation in high-As water refill (112–137 mg/kg) than in low-As water refill (58–75 mg/kg). This indicates that rhizome may be a secondary storage structures for As accumulation in *P. vittata*. Similar results were reported in previous studies where rhizomes were reported as "buffer" tissue for As storage [10,22,28].

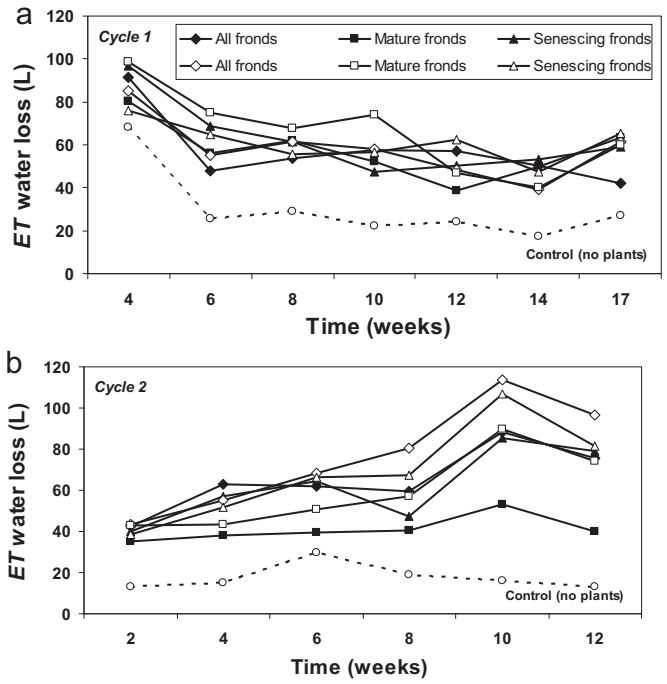

Fig. 3. Effects of arsenic levels in refill water and frond harvesting regimes on the amounts of water loss from hydroponic tanks during two experiment cycles: (a) *Cycle 1* for 17 wks with young ferns, and (b) *Cycle 2* for 13 wks reusing the same fern. The solid and open data points represent high-As water (140/180 μg/l) and low-As water (<7 μg/l) refill, respectively. Water in the control tank was initially filled with high-As water (140 μg/l) and refilled with low-As water biweekly for evaporation loss. Values are means of four replications.

3.5. Strategies for an efficient As phytofiltration using *P. vittata*

3.5.1. Water refilling for evapotranspiration (ET) loss

This is the first study showing promising results to develop cost-effective strategies for As phytofiltration from contaminated groundwater in a large-scale hydroponic system using *P. vittata*. On average, one tank with 32 ferns (one fern/ft^2) growing in 600 L of water lost ~10% (60 L) of water every 2 wks due to *ET* (Fig. 3). For healthy fern growth and regular monitoring of As concentration, water lost due to *ET* must be compensated. Depending upon the extent of As contamination, water availability, and other cultural practices, different refilling strategies may be used. Based on our results, the time needed to reduce arsenic from 140 or 180 μg/L to <10 μg/L was longer when the tanks were refilled with As-contaminated water (Fig. 2). Although theoretically refilling tanks with As-contaminated water should be more efficient (i.e., 600 L plus 60 L added biweekly) than with low-As water (i.e., 600 L). However, the throughput was greater with low-As water (i.e., 16–20% more efficient in *Cycle 1* and 29–32% in *Cycle 2*) than with high-As water refill (Table 3).

