

Review

Critical Perspectives on Soil Geochemical Properties Limiting Arsenic Phytoextraction with Hyperaccumulator *Pteris vittata*

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Abstract: Arsenic is a metalloid widely distributed in the environment and of global concern for human health. In a promising breakthrough for sustainable arsenic soil remediation, a fern, *Pteris vittata* L., was discovered to take up arsenic from the soil and accumulate it in its fronds at up to ~100 times soil concentrations. Successively harvesting the fronds removes, or phytoextracts, arsenic from the soil with potential environmental and economic benefits including low site disturbance and low cost. The practical use of *P. vittata* for soil remediation faces challenges largely stemming from the complex nature of the soil. Here, we review soil geochemical processes governing the transport of arsenic from soil to the roots of arsenic-hyperaccumulating ferns. We find that phytoextraction is a soil-dependent process, but that key soil attributes including texture are often not reported. We show that rhizosphere processes play a crucial role in arsenic phytoextraction, and that nutrient management is most successful with ecologically based approaches including sparingly soluble nutrient forms. We conclude that a multi-scale ecological approach is needed to validate *P. vittata* behavior across controlled and field conditions, and arsenic movement between soil, water, and plant compartments. Our synthesis suggests that phytoextraction as currently practiced is limited to soils with low arsenic concentrations and that *P. vittata* cultivation is climate-limited to a zone smaller than its range as a wild species.

Keywords: phytoremediation; rhizosphere; soil texture



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1. Introduction

Arsenic is a metalloid widely distributed in the environment and of global concern for human health. Arsenic can cause acute and chronic poisoning through exposure routes including inhalation and ingestion of water, food, and soil material. Arsenic causes numerous adverse health effects to humans [1]. Globally, the major concern comes from the contamination of drinking water from natural geological sources [2,3]. However, health risks from arsenic-contaminated soil should not be underestimated. In the USA, arsenic tops the Substance Priority List (SPL) created by the Agency for Toxic Substances and Disease Registry /US EPA, which ranks pollutants at Superfund sites based on toxicity and potential for exposure [4].

Conventional arsenic remediation methods are too expensive to clean up large, moderately contaminated areas, where the risk of negative environmental and health impacts is still unacceptably high [5]. In a promising breakthrough for large-scale, sustainable arsenic soil remediation, *Pteris vittata* L., a fern distributed globally in tropical and subtropical climates [6], was discovered to take up arsenic from the soil and accumulate it in its fronds at up to ~100 times soil concentrations [7]. Successively harvesting the fronds removes, or phytoextracts, arsenic from the soil with limited disturbance. Although there are costs associated with phytoextraction, for example, treating arsenic-enriched fronds, phytoextraction could be less expensive than other methods [8].

Many challenges still lie ahead for arsenic phytoextraction with *P. vittata* [9]. These challenges largely stem from the complex nature of the soil, especially spatial and temporal

variation in soil characteristics. Remediation time estimates derived from rates measured under field conditions are long, about 40 years to remove 180 kg As/ha (100 mg As/kg) from soil (0 to 15 cm depth) [10–13]. The application of soil amendments has been investigated in efforts to increase arsenic uptake rates and shorten remediation times, but results are inconsistent. Frond arsenic concentrations and biomass were higher in *P. vittata* supplied with sparingly soluble compared to soluble phosphorus [14,15], though in other cases phosphorus application did not affect arsenic uptake in *P. vittata* regardless of solubility [16–19], and soluble phosphorus increased arsenic concentrations in porewater but not in *P. vittata* fronds [20]. Furthermore, some studies have shown that the presence of other soil contaminants will negatively affect arsenic uptake by *P. vittata* [20,21]. Finally, it is not well understood how arsenic depletion from soil correlates to arsenic accumulation in *P. vittata* fronds, for example, if all arsenic depleted from soil is accumulated in the fern or lost to other processes [11,13,22], especially under field conditions where fern roots have access to a larger volume of soil than in pot experiments [23,24].

Here, we review soil geochemical processes governing the transport of arsenic from soil to the roots of arsenic hyperaccumulating ferns, especially *P. vittata*, to provide a critical perspective on factors limiting arsenic phytoextraction. Biological aspects of arsenic phytoextraction have been reviewed extensively [9,25–27]. However, the effects of soil properties including texture, arsenic availability, nutrient addition, and root exudation on arsenic uptake in *P. vittata* have only been briefly reviewed [27]. Here, we first discuss the chemical stabilization of arsenic in soils, another in situ arsenic remediation method, to give context to work on arsenic phytoextraction. We then introduce arsenic phytoextraction including mechanisms for arsenic release from soil, discuss experimental approaches, and review the effects of soil characteristics on arsenic phytoextraction by *P. vittata*, including soil texture and mineralogy and arsenic and metal concentrations. We discuss efforts to increase phytoextraction efficiency, including the use of fertilizers, compost, chelating agents, and mycorrhizal fungi inoculants. We consider remediation rates, estimates of remediation times, and the need for mass balances in phytoextraction studies. Finally, we discuss the implications of existing research for the widespread application of arsenic phytoextraction with *P. vittata* and suggest avenues for future work based on a consideration of the soil-plant system.

We find that phytoextraction is a soil-dependent process, but that key soil attributes including texture are often not reported. We show that rhizosphere processes play a crucial role in arsenic phytoextraction, and that nutrient management is most successful with ecologically based approaches including sparingly soluble nutrient forms. We conclude that a multi-scale ecological approach is needed to validate *P. vittata* behavior across controlled and field conditions and arsenic movement between soil, water, and plant compartments. Our synthesis suggests that phytoextraction as currently practiced is limited to soils with low arsenic concentrations and that *P. vittata* cultivation is climate-limited to a zone smaller than its range as a wild species.

2. Geochemistry of Arsenic Soil Contamination

The chemical forms and oxidation states of arsenic control its toxicity [28]. In aqueous environments and soils, arsenic occurs mainly as its oxidized forms, arsenic(V) and arsenic(III), more specifically as the oxyanions arsenate (present as H_2AsO_4^- and HAsO_4^{2-} in pH range 2–11) in oxidizing conditions, and arsenite (present as H_3AsO_3 for pH below 9) [29] in moderately reducing conditions. Even in oxic soils, arsenite can be present [30] due to reducing conditions in soil microsites [31,32].

The type and amount of sorbents control the mobility and phytoavailability of arsenic. In soils and sediments, arsenic sorbs readily to iron, aluminum and manganese (oxy)hydroxides as well as to calcium compounds [33], with a stronger sorption potential for arsenate than arsenite in acidic conditions, and the opposite in alkaline conditions [34,35]. In oxic soils arsenic sorbs primarily to iron(III) oxides and secondarily to aluminum oxides [29,36,37]. The form of iron oxides affects arsenic sorption, with more arsenic sorbed

to amorphous rather than crystalline phases due to larger surface area, although sorption to crystalline phases is stronger [34]. Manganese oxides can control arsenic cycling in soils [38], oxidizing arsenic, which then can resorb to iron oxides [39]. Arsenic can also be associated with clay minerals in soil [40], and with organic matter [41], although these sinks are considered less important than soil (oxy)hydroxides, due to surface charge effects. The presence of phosphate and organic matter limits the sorption of both arsenate and arsenite in soils and consequently increases arsenic mobility and leachability [42–46].

