

REVIEW ARTICLE

Pteridophytes as Natural Scavengers of Soil Arsenic: From Physiological Foundation to Environmental Maneuvering

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ABSTRACT

Arsenic (As) contamination in soil poses a serious threat to ecosystem and human health. Different physical and chemical methods are employed to mitigate this problem, but they have several inherent drawbacks including high cost, energy intensive and eco-invasive nature. Whereas phytoremediation is an eco-friendly, cost-effective and sustainable strategy for remediation of soil As contamination. Pteridophytes have evolutionary advantages that allow them to accumulate contaminants from soil, thus acting as natural scavenger of soil contaminants. Chinese brake fern (*Pteris vittata* L.) is credited with the distinction of being 'first' arsenic hyperaccumulator exhibiting remarkable potential for arsenic removal from contaminated soils up to 22,630 mg kg⁻¹ As in its dry mass. In this review starting with a brief outline of the taxonomy of phytoremediation, the molecular mechanisms of accumulation, translocation, and detoxification of As in pteridophytes have been highlighted with special reference to *P. vittata*. Further, the means and measures of maneuvering phytoremediation have been discussed with an objective of enhancement of its performance and success in environmental remediation.

KEYWORDS

• Arsenic • Bioaccumulation • Hyperaccumulators • Phytoremediation • *Pteris vittata* • Soil

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INTRODUCTION

Arsenic (As) is a redox active toxic metalloid classified as Class I category of carcinogen the International Agency for Research on Cancer (IARC, 1987) (Y. Li *et al.*, 2024). Based on abundance, As has been ranked at 20th position among the elements available in earth's crust. Pollution of As in soil and water has become a burning environmental issue in many parts of the world, especially in South and Southeast Asia. More than 60 million people are estimated to be at risk of As poisoning in Bengal delta comprising major part of West Bengal, India and Bangladesh as its hotspot (Mondal *et al.*, 2025). The sources of As contamination in soil and water can be natural (geological and biological) as well as anthropogenic. Currently, unplanned development, industrialization and resource mismanagement have added fuel to the fire of the pre-existing As contamination. In such areas, even a single hand pump exposes a large section of the population to As-contaminated drinking water and its toxicity. Main staple food of these regions is rice, cultivated twice or thrice a year, relying on rainwater as well as heavily on irrigation. Therefore, significant amount of As get deposited gradually in agricultural soil, and enters easily into the food chain. Rice and other crops grown in this soil is likely to be contaminated with As, while its prolong consumption poses pernicious human health risks and diseases such as peripheral neuropathy, cardiovascular diseases, miscarriage, prostate and skin cancer (Y. Li *et al.*, 2024).

Conventionally, different physical and chemical approaches are adopted to remove As from soil but most of them are expensive, labour-intensive, and eco-invasive in nature, which can alter soil physicochemical properties, and adversely affect the beneficial soil microbiome (Jassal *et al.*, 2025; F. J. Zhao *et al.*, 2022). Contrarily, phytoremediation offers a green, eco-friendly, sustainable, and cost-effective alternative demanding low or no external inputs but by involving (periodic growing and harvesting) plants capable of accumulating As in their body parts (Capobianco *et al.*, 2026; Huslina *et al.*, 2024). In recent years researchers have focused on employing plants to cleanup and restore arsenic-contaminated soils. Non-edible plants endowed with high growth rate and contaminant accumulation potential

are ideal candidates for remediating arsenic-contaminated soils safely and effectively. Among different plant groups vascular cryptogams (belonging to Pteridophyta) appear as the best choice as potent soil As phytoremediation agents. Arsenic hyper-accumulating pteridophytes (ferns) belong to order Pteridales. Well known As hyper-accumulating ferns are *Pteris vittata* and *Pityrogramma calomelanos*, which can accumulate up to 19,300 mg kg⁻¹ and 11,600 mg kg⁻¹ As respectively, in respective dry mass after 78 days from soils contaminated with As (Yong *et al.*, 2010). Being an inherently slow process a successful and substantial remediation outcome demands phytoremediation to become a part of long-term remediation strategy. Such disadvantages of phytoremediation can be overcome through adoption of a holistic approach known as phytomanagement (Pandey & Bajpai, 2019). The main objectives of phytomanagement is ecosystem rehabilitation with substantial land use. Phytoremediation efficiency of pteridophytes can be improved by applying chelating agents, organic amendments, beneficial bacteria, genetic engineering and omics approaches (Parida *et al.*, 2024). Nano-phytoremediation is relatively recent phytoremediation approach which combines the use of nanoparticles in phytoremediation technology, but its application is till now limited due to lack of inadequate research evidences (Prakash & Chandran, 2023; Usman *et al.*, 2025).

