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REVIEW

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# One magic pteridophyte (*Pteris vittata* L.): Application in remediating arsenic contaminated soils and mechanism of arsenic hyperaccumulation

**Abstract** One pteridophyte, *Pteris vittata* L., was found to be a suitable plant used in phytoremediation of the soils contaminated with excess arsenic. In this paper, literature on this fern's potential in remediating soils contaminated with arsenic, its possible detoxification strategies, and the measures facilitating its practical application in phytoremediation were reviewed. Some of the unresolved questions about this fern in both detoxification and phytoextraction of arsenic (As) were also listed. The aim of this paper was to introduce this magic fern to more researchers focusing on a wider field of discipline and make it more useful in both macroscopic and microcosmic science world.

**Keywords** pteridophyte, fern, arsenic, soil, *Pteris vittata* L.

## 1 Introduction

Arsenic (As) is a toxic element widely encountered in environments and organisms (Tian et al., 2007). It can enter terrestrial and aquatic environments through both natural and anthropogenic activities. The primary anthropogenic input derives from pesticides, fertilizers, wood preservatives, smelter wastes, and coal combustion (Wang and Jia, 2007). Severe As contamination in soils may cause a variety of problems such as loss of vegetation, ground water contamination, and As toxicity in plants and animals, which are threatening human health (Zhang et al., 1996; Cai et al., 2004b). For example, long term exposure to low concentration of As in drinking water can lead to skin, bladder, lung, and prostate cancers, cardiovascular disease, diabetes, anemia, as well as reproductive, developmental, immunological, and neurological disorders (Huang et al., 2009). In recent years, public concern regarding this element has increased due to new evidence that As may be toxic at lower concentrations than previously thought. EPA has decreased the drinking water As standard from previous $50\,\mu g \cdot L^{-1}$ to $10\,\mu g \cdot L^{-1}$ in 2001 in order to more adequately protect public health (Zhang et al., 2002).

Current remediation methods for As contaminated soils include soil removal and washing, physical stabilization, and the use of chemical amendments, and all of which are expensive and disruptive. Phytoremediation, cleaning up environments contaminated with heavy metals and metalloids, is an emerging technology due to its cost-effectiveness and environmental friendliness (Baker, 1989; Salt et al., 1995). A specific phytoremediation approach, called phytoextraction, makes use of terrestrial plants that extract pollutants from the soils and accumulate them in harvestable parts (Lasat, 2002). A versatile and hardy fern (*Pteris vittata* L.), as the first found As hyperaccumulator (Ma et al., 2001; Chen et al., 2002), is reported to accumulate extremely large concentration of As in its above ground biomass. *P. vittata* L., locally called brake fern, Chinese brake, or ladder brake, is widely cultivated and naturalized in many areas with a mild climate (Wu and Qin, 1991) and especially prefers sunny and alkaline soils, where As is more available.

In this paper, studies on *P. vittata* L. as an As hyperaccumulator were reviewed. Its extraordinary capability in hyperaccumulating As in fronds is depicted, the physiological mechanism of this capability is generalized, and some practical issues of application of this fern in cleaning up soils contaminated with As are also listed. Our aim of the paper was to introduce this magic pteridophyte to more researchers engaged in a wider scope of disciplines and make it possible to understand the biochemical and genetic mechanism of the phenomenon of hyperaccumulating the toxic element As in the plant body.

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## 2 High phytoextraction capacity

### 2.1 High As tolerance

It is interesting that As contaminated soils at some extent significantly stimulated fern growth. When exposed to low levels of As ($\leqslant 20\,\mathrm{mg \cdot kg^{-1}}$), the fern biomass increased with the increase of soil As concentration for both roots and fronds (Cao et al., 2004); growing in soils amended with 50 and 100 $\mathrm{mg \cdot kg^{-1}}$, brake fern biomass aboveground increased by 107% and 64% greater than that of the control (Tu et al., 2002); and the application of 200 $\mathrm{mg \cdot kg^{-1}}$ As to a sandy soil had less effect on biomass yield; however, no toxicity symptoms were observed (Tu et al., 2002). All above results implied that *P. vittata* L. has a particular high tolerance to this toxic element, and the maximum concentration in soil might be as high as up to 1500 $\mathrm{mg \cdot kg^{-1}}$ (Ma et al., 2001).