The global average concentration of arsenic in uncontaminated soils is 5–6 mg/kg [35], but background arsenic concentrations in soils vary by several orders of magnitude depending on the parent material from which they were formed [47], making it difficult to establish typical values [48]. Soil cleanup criteria for arsenic vary greatly between and within countries [49,50]. Arsenic enrichment in soils originates from agriculture, arsenical pesticide use, manure application, and irrigation with arsenic-contaminated water; use of arsenic-containing wood preservatives, such as chromated copper arsenate (CCA); and metal mining and smelting [29]. Arsenic contamination persists in soils for decades to centuries [51].

3. Methods to Remediate Arsenic-Contaminated Soils

3.1. Chemical Stabilization

Chemical stabilization, which involves applying a sorptive amendment to the soil to lower the soluble fraction of contaminants and decrease plant uptake [52], could be a less destructive alternative to conventional remediation approaches [53]. Chemical stabilization of arsenic typically involves applying precursors of iron (Fe) oxides (ferrous (FeSO_4) or ferric sulfates ($\text{Fe}_2(\text{SO}_4)_3$)) and zerovalent iron ($\text{Fe}(0)$), or directly applying poorly crystalline (ferrihydrite ($\text{Fe}(\text{OH})_3$) or crystalline iron oxides goethite $\alpha\text{-FeOOH}$). Other materials, including aluminum-based industrial by-product compounds, have also been shown to be effective [54].

Amending soil with iron compounds decreases arsenic mobility and therefore soil and porewater toxicity in ex situ studies. Amendment with iron sulfates, together with lime to avoid soil acidification, decreased the concentration of water-soluble arsenic [55] and of arsenic in leachates [56,57], as well as arsenic uptake by crops [52,58]. The addition of zerovalent iron decreased the concentration of arsenic in soil porewater, leachates, and plant tissues, as well as of extractable and bioaccessible arsenic [56,57,59–63]. Goethite and ferrihydrite amendments decreased arsenic concentrations in porewater [64–66], in leachates [56,57,65], and arsenic uptake by plants and phytoavailability [67].

However, there are limitations to using these methods in more complex field conditions [53,62,68,69]. For example, amendments with iron sulfates plus lime or zerovalent iron led to increased concentrations of metals such as cadmium, copper, zinc, and lead in leachates or in plant tissues compared to untreated soils [56,57,61]. Additionally, when used in situ, arsenic stabilization methods were much less efficient than when used in batch or pot experiments [58,59]. Finally, even if amendments effectively decrease arsenic mobility under current geochemical conditions, changing conditions, especially changing redox potential due to microbial activity [70] could lead to arsenic release. On-going site monitoring is required to ensure effective remediation over timescales of years, akin to monitored natural attenuation [71].

3.2. Phytoextraction Using *Pteris vittata* and Other Arsenic-Hyperaccumulating Plants

3.2.1. Phytoextraction with Hyperaccumulators

Phytoextraction is a soil remediation method where plants extract contaminants from the soil and concentrate them in their aboveground biomass, which is then harvested to remove the contaminants while leaving the soil in place. Phytoextraction of metal(loid)s has been investigated using naturally occurring metal(loid) hyperaccumulators and non-hyperaccumulators including genetically modified plants [72]. Here, we focus on phy-

to extraction with non-genetically modified hyperaccumulators to explore the relationships between natural hyperaccumulator ecology and soil geochemistry.

Brooks et al. [73] established the term hyperaccumulator in a study focusing on nickel to describe plants able to accumulate nickel at concentrations >1000 mg/kg (ppm) in dry leaf tissue. Since then, the term hyperaccumulator has been extended to other metals and metalloids with element-specific thresholds. Other criteria include extreme metal tolerance [74], a shoot-to-root metal(loid) concentration ratio (or translocation factor) typically >1 [75], and a ratio of metal(loid) concentrations in plant biomass to those in soils (or bioconcentration factor) typically >1. Hyperaccumulators tolerate high concentrations of metal in soils, but hyperaccumulation is a genetically distinct trait from tolerance [76]. Natural hyperaccumulation depends on both constitutive up-regulation of transporters to move metal(loid)s across membranes [77], and mechanisms that confer tolerance of the high concentrations of metal(loid)s thus taken up [78].

Several theories have been proposed to explain hyperaccumulation, including the defense theory, where high metal(loid) concentrations kill or deter pests [79,80]; allelopathy, where litterfall locally increases soil metal(loid) concentrations above those tolerable to other species [81]; and the phosphorus starvation theory, where metal(loid) uptake is a byproduct of nutrient acquisition [82].

3.2.2. Arsenic-Hyperaccumulators

As early as 1975, many plants accumulating >1000 mg/kg arsenic in their leaves and with a translocation ratio >1 were found growing in soils enriched in arsenic, mainly around mining sites. Among them *Jasione montana* L., *Calluna vulgaris* (L.) Hull, *Agrostis tenuis* Sibth. and *Agrostis stolonifera* L. were found in the UK [83], *Agrostis castellana* in Portugal [84] and *Paspalum racemosum* and *Bidens cynapiifolia* in Peru [85]. However, since those plants grew on soils extremely enriched in arsenic, the bioconcentration factor was <1, despite high concentrations in plant tissues, and those plants are consequently not hyperaccumulators, according to the bioconcentration criterion presented earlier.

The first plant to be labeled an arsenic hyperaccumulator was the fern *Pteris vittata* L. *P. vittata*, an invasive species in Florida, USA [86], was identified in 1998 as an arsenic hyperaccumulator during a survey of plants growing on a Florida site contaminated with chromated copper arsenate (CCA) [87,88]. There, *P. vittata* accumulated between 1442 and 7526 mg/kg arsenic in its fronds with no observed toxicity effects [7]. In addition, in a pot experiment with a soil spiked with 1500 mg/kg arsenic, *P. vittata* was able to accumulate as high as 22,630 mg/kg arsenic [7]. The ratio of arsenic concentrations in fronds to roots in *P. vittata* was >20 and the bioconcentration factor ranged between 5 and >100 [7]. Simultaneously, arsenic hyperaccumulation in *P. vittata* was discovered separately in populations growing in an arsenic sulfide mine in Hunan Province, China [89].

Since then, other arsenic-hyperaccumulating plants have been identified, most of them belonging to the fern family, and more specifically to the *Pteris* genus [9]. However, not all *Pteris* species hyperaccumulate arsenic [90,91]. Known arsenic-hyperaccumulating ferns include many varieties of *Pteris cretica* [90–96], *Pityrogramma calomelanos* [97,98], *Pteris longifolia* [94], *Pteris umbrosa* [90,94,99], many varieties of *Pteris multifida* [91,92], *Pteris biaurita* L., *Pteris quadriaurita* Retz and *Pteris ryukyuensis* Tagawa [95], *Pteris aspericaulis*, *Pteris fauriei*, and *Pteris oshimensis* [91]. Zhao et al. [94] suggested that arsenic hyperaccumulation is a constitutive property in *P. vittata* (i.e., it is expressed in all members of the species regardless of the presence of arsenic in the soil), similarly to what was proposed for hyperaccumulation of metals generally [100], and for zinc and cadmium in *Arabidopsis halleri* and *Thlaspi caerulescens* specifically [101]. The constitutive property of arsenic hyperaccumulation was later revealed in populations of *P. multifida*, *P. oshimensis* and *P. cretica* var. *nervosa* and confirmed in *P. vittata* [91]. *P. vittata* is the most studied of the above ferns and is considered a model arsenic-hyperaccumulating plant [102,103].