TAXONOMY OF PHYTOREMEDIATION

The phytoremediation process refers to using plants, along with their associated soil microorganisms, to minimize the concentration, mitigate toxicant's stress and/or alleviation of toxicity of environmental contaminants. The term phytoremediation is derived from two words: the Greek word phyto, meaning "plant," and the Latin word remedium, meaning "to remove or remediate". Phytoremediation is an eco-friendly, efficient, cost-effective and solar-driven technique which can be used efficiently for remediation of inorganic (viz. heavy metals, metalloids, radionucleotides etc.) and organic pollutants [viz. polychlorinated biphenyls (PCB), polynuclear aromatic hydrocarbons (PAH), Hexachlorocyclohexane (HCH), Dichlorodiphenyltrichloroethane (DDT),

Polybrominated diphenyl ethers (PBDE), Chlorpyrifos (CPF) etc.]. Hyperaccumulator plants can be grown on toxic heavy metals contaminated sites to remedy the polluted soil. Phytoremediation has earned popularity as nature-based solution (NbS) because of its innocuous nature and affordability arising from lower maintenance and installation costs compared to conventional methods (Bhat *et al.*, 2025). Thus common people termed phytoremediation as “green clean” (Pham *et al.*, 2022).

DIFFERENT PROCESSES OF PHYTOREMEDIATION

The processes involved in phytoremediation are phytoextraction/phytoaccumulation, phytodegradation, phytofiltration, phytostabilization, and phytovolatilization.

1. Phytoextraction

It is also known as phytosequestration or phytoaccumulation or phytoabsorption. In this uptake process contaminants are taken up from the environmental medium by plant roots and subsequently translocated and accumulated in above-ground plant biomass. The efficiency of contaminants accumulation in above-ground biomass of the plants, especially in shoot depends on their ability to take up that particular contaminants with high efficiency and subsequent translocation to their shoots avoiding complexation in their roots. This contaminants translocation is very crucial for the effective phytoextraction as the root biomass is not generally efficient (X. Cao *et al.*, 2025; Islam *et al.*, 2024).

2. Phytofiltration

It is also known as rhizofiltration (by plant roots) or caulofiltration (by excised shoots) or blastofiltration (by seedlings). This process is associated with contaminants removal from waste waters or surface waters by plant root system. Actually phytofiltration reduces metals and metalloids mobility in sediment by eliminating pollutants from aqueous environment. Here the movement of contaminants to underground water is minimized due to their adsorption or absorption by plant roots (Bokhari *et al.*, 2022).

3. Phytodegradation

The process involves the degradation of only organic pollutants in medium by plant

enzymes such as dehalogenase and oxygenase (Praveen and Pandey, 2020). It is also known as phytotransformation. Several organic xenobiotic compounds are accumulated in plant bodies and detoxified through their metabolic activities. Phytodegradation is independent of rhizospheric microorganisms. Due to the non-biodegradable nature of heavy metals and metalloids, phytodegradation is applicable for the removal of organic pollutants only (H. Liu *et al.*, 2024). Although toxic metal(loid)s may undergo biotransformation process.

4. Phytovolatilization

In phytovolatilization process plants absorb metals and metalloids from the soil, transform them into less toxic, volatile, or organic forms, and subsequently release the transformed compounds into the atmosphere. Phytovolatilization is only feasible for organic pollutants and certain metals and metalloids that have volatile species like Hg, Se, As. Phytovolatilization is a controversial phytoremediation technique as it releases the volatile toxic pollutants from one segment (soil) to atmosphere for further dispersion through air from where it may be redeposited, just shifting the pollution burden from one environmental medium to another (Shen *et al.*, 2022).

5. Phytostabilization

It is also known as phytoimmobilization. Phytostabilization refers to the stabilization of contaminants in contaminated soil by using certain plants, thereby, immobilizing contaminants in the medium. This technique is very much useful to minimize the bioavailability and mobility of contaminants in the environment. It also decreases the rate of metal(loid)s leaching into the ground water. The immobilization of contaminants by plants occurs through various mechanisms such as sorption onto root surfaces, complexation, precipitation, and valence reduction of metals or metalloids within the rhizosphere (Siunova *et al.*, 2025). However, phytostabilization is considered a temporary mitigatory approach because the contaminants still remain in the soil with their restricted mobility. Phytostabilization indeed is a management strategy for stabilizing the potentially toxic elements (Borbón-Palomares *et al.*, 2024).