### 2.2 Remarkable capability of extraction and translocation of As

Except for its high tolerance to As, brake fern accumulates high concentration of As in its fronds. For many crop plant species, typical phytotoxicity of As is shown when As in shoots reaches 5–20 $\mathrm{mg \cdot kg^{-1}}$ (DW). It is reported that in As polluted regions in China, As concentrations in vegetables ranged from 0. 001 to 8. 51 $\mathrm{mg \cdot kg^{-1}}$ (FW) and that in grain crops ranged from 0.007 to 6.83 $\mathrm{mg \cdot kg^{-1}}$ (Xiao et al., 2009). However, ferns posses a remarkably ability to tolerate exceedingly high concentrations of As ($> 10000\,\mathrm{mg \cdot kg^{-1}}$) in the fronds (Lombi et al., 2002). Arsenic concentration in the fronds increased rapidly from 12.1 $\mathrm{mg \cdot kg^{-1}}$ to 6000 $\mathrm{mg \cdot kg^{-1}}$ (DW) at the eighth week of transplanting to contaminated soil with 98 $\mathrm{mg \cdot kg^{-1}}$ As, and it increased to 7230 $\mathrm{mg \cdot kg^{-1}}$ after 20 weeks (Ma et al., 2001).

Brake fern is efficient in extracting As from soil and then translocates most of them from underground part to aboveground fronds. Bioconcentration factor (BCF), defined as the ratio of As concentrations in plant tissue to those in soil, can be used to compare the effectiveness of the plant in concentrating As from soil into its biomass. The BCF of brake fronds increased with an increase in water soluble As, which varied from 2.68 to 51.6 among different As amendments into soil (Tu and Ma, 2002). The translocation factor (TF), defined as the ratio of As concentrations in fronds to those in roots, depicts the effectiveness of a plant in this translocation. The TF values showed that As concentrations in aboveground biomass were 4 to 25 times greater than those in roots.

### 2.3 Considerable biomass and its biological characteristics

Successful application of phytoextraction depends on many factors, among which that is most important is the As concentration in above ground part and plant biomass. Brake fern has considerable biomass, for after transplanting to As contaminated soil for 20 weeks, its biomass reached 18 g per plant (Tu et al., 2002). Moreover, it is fast growing and easy to propagate, which makes it possible to be cultivated in most places in the world. Its perennial nature makes the phytoextraction process even more cost-effective since no replanting after harvest is needed.

## 3 How As is detoxified in *P. vittata* L.

### 3.1 Arsenic distribution

To use phytoremediation efficiently, it is important to obtain a detailed understanding of the biochemical pathways and mechanisms that operate the translocation of As from soil to shoots. In terms of the As distribution, for the whole plant, up to 93% of the total As accumulated was concentrated in fronds and the majority of As was concentrated in the pinnae (96% of total As), while the concentration in spores were much lower (Ma et al., 2001).

Arsenic concentration, especially of water soluble As in soils, significantly affects its distribution in fronds of different ages. At low As levels ($< 50\,\mathrm{mg \cdot kg^{-1}}$), As tends to be preferentially supplied to actively growing parts of the fern, which is much like a case of nutrient, especially of phosphorus; when adequate levels of As are present in the soil ($> 50\,\mathrm{mg \cdot kg^{-1}}$) (Tu and Ma, 2002; Cao et al., 2004), As will be translocated to all fronds with little discrimination, leading to a higher concentration in mature fronds that have been receiving As for a longer time.

Root As concentration is positively correlated with soil As concentration, whereas fronds As concentration increased linearly with soil As concentration $< 100\,\mathrm{mg \cdot kg^{-1}}$ and decreased when the soil As concentration $> 100\,\mathrm{mg \cdot kg^{-1}}$ (Tu and Ma, 2002). This indicates that the As translocation to the fronds reduced owing to the changes of toxic levels of As in the roots.