3.2.3. Arsenic Hyperaccumulation in *P. vittata*

The mechanisms of arsenic tolerance, translocation and transformation in *P. vittata* have been extensively reviewed [9,25,26] and will be discussed here briefly in regard to implications for arsenic cycling in the whole soil-water-plant system. *P. vittata* absorbs arsenic from soil through its roots, which are primarily found in the 0–10 cm soil depth interval [104]. *P. vittata* primarily takes up arsenate and only limited arsenite, with the rate of arsenate uptake being 10 times that of arsenite uptake [105]. Like many plants, *P. vittata* takes up arsenate, a chemical analogue of phosphate, through the phosphate intake pathway, specifically through phosphate transporters in the root plasmalemma [82,105]. Arsenite uptake is also efficient and occurs through an active transport process [106]. *P. vittata* translocates and sequesters most accumulated arsenic in its pinnae [107], though arsenic translocation decreases under metal stress [108] and the rhizome can be a secondary storage organ when soil arsenic is highly available [104]. In pinnae, arsenic is compartmentalized in the vacuoles of pinnae epidermal cells [107], located on pinnae surfaces as crystalline deposits [109], and in trichomes on pinnae surfaces [110]. Between 47 and 94% of the arsenic in *P. vittata* fronds is present as arsenite and the rest as arsenate [7,107,111,112]. Indeed, during arsenic translocation in the fern, arsenate is reduced to arsenite [7], although there is disagreement about whether reduction occurs in roots [113], stipes [114] and/or pinnae [115]. Arsenite reoxidation was found in older fronds 18 weeks of age [112] and in senesced fronds [103].

3.2.4. Mechanisms for Arsenic Release from Soil

Arsenic uptake, and therefore hyperaccumulation, is likely linked to nutrient uptake in *P. vittata*. According to the phosphorus starvation theory, arsenic could be released from soil and taken up into the fern as a byproduct of rhizosphere-based nutrient scavenging processes [82,116]. In response to nutrient deficits [117], *P. vittata* likely releases root exudates [14,118–120] that mobilize phosphorus, iron, and arsenic from soil through ligand-enhanced dissolution of iron minerals [118,121,122].

P. vittata exudates include oxalic ($C_2H_2O_4$), malic ($C_4H_6O_5$), and phytic ($C_6H_{18}O_{24}P_6$) acids [14,119,123]. Oxalic and malic acids are common root exudates across plant communities [124], and oxalic acid is a well-known effective metal complexer [125,126]. Phytic acid, also a metal complexer [127] is less well-known as a root exudate but is released by fern roots [119,123]. Iron-oxalate and -phytate complexes lead to dissolution of iron oxides [128].

Both oxalic and phytic acid supplied hydroponically to *P. vittata* lead to release of arsenic from iron oxides and to higher *P. vittata* frond arsenic concentrations [123,128], but phytic acid could be more important to arsenic uptake in the fern [128]. *P. vittata* produced 4 to 7 times more phytic acid than oxalic acid [119], phytic acid released 1.2 to 50 times more arsenic from $FeAsO_4$ than oxalic acid [119,123], and *P. vittata* frond arsenic concentrations were 3–10 times higher when phytate compared to oxalate was supplied [128]. The ability to sustain phytic acid production in the presence of arsenic could be a characteristic of arsenic tolerance in *P. vittata*, as phytic acid release from non-hyperaccumulating ferns decreased in the presence of arsenic [119,123].

The importance of root exudates to *P. vittata* arsenic uptake suggests the rhizosphere is a key zone for arsenic phytoextraction. Indeed, 66–95% of arsenic accumulated in *P. vittata* was shown to be extracted from rhizosphere soils, depending on soil texture, based on calculations of the mass of arsenic taken up in the fern through transpiration of bulk soil porewater [129]. The importance of rhizosphere processes suggests that is primarily rhizosphere, not bulk, soils that are effectively phytoextracted.

Even if arsenic uptake in the fern is closely associated with root exudate activity, the link between exudate compounds and nutrient budgets, especially phosphorus and iron, in *P. vittata* needs further elucidation. *P. vittata* frond phosphorus concentrations increased when ferns were supplied with higher (500–1000 μM) [123,130] but not lower (50 μM) [128,130] concentrations of phytic acid. It remains unclear how phytic acid release

relates to *P. vittata* phosphorus budgets. *P. vittata* could use externally supplied phytic acid as a phosphorus source [123]. Phytase present in *P. vittata* root exudates [14,123] could help the fern recover released phytic acid [131]. Iron and arsenic cycling in *P. vittata* are likely related, given that arsenic in oxic soils is usually associated with iron minerals. Frond iron concentrations increased when ferns were supplied with phytic or oxalic acid in some cases [123] but not in others [128,130].

In addition to acting through ligand-exchange mechanisms, root exudates could affect rhizosphere pH and therefore arsenic availability. *P. vittata* rhizosphere pH, a key factor in arsenic release from iron oxides, is not well understood. Compared to bulk soil, *P. vittata* rhizosphere pH has been found to be lower [132], similar [118], or higher [120,133]. Counterintuitively, decreasing pH below neutral would increase iron oxyhydroxide solubility [134] but also increase the amount of arsenic sorbed [34]. pH also affects root exudate behavior. Oxalate has a higher affinity for iron at lower pH [135], so decreasing pH could increase ligand-enhanced dissolution of iron oxides, increasing arsenic release. Alternately, oxalic and acetic acid release was shown to increase soil pH adjacent to artificial roots, possibly due to mineral dissolution or dissimilatory metal reduction [125], which would also increase arsenic release.

4. Experimental Approaches

Arsenic phytoextraction research employs a variety of experimental approaches, from hydroponic and greenhouse studies to field studies and surveys, each with advantages and drawbacks (Table 1). Simple systems allowing detailed investigation of the mechanisms underlying arsenic phytoextraction must be combined with work approximating real-life phytoextraction conditions (i.e., work in historically contaminated soils under in situ conditions) to (a) develop a mechanistic understanding of phytoextraction and (b) apply this understanding to develop viable remediation methods. Here, we synthesize the results of a wide body of work on arsenic phytoextraction consisting mainly of hydroponic and greenhouse/pot studies with fewer in situ studies. Extrapolating the results of controlled conditions to field performance, characterized by inherent heterogeneity, must be performed with caution.

Hydroponic studies provide mechanistic information on *P. vittata* arsenic uptake into roots, but typically use much higher concentrations of arsenic (10^1 – 10^5 µg/L) than those found in the soil solution (1–4 µg/L) [74,136–138], limiting their relevance to real-life soil conditions. Additionally, hydroponic study results cannot be easily extrapolated to field conditions because soil properties influence (and likely decrease) the solubility and therefore the supply of nutrients and arsenic to the fern [136].

Greenhouse pot experiments with arsenic-spiked soils also likely overestimate arsenic accumulation due to high arsenic availability and ideal greenhouse conditions. Metal(loid)s added to soil typically as soluble salts (e.g., NaH_2AsO_4) under laboratory conditions are more plant-available and less heterogeneously distributed than metal(loid)s that have been present in soil for longer times (i.e., years-centuries) [139], the case for most geogenic and anthropogenic soil arsenic. For example, total arsenic accumulation *per plant* was 4 times larger in *P. vittata* grown in soil spiked with arsenic, than in historically contaminated soil with similar arsenic concentrations [17]. Greenhouse pot studies with historically contaminated soils offer more realistic available arsenic concentrations, though can still overestimate arsenic accumulation in *P. vittata* due to ideal climate conditions.