6. Phytodesalination

Phytodesalination is associated with salts removal from saline soil by using halophytic

plants to ensure normal plant growth and development. Naturally halophytic plants are adapted better to salt stress conditions than salt-sensitive glycophytic plants to cope with heavy metal(loid)s in the soil (Manousaki & Kalogerakis, 2011).

7. Rhizodegradation

This technique is associated with rhizospheric microorganisms mediated organic contaminants breakdown in the soils. Plants release exudates rich in nutrients such as simple carbohydrates, amino acids, flavonoids etc., in the rhizosphere helps to increase metabolic activities of microbes that enhance the degradation of organic pollutants in the rhizosphere. Apart from these plant roots also secrete certain exoenzymes which is capable of degradation of organic contaminants in soils (K. M. Yang *et al.*, 2023).

EFFICIENCY OF PHYTOREMEDIATION

The main and most useful technique of phytoremediation is phytoextraction to remove the contaminants from polluted soils and water (Praveen & Pandey, 2020).

Different factors like bioavailability of the contaminants in soils, properties of soils, redox state of the contaminants, concern plant species etc. regulate the efficiency of the phytoextraction. The screening and selection of hyperaccumulators for the phytoextraction of target metal(loid)s solely depend on the values of bioconcentration factor (BCF) and translocation factor (TF) (Huang *et al.*, 2025), BCF bears much significance in assessing the potential of a plant species for phytoextraction rather than the bulk quantum of metal(loid) concentration in shoot. The value of TF more than 1 refers the efficient translocation of accumulated metal(loid) from root to above ground parts (Cakaj *et al.*, 2024). Plant species with both BCF and TF being greater than one (>1) should be ideal for phytoextraction (Sharma *et al.*, 2023). Sometimes hyperaccumulators have BCF even up to 50 – 100 (Cluis, 2004).

1. Bioconcentration factor (BCF)

The efficiency of a plant species for a metal(loid) accumulation into its biomass from the surrounding environment is particularly determined by bioconcentration factor (BCF). It is calculated as follows (Gupta *et al.*, 2022).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration of the target metal(loid) in the harvested plant part(tissue)}}{\text{Concentration of the same metal(loid) in the soil}}$$

2. Translocation factor (TF)

The efficiency of a plant species in translocation of accumulated metal(loid) from

roots to shoots is determined by translocation factor (TF), which is calculated as stated below (Gupta *et al.*, 2022).

$$\text{Translocation factor (TF)} = \frac{\text{Concentration of the target metal(loid) in plant shoot}}{\text{Concentration of the target metal(loid) in plant root}}$$

ARSENIC

Arsenic is considered as one of the most enigmatic elements known to humankind. It is believed that Albertus Magnus, a German scholar and alchemist of the 13th century, was the first to discover and isolate elemental As from its mineral sources (Paul *et al.*, 2022). Arsenic is ranked as the 53rd most abundant element in the Earth's crust. Inorganic arsenic continues to hold the highest position in the priority list of hazardous contaminants as enumerated by the Agency for Toxic Substances and Disease Registry (ATSDR, 2023). Arsenic is a metalloid having an atomic number of 33 and an atomic mass of 74.92 g mol⁻¹. Arsenic belongs to pnictogen group i.e. under group

15 and period 4 of the modern periodic table. The average As concentration in the earth's upper continental crust approximately 4.8 µg g⁻¹ (Schlesinger *et al.*, 2022). However, in many contaminated areas As concentration exceeds 300 µg g⁻¹ (Michopoulos, 2021). In the environment, As exists in four major oxidation states: -3, 0, +3, and +5, which vary according to the prevailing redox conditions and pH. Under oxic and generally alkaline groundwater conditions, arsenate [As(V)] remains as the dominant species whereas, arsenate [As(V)] gets reduced to arsenite [As(III)] under reducing redox condition of suboxic groundwater within deltaic aquifers. As(V) is 60 times less harmful than As (III)

(Mukherjee *et al.*, 2024). Under both oxidized and reduced conditions within the normal pH range (6.5-8.5) As mobilization in soil can take place (Q. Y. Chen & Costa, 2021; Rae, 2020). Arsenic exhibits chemical similarities with N and P because both belong to the same group 15 in the periodic table.