3.5.2. Frond harvesting regimes to minimize operation cost and maximize As uptake

One of the most important aspects of phytofiltration is that, at some point, the aboveground biomass must be harvested for disposal. However, method, extent and frequency of biomass harvesting were not well addressed previously for *P. vittata* used in As phytofiltration. Results from this study indicate that harvesting practice may drastically affect fern regrowth and phytofiltration efficiency (Fig. 2b). Fern growth was greatly affected by frequent harvesting (mature fronds) and continuous As addition (high As water), thereby delaying the phytofiltration by several weeks (Fig. 2b). Among the three harvest regimes, senescing-frond harvest was the best practice for fern growth and As-removal efficiency. However, when ferns are allowed to grow without harvest, mature fronds will grow old and senesce after a period of time. Results indicate that the biomass harvested from senesced fronds (Table 1; *2nd harvest*) was lower than that from mature fronds. Except for mature-frond harvest coupled with high-As water, As concentrations in the fronds in all treatments were below 73 mg/kg (Table 2; *2nd harvest*). Hence, removing only senesced fronds is recommended to minimize operation cost, maintain the site and prevent unwanted dispersal of As-rich fronds. Also, while harvesting the fronds care should be taken that rhizomes and croizers (young unfurling fronds) should not be damaged, this may help the regrowth of ferns after harvesting.

From this study, it can be further speculated that, in areas where clean water is limiting, it may be possible to refill with As-contaminated water; however, ferns should not be further stressed by harvesting its actively growing fronds. In areas where clean water is not limiting, refilling with clean water and frequent frond harvesting may be practiced. This can expedite the clean-up process and keep the tissue-As concentration below 73 mg/kg for disposal as non-hazardous material. In any case, labor costs for harvesting and biomass for disposal can be significantly reduced by removing only senesced fronds, while refilling tanks with either contaminated water or clean water.

3.5.3. Nutrients to maintain fern growth and quality of remediated water

One of the major concerns about using plants for phytofiltration was secondary contamination in the solution, particularly nitrates (NO_3-N) and phosphates (P) from fertilizer use. Such contamination would make the release of the As-decontaminated

Table 3
Effects of arsenic levels in refill water and frond harvesting regimes on phytofiltration efficiency of *P. vittata* in two experiment cycles using same plants.

Treatments		*Cycle 1*			*Cycle 2*		
Arsenic levels in refill water	Frond harvest regimes	Amt. of water cleaned (liters/tank)[a]		Phytofiltration efficiency[b]	Amt. of water cleaned (liters/tank)[a]		Phytofiltration efficiency[b]
		8 wks	17 wks		6 wks	12 wks	
High-As water (140–180 μg/L)	All fronds	–	1004	–	–	880	–
	Mature fronds	–	1006	–	–	[c]	–
	Senescing fronds	–	1041	–	–	860	–
Low-As water (<7 μg/L)	All fronds	600	1200	20% more	568	1136	29% more
	Mature fronds	600	1200	19% more	568	1136	[c]
	Senescing fronds	600	1200	15% more	568	1136	32% more

Water in high-As water refill treatments was not cleaned until 17 and 12 wks in *Cycle 1* and *Cycle 2*, respectively, and *ET* water loss was replaced biweekly.

[a] Values were based on the assumptions that hydroponic tanks were filled twice with fresh As water at the end of 17 and 12 wks in *Cycle 1* and *Cycle 2*, respectively.

[b] Phytofiltration efficiencies were compared based on the same above assumptions.

[c] No values were obtained since phytofiltration duration was longer than 12 wks.

water inappropriate. Fortunately, *P. vittata* is a hardy fern and can thrive in a nutrient-poor environment. Based on our preliminary results, the weak nutrient solution (0.25 strength Hoagland solution with N = 21 mg/L and P = 1.2 mg/L) was sufficient to maintain fern growth. In the two cycles tested, NO_3-N and P concentrations in the remediated water were below the maximum contamination limits prior to discharge (<10 mg/L NO_3-N and <0.75 mg/L P; data not shown). Hence, for practical application of these ferns for large-scale As phytofiltration, a weak fertilizer solution can be safely used.

In conclusion this study established the basic cultural practices to operate a large-scale cleanup project of As-contaminated groundwater using *P. vittata*. Further studies are needed to develop a less labor-intensive technique to refill tanks for *ET* loss. For instance, a dynamic approach of emptying and refilling hydroponic tanks in a continuous fashion (automatic or manual) may be developed to maintain constant water levels in phytofiltration tanks.

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