In situ conditions, characterized by heterogeneous distribution of soil arsenic, low and varied arsenic and nutrient phytoavailability, and climate stress, most closely approximate *P. vittata* cultivation for phytoextraction and therefore provide the best estimates of *P. vittata* phytoextraction rates. The number of field studies is increasing [8,10–13,22,140–148], especially studies investigating *P. vittata* field performance outside of the humid subtropics (e.g., Figure 1) [10,12,13,140,146–148]. However, the complexity of in situ systems, especially high variability in arsenic concentrations, can mask treatment effects and changes in soil arsenic during phytoextraction [11,13], leading to the underestimation of phytoextrac-

tion progress. In some studies, replication at the fern but not plot scale [11,13,140,146] could lead to autocorrelation in results. Furthermore, weather and climate factors can confound experimental treatment effects.

Table 1. Characteristics of experimental approaches commonly used in arsenic phytoextraction studies.

Experimental Approach	Arsenic Source/Form	Arsenic Concentrations and Distribution	Advantages	Drawbacks	Complexity
Hydroponic	Aqueous arsenic	Typically, higher concentrations of arsenic (10^1 – 10^5 µg/L) than those found in the soil solution (1–4 µg/L).	Easy replication. Mechanistic information on arsenic uptake into roots.	Results cannot be easily extrapolated to behavior in soil. Soil properties influence the solubility and therefore the supply of nutrients and arsenic to the fern.	Simple
Greenhouse pot study	Spiked soils	Typically, higher concentrations of plant-available arsenic than historically contaminated soils. Uniform soil arsenic distributions.	Easy replication. Controlled conditions help avoid confounding factors (e.g., effects of weather/climate).	Overestimate arsenic accumulation due to high arsenic availability and ideal greenhouse conditions.	Simple
Greenhouse pot study	Historically contaminated soils	Easier to mix soil well and decrease soil arsenic heterogeneity, compared to field studies.	Easy replication. Controlled conditions help avoid confounding factors (e.g., effects of weather/climate).	Overestimate arsenic accumulation due to ideal greenhouse conditions.	Moderate
Field study	Historically contaminated soils	Often 10^1 – 10^2 mg As/kg, heterogeneously distributed over large scales even if soil is tilled to decrease heterogeneity at small scales.	Best approximate of practical application of phytoextraction.	Soil arsenic heterogeneity masks changes in soil arsenic concentrations during phytoextraction. Weather/climate effects confound treatment effects.	Complex
Field survey	Historically contaminated soils	Often 10^2 – 10^4 mg As/kg, heterogeneously distributed at small to large scales.	Well-established populations allow investigating long-term arsenic uptake. Indicate the range of environmental conditions in which <i>P. vittata</i> is hardy.	<i>P. vittata</i> can hybridize and arsenic uptake and fern behavior can be population-dependent. Not representative of cultivated fern arsenic uptake.	Complex

Surveys of wild *P. vittata* populations [92,104,149] provide insight into behavior of well-established populations growing in historically contaminated soils where arsenic distributions are likely heterogeneous, potentially even more so than in field experiment soils which could be tilled. However, *Pteris* could be more than one genus and hybridizing is common among fern species [150], complicating comparisons across ecotypes. Survival of wild populations in certain (e.g., Mediterranean) climates does not guarantee survival under cultivation for phytoextraction [146].

Rigorous in situ studies in a variety of climates coupled to process-based experiments under controlled conditions are required to determine if arsenic phytoextraction can fully “emerge” as a viable remediation technology [151].



Figure 1. Field-scale phytoextraction of arsenic with *Pteris vittata* over one year in a Mediterranean climate (Richmond, CA, USA). (A) A season extension hoop house was constructed over the site, soil tilled, soil treatments mixed in, and (B) soil sampled before (C) ferns were planted. (D) Ferns were approximately 30–50 cm high at 26 weeks and (E) up to 2 m tall at harvest (58 weeks). (F) Randomly selected ferns were cut for analysis and senesced fronds separated from living fronds. (G) Field was fully harvested and rhizomes removed before (H) final soil sampling. Full details are available in Matzen et al., 2020.

5. Effects of Soil Conditions on Phytoextraction with *P. vittata*

5.1. Effect of Soil Texture and Mineralogy

Soil texture is the percent sand, silt, and clay-sized particles in soil, leading, more generally, to coarse-, medium-, and fine-textured soils, respectively. Soil texture affects soil chemical aspects including mineralogy and arsenic and nutrient availability, and soil physical aspects including aeration and drainage. Soil texture is a useful proxy for considering the effects of the soil chemical and physical attributes on *P. vittata* frond arsenic concentrations and biomass and should always be reported.

P. vittata hyperaccumulates arsenic from soil textures ranging from loamy sand (2 to 4.5% clay) to silty clay (59% clay) [14,17,20,118,129,146,152–154]. However, *P. vittata* frond arsenic concentrations [104,129,146,155] and arsenic accumulation *per plant* [129,146,155] decreased when soil clay content increased. Arsenic is less mobile, and consequently less plant available in fine- than in coarse-textured soils [36,156] because it strongly associates with the clay particle size fraction, including iron oxides [30,157]. *P. vittata* removed arsenic associated with iron and/or aluminum oxides [158], but uptake of these forms was lower compared to more soluble forms of arsenic [7,155,159], confirming arsenic would be less available to *P. vittata* in soils with higher clay content. The effects of clay content on *P. vittata* biomass depended on percent clay. When clay content increased from 20 to 28%, biomass increased, likely due to decreases in arsenic phytoavailability and increases in nutrition [129], though with increases to higher clay contents biomass decreased [104,146].

Effects of soil moisture depend on soil type, likely due to the relation between soil texture and aeration. In a sandy loam [160] and a (presumably well aerated) farmland soil [143], arsenic uptake in *P. vittata* was positively correlated with soil moisture content. However, *P. vittata* was less tolerant of long-term field stress of high soil moisture content associated with seasonal heavy rain conditions in poorly drained heavy clay (silty clay loam) soils compared to sandy loam soil [146].

Overall, arsenic phytoextraction was optimized in coarse- to medium-textured soils, likely due to moderate arsenic phytoavailability and good nutrient access and aeration.

5.2. Effects of Soil Arsenic Concentrations

Arsenic hyperaccumulation in *P. vittata* depends on a soil arsenic threshold and might not be required for efficient phytoextraction of soils with low (e.g., <35 mg/kg) arsenic concentrations [161]. Frond arsenic concentrations and biomass increased when soil concentrations increased from low to moderate [12]. In soils with historical arsenic concentrations lower than 70 mg/kg, hyperaccumulation has only rarely been reported [153], with *P. vittata* frond arsenic concentrations typically lower than 600 mg/kg [12,14,20,158]. Hyperaccu-

mulation is well established in *P. vittata* grown in sandy soils with 105–130 mg arsenic/kg, e.g., [14,17,152].

P. vittata tolerates very high concentrations of arsenic in soil, but at upper limits restricts biomass [104,162] and dies [159]. *P. vittata* grown in soils spiked with arsenic tolerated 500 mg/kg arsenic with phytotoxic effects but died when exposed to 1000 mg/kg arsenic [159]. In this study, the soil spiked with 500 mg/kg arsenic contained 78–89 mg/kg water-soluble arsenic [159], about 4 times the soluble arsenic predicted for historically contaminated soils [138], suggesting the fern could tolerate higher geogenic or anthropogenic soil arsenic concentrations. Naturally occurring populations of *P. vittata* hyperaccumulated arsenic from soils with more than 38,000 mg/kg arsenic and up to 1000 mg/kg available arsenic [104,162].