Methylated and organic As species represent key intermediates in the biogeochemical cycling of As. Biomethylation of inorganic As mediated by plants and microbes results in the formation of methylated As species such as monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) etc. Different organic As compounds including arsenobetaine, arsenosugars, and arsenolipids are found in marine organisms, and originate through complex biosynthetic processes. Inorganic forms of As are reported to be about 100 times more toxic than organic As compound (Mukherjee *et al.*, 2024). Although organic As species are more mobile than inorganic arsenicals, governing As transport and bioavailability in soil–water systems.

1. Sources of arsenic in environment

Arsenic is naturally found in the air, rocks, soil, sediment, water, plants, animals, and sediments, released into the environments through different process such as volcanic eruptions, weathering of rocks, erosion, and forest fires etc. Arsenic occurs naturally in more than 200 types of minerals, with nearly 99% of the total As present in rocks and minerals (Q. Y. Chen & Costa, 2021). Sulfidic minerals are the main source of As, predominantly exists in anaerobic conditions. However, As is associated with several rock-forming minerals, including phosphates, silicates, sulfides, and oxides. Apart from this arsenolite (As_2O_3), cobaltite (CoAsS), orpiment (As_2S_3), and olivenite ($\text{Cu}_2\text{OHAsO}_4$) are the important As containing minerals. Arsenic can be released into ground water through the dissolution of As-bearing minerals (Polizzotto *et al.*, 2006). Arsenic may also be released from river sediments to ground water under reducing redox condition. The As mobilization from iron oxides located in aquifer sediments has been reported to major causes of groundwater contamination. The sources of As due to anthropogenic activities are mining; burning fossil fuels; application of arsenical pesticides, fungicides; and also pharmaceutical drugs (Nriagu *et al.*, 2007).

2. Global status of As contamination

Both organic and inorganic form of As become a major public health concern, affecting hundreds of millions of people worldwide. The World Health Organization (WHO) and the Environmental Protection Agency (EPA) have limited maximum As concentration in drinking water $10\mu\text{g L}^{-1}$ while, As concentrations even below $40\mu\text{g L}^{-1}$ can pose serious health risks to humans (Mukherjee *et al.*, 2024). Globally, the highest concentrations of arsenic have been reported in metalliferous mining areas. As level reaches up to 2500 mg kg^{-1} as recorded in estuarine sediments in southwest England and 484 mg kg^{-1} in stream sediments from the Surata river in Colombia (Ozturk *et al.*, 2021; Patel *et al.*, 2023). Both surface and ground water are contaminated with As in several countries of African subcontinent. Arsenic concentration reaches up to $6150\mu\text{g L}^{-1}$ in Rift valley of Ethiopia and Johannesburg in South Africa (B. K. Thakur *et al.*, 2021). Similarly, in North America and Latin America, several countries including Brazil, Argentina, Bolivia, Colombia, Chile, Ecuador, Cuba, Guatemala, Honduras, Peru, Nicaragua, Uruguay and Mexico have been reported to be affected by As contaminated ground water. Western part of the USA and many European countries including Turkey, Spain, Siberia, Romania, Hungary, and Greece also suffer from As contaminated ground water which has been reported to range between 30 and $4500\mu\text{g L}^{-1}$ (Q. Y. Chen & Costa, 2021; Patel *et al.*, 2023). In this count Bangladesh stands as a remarkable country, where 77 million people are exposed to mass As poisoning via drinking of As-contaminated ground water (Rehman *et al.*, 2021). Highest As concentration reported in ground water of Bangladesh is $4,730\mu\text{g L}^{-1}$ (Chowdhury, 2023). In India, occurrence of As in groundwater contaminated region is mainly distributed across two distinct zones: (i) the hard-rock regions of Karnataka and Chhattisgarh; (ii) alluvial region of Punjab, Uttar Pradesh, Bihar, Jharkhand, Manipur, Assam, and West Bengal (Chakraborti *et al.*, 2017). The surface soil of Korba basin area and Ambargarh Tehsil of Chhattisgarh state have recorded exceptionally high levels of As up to 164 mg kg^{-1} and 4600 mg kg^{-1} respectively (Patel *et al.*, 2023). Ground water contamination by As in West Bengal was first reported in 1978, and now it has become one of the severely affected areas in the world (Chakraborti *et al.*, 2017). According