### 3.2 Arsenic speciation

Speciation of As in plants can provide important information helpful to understand the mechanisms of As accumulation, translocation, transformation, and detoxification.

It is known that both inorganic and organic As compounds are toxic to most living organisms, but inorganic As compounds tend to be more toxic than organic As, and arsenite (As(III)) is more toxic than arsenate (As(V)). The Chinese Brake fern that can remove As from soil contains different As species As(III)/As(V); organic/inorganic), with a little effect on the As concentrations in fronds (Tu and Ma, 2002).

As(V) is the predominant species in the fern roots, whereas As(III) is the predominant species in the fronds.

Using HPLC-ICP-MS, Chen et al. (2004) found that approximately 60%–70% of As(III) was present in the fronds, while there was only 8.3% As(III) in the roots. The X-ray absorption spectroscopy results also showed that primarily 75% As in the fern fronds proved to be As(III), and the rest was As(V) (Lombi et al., 2002; Webb et al., 2003).

Although As was stored in fronds of the fern mostly in the form of As(III), As(V) was the main species that existed in the root when supplied in the inorganic form under a hydroponics system (Kertulis et al., 2005). As(III), before being taken up from the soil, might be oxidized to As(V) that was taken up via the phosphate uptake system, but As(III)) uptake did not share the same transport system as phosphate (Wang et al., 2002). Kertulis et al. (2005) found that As(V) was the main speciation in the xylem sap when supplied with As(III) and As(V). Regardless of the species supplied, As may be transported in form(s) that are least harmful to the plant. However, in the fern fronds, As (III) was the main species as well as the more toxic style of As compared with As(V). Arsenic reduction may serve as one of the strategies for Chinese Brake fern to accumulate a large quantity of As in fronds without toxicity (Tu and Ma, 2003a). Shoji et al. (2008) reported that *P. vittata* L. callus could efficiently reduce As(V) to As(III) by the rapid introduction of reductase and synthesize thiols leading to phytochelatins production. As we know, As(V) acts as a phosphate analog and affects the phosphate metabolism. Brake fern may make use of a difference between As(V) and phosphate to avoid As(V) toxicity, with greater reduction of As(V) to As(III) than that of phosphate to phosphite.

### 3.3 Chelation

It is known that As(III) can react with sulfydryl group of enzymes and tissue proteins, leading to inhibition of cellular function and death, and a further process may be involved in making As(III) less toxic. After reduction to As (III) in cytoplasm, As(III) must be chelated by ligand to avoid the consequences of cellular toxicity. Then, arsenic complexes are eventually sequestrated into vacuoles to be stored.

Arsenite has a strong affinity for thiols, and at high As concentrations in the fern fronds, As displayed a significant degree of coordination with sulfur atoms (Webb et al., 2003). The contents of GSH and-SH were significantly increased at high levels of As exposure ($>20\,\mathrm{mg \cdot kg^{-1}}$). Moreover, high concentrations of GSH and-SH were associated with high plant As concentrations (Cao et al., 2004). Nonenzymatic antioxidant glutathione (GSH) and phytochelatins (PCs) are considered to be responsible for the observed As(III)-sulfur coordination (Zhao et al., 2003). Cai et al. (2004b) reported that in addition to cysteine and glutathione, an unidentified thiol was observed in the fern exposed to As, which was not found in the control. The concentration of the unidentified thiol showed a very strong and positive correlation with As concentration in the fronds. Chelation with PCs in cytoplasm is essential for As detoxification but seems to play a limited role in extraordinary As tolerance in *P. vittata* L. (Zhang et al., 2004b). Cai et al. (2004a) also found that the acid-soluble thiols synthesized were not sufficient to compound all As(III) accumulated in the fern. Therefore, the mechanism of As(III) detoxification in Chinese Brake fern may be more complex than simple chelation of As(III) by the thiols. A PC-independent sequestration of As into vacuoles was suggested to play a major role in As tolerance in *P. vittata* L. For example, the low molecular weight thiols may play a transport role by facilitating the transportation of As into the vacuoles, where As may form a more stable aggregation with sulfide and organic acids. Zhang et al. (2004a) reported that except for phytochelatin (PC2) in the fern found under As exposure, one unknown As complex was found. This complex was sensitive to temperature and metal ions but relatively insensitive to pH. It probably functions as a shuttle to transport As(III) from cytoplasm into vacuoles, and most of As(III) are stored in the vacuoles.