Tolerance mechanisms could depend on soil arsenic concentrations and affect phytoextraction efficiency. At low soil arsenic concentrations (<50 mg/kg), arsenic concentrations were higher in young fronds, whereas at moderate concentrations (>100 mg/kg), arsenic concentrations were higher in older fronds [159,163]. At very high soil arsenic concentrations, aboveground biomass of naturally occurring populations was smaller than in populations growing at sites with lower total and available arsenic [104], suggesting high concentrations of total and/or available soil arsenic cause phytotoxicity [104] or energy reallocation away from biomass production towards arsenic tolerance [164].

P. vittata populations adapt to exclude arsenic from aboveground biomass in high-arsenic habitats [162]. When exposed to the same arsenic level, translocation was greater in ferns from low-arsenic habitats [162]. Translocation decreased and rhizome storage increased in some *P. vittata* populations growing in soils with moderate (10^2 to 10^3 mg/kg) as well as high (10^4 mg/kg) soil arsenic levels [104]. Arsenic phytoextraction could be optimized using a population from an area with low arsenic concentrations in soil.

Even when frond arsenic concentrations are high, lower biomass can lead to lower phytoextraction efficiency. Frond arsenic concentrations were the same in ferns grown in soils with high and dilute arsenic concentrations, but more arsenic by mass was removed by larger ferns in the diluted soil [165]. In soils with high phytoavailable arsenic, high frond arsenic concentrations can be offset by low biomass restricted due to arsenic stress. Frond arsenic concentrations were up to 10 times higher but frond biomass was much lower, leading to only 2 times higher mass arsenic accumulated, in *P. vittata* growing in a coarse-textured (95 mg/kg As) compared to medium-textured soil (138 mg/kg As), indicating that even in soils with moderate total arsenic concentrations, soil texture affects arsenic phytoavailability and *P. vittata* allocates more energy to arsenic tolerance and less to biomass production [129].

5.3. Effects of Metals

Soils contaminated with arsenic often also contain elevated total metal concentrations. Instead of hyperaccumulation, *P. vittata* uses a different tolerance mechanism for metals, excluding lead, copper, nickel, chromium (VI), cadmium and zinc from aboveground biomass such that concentrations are higher in roots than fronds [75,108,152,166–172] and *P. vittata* could be used for metal phytostabilization [173]. *P. vittata*'s tolerance mechanisms for metals (exclusion) and arsenic (hyperaccumulation) function simultaneously up to certain metal concentrations, but at higher metal concentrations arsenic hyperaccumulation is inhibited. Arsenic phytoextraction occurred in the presence of moderate concentrations of metals in soil [20], but frond arsenic concentrations decreased as soil metal concentrations increased [19,108,152,169]. High concentrations of metal(loid)s in soil (arsenic, copper, zinc, manganese all on the order of 10^3 mg/kg and lead up to 10^4 mg/kg) [20,165,168] inhibited arsenic hyperaccumulation in *P. vittata* [20,165], mainly due to decreases in root biomass [20]. Metal exclusion [166] and phytotoxicity [20,147,152] can lead to lower *P. vittata* biomass, higher mortality [147], and lower phytoextraction efficiency.

Notably, lead inhibited arsenic hyperaccumulation in naturally occurring populations of *P. vittata* growing on mine wastes containing higher levels of lead than arsenic [168,171],

but had no effect in studies with spiked soils and solution culture [152,171]. Precipitation of arsenic-lead mineral phases over long equilibration times could cause low arsenic availability in situ conditions [174], decreasing the phytoextraction potential of soils historically contaminated with lead and arsenic.

Metal tolerance and hyperaccumulation of arsenic in the presence of metals vary with fern population. The ability to tolerate metals could be constitutive in *P. vittata*, since metallicolous fern populations growing in soils with elevated metal concentrations were not more tolerant to metals than the nonmetallicolous ferns [165]. However, arsenic uptake in the presence of metals was higher in ferns from nonmetallicolous than metallicolous ecotypes, suggesting low arsenic uptake in metallicolous populations could be interpreted as an adaptive tolerance mechanism, instead of indicating interference of metals with uptake [165]. Arsenic phytoextraction could be optimized using a nonmetallicolous *P. vittata* ecotype.

6. Soil Treatments to Increase Phytoremediation Efficiency

Because nutrient and arsenic uptake in *P. vittata* appear related [14], considerable research has explored the use of soil amendments, including fertilizers, compost, chelating agents, and/or mycorrhizal fungi, to increase phytoextraction rates. Increasing nutrient supply to *P. vittata* could increase fern biomass and/or its ability to tolerate arsenic toxicity, but could be ineffective or even counterproductive because the fern appears to be well-adapted to low-nutrient soils [154,175] and have low nutrient requirements [176,177]. However, under field conditions, *P. vittata* frond concentrations of macro- and micronutrients decreased over four years, as did arsenic phytoextraction rates, suggesting nutrients should be managed for long-term efficient phytoextraction [146]. Furthermore, the goal of soil amendment is not necessarily to meet *P. vittata*'s nutritional needs, but also to increase arsenic availability in soil.

6.1. Fertilization with Phosphorus

Phosphorus application increased arsenic phytoextraction from historically contaminated and arsenic-spiked soils with a range of textures from sandy to silty clay [14,16,17,19,153,178], though in other cases phosphorus application decreased [21,129,146,179] or had no effect on arsenic phytoextraction [16,17,19,20,178,179] (Table 2). Sparingly soluble phosphorus more successfully promoted phytoextraction than soluble phosphorus [14,17].

Phosphorus application in arsenic phytoextraction is compelling because arsenate and phosphate have similar chemical behavior in soil, so phosphate addition could stimulate arsenic uptake. In soil, phosphate can compete with arsenate for sorption sites and release arsenic to solution [180], increasing the mobility of arsenate in the soil solution [44,180,181], which could make arsenate more available for plant uptake. Because phosphate has a smaller ionic radius and a denser charge distribution, it could have a higher binding affinity for soil than arsenate [182]. However, the desorption of arsenic from soil in the presence of phosphorus is incomplete [183] and could be rate-limited due to the diffusion of arsenic into the sorbent [184]. In plants, phosphate can compete with arsenic for uptake at the phosphate intake pathway [82], though it is unclear whether *P. vittata*'s phosphate transporter system has a higher affinity for phosphate or arsenate [105,185]. Consequently, it is possible that high concentrations of phosphate in soil could competitively desorb arsenic from mineral surfaces, increase arsenic availability for the fern, limit arsenic uptake by *P. vittata* due to competition for the transporters, and/or promote fern growth leading to increases in arsenic removal from soil.

Phosphorus outcompeted arsenate, and to a lesser extent arsenite, for uptake in *P. vittata* over timescales of hours to months in hydroponic studies [18,105,186–188]. This apparent competition was sometimes not observed over longer time scales under hydroponic conditions or within field-relevant concentration ranges in artificial growth media [113,188], suggesting the fern could express different phosphorus transporters to maintain phosphorus uptake even under competition from arsenic [113]. However,

in historically sludge-amended soil with very high available phosphorus concentrations, phosphorus successfully competed with arsenic for uptake [21]. Under field conditions, arsenic uptake in *P. vittata* was lower in the presence of supplied phosphorus [146].

Conversely, *P. vittata* took up arsenic made available by phosphorus addition [153], suggesting in some cases phosphorus competes with arsenic more effectively at soil sorption sites than at the root intake pathway. In other cases, the fern did not take up arsenic made available through phosphorus addition [17,20], indicating phosphorus competition can occur at both soil and root uptake sites. In other cases, supplying *P. vittata* with phosphorus did not affect arsenic accumulation [16,17,19,178], perhaps due to high arsenic availability or a large soil sorption capacity for phosphorus and arsenic.

P. vittata is sensitive to phosphorus availability and requires only a small amount of phosphorus, though demand could increase with stress. In coarse-textured soil, soluble phosphorus application kept *P. vittata* alive, compared to in the absence of supplied phosphorus, but did not promote growth or arsenic accumulation to the extent sparingly soluble phosphorus did [14]. In fine-textured soils, soluble phosphorus appeared to exchange for arsenic on sorption sites, mobilizing arsenic to increase *P. vittata* uptake but did not affect biomass, suggesting phosphorus itself was not available for uptake [16,153] or that increasing phosphorus uptake does not result in increased biomass. Under conditions of arsenic phytotoxicity, increasing soluble phosphorus supply alleviates stress and promotes growth [178]. However, in medium- and coarse-textured soil supplying *P. vittata* with sparingly soluble phosphorus delayed growth, presumably due to phosphorus sensitivity, and resulted in increased arsenic leaching from the medium-textured soil due to higher infiltration associated with lower transpiration from the smaller biomass [129].

Table 2. Effects of phosphorus on arsenic phytoextraction.

Study	Chemical Form of Phosphorus	Soil	Type of Study
<i>Increased arsenic phytoextraction</i>			
Chen et al., 2002 [16]	NaH ₂ PO ₄	26% clay, spiked with As	Greenhouse pot
Cao et al., 2003 [17]	Phosphate rock	Sandy, contaminated with chromated copper arsenate	Greenhouse pot
Tu and Ma, 2003 [18]	NaH ₂ PO ₄	Sandy, spiked with As	Greenhouse pot
Fayiga and Ma, 2006 [19]	Phosphate rock	Sandy, spiked with As and metals	Greenhouse pot
Mandal et al., 2012 [153]	Monocalcium phosphate, diammonium phosphate	Silty clay, historically contaminated	Greenhouse pot
Lessl and Ma, 2013 [14]	Phosphate rock	Sandy, contaminated with chromated copper arsenate	Large outdoor container
<i>Decreased arsenic phytoextraction</i>			
Shelmerdine et al., 2009 [21]	Not reported	Sewage sludge-amended soil (texture not reported), high phosphorus	Greenhouse pot
Matzen et al., 2022 [129]	Calcium phosphate	Sandy clay loam, historically contaminated	Greenhouse soil column
Matzen et al., 2022 [129]	Inorganic P (superphosphate) and organic P (blood meal)	Sandy loam, silty clay loam, both historically contaminated	Field
Hua et al., 2020 [179]	KH ₂ PO ₄	Farmland (texture not reported), historically contaminated	Greenhouse pot
<i>No effect on arsenic phytoextraction</i>			
Chen et al., 2002 [16]	NaH ₂ PO ₄	26% clay, spiked with As	Greenhouse pot
Cao et al., 2003 [17]	Phosphate rock	Sandy, spiked with As	Greenhouse pot
Tu and Ma, 2003 [18]	NaH ₂ PO ₄	Sandy, spiked with As	Greenhouse pot
Caille et al., 2004 [20]	NaH ₂ PO ₄	Loam, historically contaminated	Greenhouse pot
Fayiga and Ma, 2006 [19]	Phosphate rock	Sandy, spiked with As	Greenhouse pot
Hua et al., 2020 [179]	Phosphate rock	Farmland (texture not reported), historically contaminated	Greenhouse pot
Matzen et al., 2020 [12]	Calcium phosphate	Sandy loam, historically contaminated	Field

6.2. Fertilization with Nitrogen, Sulfur, Potassium, and Calcium

Nitrogen is well known to increase above and below-ground plant biomass and affect soil pH [189]. Surprisingly, contrary to expected anion-balancing effects where uptake of a cation would balance uptake of the oxyanion arsenate, nitrate more effectively promoted frond biomass and arsenate uptake from solution culture than ammonium [187]. However, in sandy soil, both ammonium-N and nitrate-N equally increased arsenic accumulation in *P. vittata* through an increase in frond biomass, though frond arsenic concentrations did not increase, possibly due to a dilution effect [190]. Under field conditions, ammonium or organic nitrogen did not affect [12] or decreased arsenic phytoextraction rates [146], possibly due to the negative impacts of supplied nitrogen on soil mycorrhizal fungi communities that could contribute to phosphorus and therefore arsenic transport to *P. vittata* [191–194].

Sulfur is a component of the antioxidant glutathione (GSH), which helps neutralize arsenic toxicity in plants [82]. Consequently, sulfur application could increase the fern's ability to withstand arsenic stress. Positive effects of sulfur application on arsenic phytoextraction in simple systems, including increases in GSH [195] and arsenic [113,170,195] concentrations in *P. vittata*, suggests sulfur application should be further investigated in soils. Like phosphate, sulfate can decrease arsenic sorption in soil [42] and could increase arsenic availability to *P. vittata* in the soil solution.

Few studies have investigated the effects of potassium, a major plant nutrient, on arsenic uptake in *P. vittata*. Potassium presence in solution culture increased *P. vittata* biomass and sometimes arsenic uptake [187]. Frond potassium and arsenic were co-located in *P. vittata* pinnae [107] and concentrations were positively correlated in *P. vittata* grown under greenhouse conditions in sandy soil [163] and under natural conditions in clay soil [149]. Many plants use potassium to maintain charge balance during anion transport and storage in vacuoles [189]. Both *P. vittata* frond potassium and arsenic concentrations were higher in ferns grown in a coarse- compared to a medium-textured soil [146]. The correlation between potassium and arsenic in *P. vittata* pinnae suggests that at least some arsenic is still present as the arsenate anion during xylem transport and when taken into pinnae cell vacuoles, and only later reduced to the neutral arsenite species.

The effects of calcium on arsenic uptake in *P. vittata* are inconsistent. Although calcium is associated with maintaining charge balance in vacuoles [196], this does not appear to be the case in *P. vittata*, as calcium was negatively correlated with frond arsenic concentrations in a naturally occurring population [154] and under field conditions [146]. Calcium promoted arsenic uptake in *P. vittata* when supplied hydroponically [187]. In soils, highly variable solubility of calcium-arsenate phases [197] might explain the inconsistent effects of calcium on *P. vittata*. *P. vittata* took up arsenic as calcium-arsenate more effectively than other forms of arsenic [159], and the addition of calcium-phosphate increased frond arsenic and calcium concentrations, indicating calcium-phosphate can be solubilized during *P. vittata* growth [19]. However, in other cases, frond arsenic concentrations were lower in the presence of calcium-phosphate [129] perhaps due to the sorption of arsenic onto the calcium phases. Calcium supplied as lime did not increase *P. vittata* arsenic uptake and instead decreased arsenic availability, perhaps due to the formation of sparingly soluble calcium arsenate phases [20,198].

6.3. Compost Addition

Compost could be an important amendment for promoting *P. vittata* growth in very low-fertility matrices such as some mine wastes. In poor-quality soils under field conditions, compost application greatly increased *P. vittata* survival across soil textures [146,147], suggesting compost is associated with processes that increase stress tolerance in *P. vittata*, processes possibly related to enhanced soil microbial activity [199].