Compartmentation of As in the vacuoles makes it possible for the plant to concentrate a large quantity of As in the fronds. For the cellular level, EDXA analyses reveals that As is compartmentalized mainly in the upper and lower epidermal cells, probably in the vacuoles (Lombi et al., 2002). Most As are captured in the vacuoles in the cytoplasm and thus keeps As(III) away from sites of metabolism in the cytoplasm.

Except for the nonenzymatic antioxidants, the enzymatic antioxidants (SOD, CAT, GPX, and APX) were found to be important for As detoxification (Cao et al., 2004). The activities of enzymatic antioxidants were increased at low levels of As exposure but decreased or leveled off at As exposure of $>20\,\mathrm{mg \cdot kg^{-1}}$, which is consistent with the changes in the biomass of Chinese Brake fern.

### 3.4 Function of root excretion

According to the investigation of the effect of plant As uptake on redistribution in soil under pot experiment, all fractions of As in soil were mobilized (Gonzaga et al., 2008b). The ability of *P. vittata* in solubilizing As from all fractions in the soil might be one mechanism and affiliate its high tolerance and hyperaccumulation of As. Gonzaga et al. (2008a) also found that large amounts of As taken up by *P. vittata* were from other nonbioavailable As fractions. This ability of mobilizing As of low availability from soils makes it available for As uptake by the fern. Tu et al. (2004) found that *P. vittata* has the ability to exude large quantities of dissolved organic carbon (DOC) and to change the rhizosphere pH, which may enhance the As bioavailability in soils, thereby increasing its As uptake.

Other possible strategies like older leaves abscission, As hyperaccumulation in the trichome (Li et al., 2004) were also suggested as the supplementary explanation of the ways for *P. vittata* to detoxify the toxicity of As.

## 4 Application of *P. vittata* in phytoremediation of As contaminated soil

### 4.1 Factors to optimize its efficiency of phytoextraction

### 4.1.1 pH value

Arsenic contamination of soil and water varies with the pH value and concentration of As and phosphate. Tu and Ma (2003b) reported that under the hydroponics condition, the optimum fern growth could be achieved by adjusting pH value correspondingly to As levels in the growth media and maximum plant As hyperaccumulation by maintaining a minimum P concentration in the medium at $\mathrm{pH} < 5.21$. This is useful for developing strategies to remediate As contamination in water by Brake fern.

### 4.1.2 Phosphorus

Unlike hydroponics, under the soil condition, phosphate application significantly enhanced As uptake by Brake fern, with As concentration in the frond increasing up to 265% as compared with the control (Cao et al., 2003). The phosphate increased the available As in the soil by replacing adsorbed As(V), thus resulting in elevating As uptake. Therefore, growing ferns with the application of phosphate rock is more effective for the remediation of the As contaminated soils. The plant biomass was mostly likely to be enhanced by increasing phosphate because As(V) added to the soil could induce more P uptake from the soils as more P was replaced by As(V). A field experiment showed that yields of *P. vittata* were enhanced with increasing P addition when the rate of P addition was $< 200\,\mathrm{kg \cdot hm^{-2}}$. As concentration increased after the application of P fertilizer and depressed under excessive P addition. The highest efficiency of As removal in theory could be achieved at $369\,\mathrm{kg \cdot hm^{-2}}$ P addition. (Liao et al., 2004).