However, caution should be taken when working with a negatively charged substrate such as compost to avoid competitive desorption and leaching of anionic arsenic. Compost addition increased arsenic mobilization in the soil solution [17,200,201], likely because compost increases anionic organic compounds in soil, which compete with anionic arse-

nate for soil sorption sites on iron and aluminum oxides/hydroxides [42], and supplies phosphorus. Under greenhouse conditions, *P. vittata* appears to not take up this mobilized arsenic, perhaps because phosphorus in compost competes with arsenic for uptake [17,200]. Treating soil with compost did not increase frond arsenic concentrations and total arsenic accumulation in *P. vittata* [17,146,200], and had no effect on *P. vittata* biomass, leaving labile arsenic higher in compost-treated soil than in untreated controls after fern growth [200].

6.4. Chelating Agents

Although chelating agents have been explored extensively to increase efficiency in the phytoextraction of metals (i.e., lead [77]), few studies have explored treating soil with chelating agents to increase arsenic (and phosphorus) availability to *P. vittata* [200]. Chelating agents could increase arsenic availability via surface complexation with iron- and aluminum-oxides, releasing inorganic arsenic to solution. The abiotic chelating agent sodium polyacrylate ((C₃H₃NaO₂)_n) increased arsenic availability in soil and accumulation in *P. vittata*, though EDTA (C₁₀H₁₆N₂O₈) only increased arsenic availability in soil [200].

Some siderophores (biological chelating agents) were highly effective at facilitating arsenic uptake in the fern, while others led to higher soluble arsenic concentrations but did not facilitate arsenic uptake. In partnership with organic acids, siderophores can increase iron oxyhydroxide dissolution rates [122], which could lead to increased arsenic availability in soil. Arsenic accumulation increased dramatically to 34 times the control in *P. vittata* supplied with phytate, a siderophore (PG-12) and arsenic-goethite, while iron increased 1.5 times [128], indicating phytate and the siderophore together were very effective in releasing arsenic and iron through ligand-enhanced dissolution of goethite. However, in the same study, a different siderophore (DFO) led to increases only in soluble arsenic but not in *P. vittata* arsenic uptake, perhaps because the DFO affected arsenic speciation and reduced arsenic, limiting uptake [128]. Concern persists that amending soil with chelating agents leads to contaminant leaching instead of phytoextraction [77,200].

6.5. Inoculation with Mycorrhizal Fungi

Mycorrhizal associations benefit host plants by improving access to nutrients including nitrogen [202] and phosphorus [203], and to water, by increasing the soil volume accessible to the plant, thus potentially increasing remediation efficiency. *P. vittata* forms associations with many mycorrhizal fungi [168,204], especially arbuscular mycorrhizal fungi species, primarily in the *Glomus* genus [168].

Inoculation with mycorrhizal fungi increased *P. vittata* biomass [160,192–194,204,205], which could indicate the mycorrhizae were helping the plants access more nutrients. Species shown to increase *P. vittata* biomass include *Funnelformis mosseae* (previously *Glomus mosseae*) [192–194], *Rhizophages intraradices* (previously *G. intraradices*) [192], *Gigaspora margarita* [194], and indigenous mycorrhizal fungi (IM) associated with *P. vittata* growing at contaminated sites [160,192,204,205]. *F. mosseae*, alone or mixed with other mycorrhizal fungi, was the most effective at promoting biomass production in *P. vittata* [160,192–194]. In particular, inoculation with a mixture of *F. mosseae*, IM, and phosphate rock was most effective at increasing biomass of ferns planted in a highly contaminated mine soil (9600 mg/kg arsenic) [160].

In ferns planted in soil spiked with arsenic, frond arsenic concentrations increased when ferns were inoculated with mining site IM [192,204], *G. intraradices*, *F. mosseae*, or mixtures of the IM with either *Glomus* species [192]. Mixtures of mining site IM with either *F. mosseae* or *G. intraradices* were equally or more effective than the individual treatments at increasing frond arsenic concentrations and arsenic accumulation [192], suggesting that different species fill different roles with the net effect of increased arsenic uptake in the fern. For example, mixtures of the IM with either *F. mosseae* or *G. intraradices* increased arsenic reductase activity in root extracts, more than the individual species or IM alone [192].

The effects of fungi inoculation on *P. vittata*'s biomass and therefore arsenic uptake could be related to arsenic availability. Arbuscular mycorrhizal fungi increased arsenic

uptake (total uptake and/or translocation) in *P. vittata* when the ferns were grown in media with potentially higher arsenic availability (solution culture, spiked soils, highly contaminated soils) [160,192–194,204,205], though uptake was not affected when ferns were grown in a moderately contaminated soil where arsenic could have been less available [193] or at lower spiked concentrations [205]. In cases where fungi inoculation did not lead to increases in arsenic uptake, fungi could have transported phosphorus instead of arsenic, and therefore contributed to increases in biomass [193]. Phosphorus and nitrogen content increased in *P. vittata* fronds and roots associated with mycorrhizal fungi [192–194].

Because most studies have occurred in arsenic-spiked soils, more work is required to elucidate whether mycorrhizae preferentially transport phosphorus over arsenic to the fern under conditions of low arsenic and phosphorus availability in soil. Furthermore, as with other fertilizers, the promising results obtained with mixtures of mycorrhizal fungal species, especially with phosphate rock, suggests that complex assemblages and systemic approaches are required to optimize arsenic accumulation in *P. vittata*.

7. Remediation Efficiency

Linear or modeling-based estimates of changes in soil arsenic as a function of time can be used to predict time to achieve cleanup goals, but uncertainty in estimates limits the practical application of phytoextraction [146,151,206]. Three factors complicate estimates of remediation rates and time: uncertainty around changes in soil arsenic availability as soil arsenic concentrations decrease, growth of *P. vittata* roots and aboveground biomass, and spatial heterogeneity of arsenic distribution in soil [151,206].

Phytoextraction efficiency can be evaluated based on (1) fern arsenic uptake rates [14,146] and/or (2) depletion of total or extractable arsenic from soil as a function of time [207]. Fern arsenic uptake is the easiest phytoextraction metric to measure, but extrapolating fern arsenic uptake over longer timeframes can be difficult due to decreases in arsenic uptake over time [13,146,206].

Arsenic phytoextraction rates did not change over 5 years in a container study [14] but decreased under in situ conditions [13,146]. In a 5-year container study where ferns were supplied with phosphate rock, arsenic uptake rates fluctuated around an average of 147 mg/plant/year (36.9 kg/ha/yr at 15 cm spacing) with no clear trend of increases or decreases with time [15]. Based on average and maximum plant arsenic uptake in the first 2.5 years of this same study, the authors calculated 6–7 years to remediate soil containing 125 mg/kg arsenic down to a local target cleanup level of 2.1 mg/kg [14], assuming a linear decrease in arsenic from soil.

However, several field studies indicate that arsenic accumulation in *P. vittata* decreases over time, which would invalidate linear remediation time estimates and lengthen remediation times. Over 27 months in an in situ field study, *P. vittata* arsenic accumulation decreased from 57 to 7 mg/plant due to decreases in frond arsenic concentrations and biomass [13]. Similarly, *P. calomelanos* arsenic accumulation decreased over 27 months from 124 to 40 mg/plant, even though that species appeared better suited than *P. vittata* to the local climate [13]. Additionally, in a four-year field study, *P. vittata* arsenic uptake rates decreased over time across soil treatments and textures [146]. Based on *P. vittata* and *P. calomelanos* arsenic uptake trends over 3 years, the authors estimated 55 and 143 years, respectively, to remediate arsenic in surface soils from about 900 mg/kg to the threshold of 20 mg/kg, at 30 cm spacing [13]. Assuming a linear decrease in arsenic from soil greatly overestimated arsenic depletion from soil by *P. vittata*, which was modeled to plateau after 2–3 years due to decreasing fern arsenic uptake [146].