### 4.1.3 Organic matters

Organic matter (OM) content was important in controlling plant As uptake. Studies on dissolved organic matters indicated that organic compounds tended to displace As that is bound to iron oxides/hydroxides, resulting in the release of the dissolved As into the soil to increase its availability (Redman et al., 2002; Saada et al., 2003).

### 4.2 Disposal

The disposal technology of Chinese Brake fern accumulating As is complex. The main processes of its resource reuse include preparation, incineration, and extraction (Xiao, 2007). Because the thermodynamically stable oxidation of As under oxic condition lies in As(V). The Brake fern must continually supply reductants in order to maintain As in As(III) state. Once the fronds are harvested and allowed to slowly dry up, the As slowly oxidizes back to As(V). Drying fronds can lead to permeability of cell membranes. To minimize secondary contamination with As because of leaking of inorganic ions, the harvested Brake fern should not come into water supplies (Tu et al., 2003a).

### 4.3 Potential application in co-contaminated soils with heavy metals and As

Exposed to As and other trace elements such as Cd, Cu, Cr, Zn, Pb, Hg, and Se, the concentrations of all the elements in fronds were moderately elevated compared with the controls, and the BCF for all other tested elements was below one (Cai et al., 2004a). Therefore, the Brake fern is the specific hyperaccumulator for As. However, *P. vittata* shows a high tolerance for Zn, Pb, and Cu. An et al. (2006) found that *P. vittata* had a very high tolerance to Zn and could grow normally at sites with high Zn concentration. A greenhouse experiment showed that *P. vittata* had a high tolerance to Pb and Zn and could efficiently extract As from the soils cocontaminated with Pb, Zn, and As (An et al., 2003). Zheng et al. (2008) found that As can stimulate growth and reduce Cu phytotoxicity in gametophytes of *P. vittata* through *in vitro* model system. These studies suggested the possibility of application of *P. vittata* as a plant used in phytoremediation of the soils cocontaminated with As and other metals.

### 4.4 Recommended agronomic measurements

Gonzaga et al. (2006) suggested that remediation of As-contaminated soils may be better accomplished by using higher dense plantation and a management practice that would narrow the distance of plant rhizosphere, because it was found that As transfer from less-available fraction to fraction more available was slower than the As depletion by *P. vittata*. Arsenic concentration in *P. vittata* in the first harvesting was significantly lower than that of the second and third harvesting. Therefore, it showed that more harvesting would increase the As accumulation and phytoextraction efficiency of As (Li et al., 2005).

## 5 Some unresolved issues about the magic hyperaccumulator of As

1) Phosphate was elevated when As was present in soil, and more phosphate was retained in the roots to cope with the increase of As (Cao et al., 2004). Under hydroponics condition, the addition of phosphate enhanced As(V) reduction (Tu et al., 2004). Phosphate and As are

chemically similar, and more work should be done in understanding whether phosphate be constitute a part of As-detoxificating mechanism of Brake fern.

2) The fact that As was stored mostly in the form of As(III) in the frond but transported in the form of As(V) indicates that the majority of the As reduction takes place in the frond pinnae. However, the process of reducing As(V) remains unclear and awaits further research.

3) XAS (X-ray Absorption Spectroscopy) gives an initial insight into the native speciation of As, and XAS experiments need to be performed to explore more hypothesis about the detoxification mechanism of *P. vittata*.

4) It is important to test the growth of *P. vittata* in different soils and its efficiency in taking up As from different sources of As contamination. Practical issues such as the time required to achieve a given target level, the long term efficiency of the process, and the As pools depleted by the plant still need further argumentation.

5) In order to achieve an effective phytoextraction of As, it is crucial that proper agronomic techniques and fern management be supplemented.

6) It has been proposed that *P. vittata* L. may enhance the metal solubility in the rhizosphere via root exudation as it is known for other plants. However, the assessment of root exudates derived from microbial metabolites in the rhizosphere is a difficult task because the root exudates are hard to collect. Therefore, more work is needed to affirm the actual efficiency of root exudates of Chinese Brake fern.

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