Basing remediation rates and remediation time estimates on changes in soil arsenic can be more challenging than on fern arsenic accumulation, due to in situ spatial heterogeneity of soil arsenic distribution at multiple scales including with depth, and the potential for arsenic concentrations to change in soil due to other processes, including leaching [12] and aerial deposition [22]. Soil arsenic decreased by 47% (from 129 to 69 mg/kg) over 5 years when *P. vittata* was grown outdoors in large containers filled with CCA-contaminated

soil and supplied with phosphate rock [15]. However, in in situ field studies, no significant change was observed in surface soil arsenic concentrations over 2 years of *P. vittata* growth [11,13], though concentrations significantly decreased over 2 years of *P. calomelanos* growth [13]. Soil arsenic heterogeneity could explain the differences in phytoextraction rates and times found in the container and in situ field studies. Distribution of arsenic in soil in the container study could have been less heterogeneous than in in situ conditions, such that changes in concentrations could have been significant in the container study but masked by high variability such that they could not be statistically detected in in situ studies.

Changes in root growth over time further complicate our ability to forecast phytoextraction efficiency. Under in situ field conditions, density and depth distribution of roots are likely to be heterogeneous and limited to the 0–10 cm depth profile [104], contributing to slower phytoextraction rates and limited phytoextracted volume of soil compared to potted plants [206]. It is not well understood from what distance in soil arsenic is transported to the root, and arsenic depletion could be limited to rhizospheric soil [129], as with phosphorus [208], given the importance of rhizosphere processes mobilizing arsenic. In natural *P. vittata* populations, root density was the highest in the 0–10 cm depth interval [104], which would have limited phytoextraction to this depth if arsenic is only phytoextracted from rhizospheric soil, as shown under controlled conditions [129]. In contrast, the high phytoextraction rate found in the 5-year container study [14,15] could be due to *P. vittata* being root-bound by the end of the study [15], such that roots accessed the entire 35 cm soil depth and most soil could have been under rhizosphere influence. Root turnover will affect the volume of extracted soil over time and deserves investigation for *P. vittata*, as for phytoremediation of petroleum-contaminated soils [209], including whether arsenic, like nutrients, is taken up primarily from young roots [210], especially the root tip [189], or also from older roots [189,211].

Because plant arsenic uptake is the easiest way to quantify remediation rates once phytoextraction has started, it would be useful to predict plant arsenic uptake in advance based on soil properties, based on correlations of plant arsenic accumulation and soil depletion. Nonlabile arsenic, the pool from which labile and soluble arsenic are derived, could be better correlated with arsenic uptake during active growth [212]. A Free Ion Activity Model [21] that captures the ability of soil to resupply soluble arsenic from recalcitrant fractions in the absence of fern growth could better predict arsenic uptake in advance of fern growth.

Importance of Mass Balances

Estimating phytoextraction efficiency based on either fern arsenic accumulation or soil arsenic depletion assumes all arsenic depleted from the soil is taken up in the fern. Changes in soil arsenic concentrations should be linked to fern arsenic accumulation through soil-plant mass balances, but arsenic budgets are rarely calculated in arsenic phytoextraction studies [151]. Several studies have shown discrepancies when comparing soil arsenic depletion to fern arsenic uptake. Arsenic depletion from soil was 2.6 times greater than arsenic accumulation in *P. vittata* after one year of field-scale phytoextraction, such that 8.7% of original arsenic could not be accounted for in a soil-plant mass balance [12]. In another field study, the change in mean soil arsenic concentrations (404 kg/ha), although not statistically significant, grossly outweighed the uptake in *P. vittata* (9.7 kg/ha), with an even greater disparity for *P. calomelanos* [13]. After 5 years of container-based phytoextraction [15], we calculated that 29% of initial arsenic could not be accounted for.

Arsenic leaching from soil during phytoextraction could account for the missing arsenic in these arsenic budgets. Arsenic leached from soil during [129] and after *P. vittata* growth [142]. Under controlled conditions, the mass of arsenic lost in leachate was only 2.5–4 percent of arsenic accumulated in *P. vittata* [129], though it could have been up to 161 percent of that accumulated in *P. vittata* under field conditions [12]. The importance

of arsenic leaching under field conditions must be evaluated to prevent environmental damage during phytoextraction.

Inputs to soil arsenic stocks should also be assessed during phytoextraction. Arsenic inputs from air pollution explained the increase in soil arsenic concentrations during phytoextraction [22]. Phytoenrichment of arsenic in surface soil due to arsenic leaching from fronds [213] or litterfall, as observed with other hyperaccumulators [214], could explain greater decreases in soil arsenic at depth than in surface soils [11,12,145,146].

8. From Research to Practical Application

This review focused on soil geochemical processes that affect arsenic phytoextraction with *P. vittata*. More research is needed to determine from what distance in soil arsenic travels to roots for uptake, and if arsenic released from soil might leach instead of being taken up by *P. vittata*. Evidence suggests root exudates, especially phytic acid, release arsenic from soil through the ligand-enhanced dissolution of iron minerals. Efforts to increase remediation rates seem most successful when nutrients are supplied in sparingly soluble forms or with an ecologically based approach combining nutrient sources and biological inputs (for example, mycorrhizal fungi and phosphate rock), and more work is needed in this direction. Nonetheless, in the absence of easy solutions to increase in situ uptake rates dramatically, phytoextraction appears limited to soils with low arsenic concentrations, according to the “gentle remediation” or “soil polishing” approach [24]. The successful remediation of soils with less than 50 mg/kg As in 5 years is consistent with this gentle remediation approach [207].

Indeed, there is considerable debate about whether phytoextraction can be broadly applied [151,206]. In China, the jump from research to practice occurs on the hectare scale in farmland trials [141,161,207]. These successful trials suggest arsenic hyperaccumulators could grow best in their native habitat, but even then, fern arsenic uptake can decrease dramatically over 27 months [13], perhaps due to field stress exacerbating uptake already low due to root and soil arsenic heterogeneity.

These results point to a paradox where arsenic hyperaccumulators are niche plants very hardy in certain conditions (e.g., limestone walls) to the point of being considered weedy/invasive in some climates. However, outside of these conditions, arsenic hyperaccumulators are challenging to cultivate on the long timescales needed for remediation [146]. Other species could be better suited for arsenic phytoextraction under certain circumstances. *Pityrogramma calomelanos* showed a greater ability to accumulate arsenic than *Pteris vittata* [13,94,98]. Native species, such as *Pteris umbrosa* in Australia, could be better candidates for phytoextraction than naturalized and/or non-native invasive species such as *P. vittata* [99].

The inherent heterogeneity of arsenic distributions in soil needs to be addressed to determine mass balances comparing fern accumulation to soil arsenic depletion, perhaps using smaller well-mixed plots within larger field applications. The discrepancy between fern arsenic uptake and soil arsenic depletion in long-term studies is concerning and suggests we need to further quantify parts of the arsenic cycle during phytoextraction, including leaching and phytoenrichment. To advance arsenic phytoextraction, we need systematic studies investigating arsenic cycling in the soil-water-plant system at root-, plant-, and field-scales, to integrate soil biogeochemical and plant physiological perspectives on arsenic cycling and inform practical cultivation methods.

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