to Central Ground Water Board (CGWB), As contamination in groundwater was detected in nine out of the twenty-three districts of West Bengal, approximately 7 million people are suffering of arsenicism having chronic symptoms (Chakraborti *et al.*, 2017). Nadia is the most affected district in West Bengal, with highest levels of As contamination in ground water. Besides, several districts in West Bengal, namely Malda, Murshidabad, North 24 Parganas, South 24 Parganas, Hooghly, Purba Bardhaman, Howrah and Kolkata have been reported to have As concentration in groundwater exceeding the WHO recommended permissible limit ($10 \mu\text{g L}^{-1}$) (Chakraborti *et al.*, 2017). The highest As concentration ($3,700 \mu\text{g L}^{-1}$) in groundwater has been recorded from a tubewell water sample in West Bengal, which is approximately 370 times higher than the WHO's recommended value of $10 \mu\text{g L}^{-1}$ (Chakraborti *et al.*, 2017).

3. Toxic effects of As on human

Arsenic is a non-essential toxic metalloid causing deleterious effects on every organ of human body as a result of interference in normal metabolic processes (Q.Y. Chen & Costa, 2021). Arsenic poisoning may be acute and chronic. Acute toxicity of As results in vomiting, nausea, severe diarrhoea; whereas prolong exposure (5 – 10 years or more) causes chronic toxicity of As resulting in multisystem diseases with typical arsenical symptoms such as black foot disease, melanosis, and keratosis. According to National Research Council [NRC (1999)], in many As affected regions of India, children under 11 years have no arsenical symptoms though their biological samples exhibit high As levels. However, characteristic As induced skin lesion was manifested among children chronically exposed through drinking of higher As contaminated water ($\geq 1000 \mu\text{g L}^{-1}$) while nutritionally poor subjects showed such typical symptoms upon chronic exposure to relatively lower contamination level of As ($\geq 500 \mu\text{g L}^{-1}$) in drinking water (Biswas *et al.*, 2020; Rahman *et al.*, 2001). Arsenic tends to accumulate predominantly in the liver, kidneys, and muscles. Arsenic also a group 1 carcinogen promotes cancers of the lung, skin, kidneys, bladder, and prostate (NRC, 1999). Arsenic is a well-recognized human carcinogen, and no specific treatment exists for chronic arsenic poisoning, so effective management strategies primarily involve

minimizing exposure to As by controlling it at its source (Moulick *et al.*, 2021).

4. Effects of As on plants

Arsenic is toxic to plants depending on the types, nature and environmental factors. Plants show differential phenotypic features subject to dose and time of exposure of As. Chronic exposure of plants to As initially results in symptomatic whitish appearance, which subsequently leads to interveinal necrosis. It causes several detrimental effects on crops such as rice, wheat, maize, barley, and vegetables. Arsenic shows accumulation and biomagnification in human population along the food chain via the consumption of crops (Garg & Singla, 2011; Upadhyay *et al.*, 2019). Arsenic alters various metabolic pathways through inactivation of enzymes or competitive inhibition of phosphate (Pi). Excess generation of reactive oxygen species (ROS) and oxidative burst stand as key biomarker for As induced stress response in plant. The As stress is defended naturally by plants as reflected in higher activities of the antioxidative enzymes or mitigation through exogenous administration of amino acid proline. Apart from this, plants operate several adaptive mechanisms to cope with As toxicity such as up regulation of antioxidative defense system, hyperaccumulation, phytochelation, and reductive transformation [As(V) to As(III)], and subsequent sequestration in cell vacuoles (Biswas *et al.*, 2024; Patel *et al.*, 2023). Besides arsenate reductase enzymes, As(V) can also be reduced to As(III) by certain other enzymes such as glyceraldehyde-3-phosphate dehydrogenase and mitochondrial F1F0-ATP synthase. Furthermore, in place of inorganic phosphate, these enzymes may incorporate As(V) into biological molecules, leading to the formation of arseno-esters (Bakhat *et al.*, 2017). High As levels in soil or water damage vital biomolecules, inhibit biosynthesis of chlorophyll and antenna pigments, disrupt photosystems I and II, light harvesting complex II, and interfere Calvin cycle enzymes, thereby reducing photochemical efficiency and photosynthetic rate, which are key indicators of As stress in plants (Zulfiqar & Ashraf, 2022). Arsenic toxicity in plants reduces germination and respiration rate, decreases biomass and yield, reduces plant growth, accelerates senescence of leaves and sometime apoptosis under lethal dose (Bali & Sidhu, 2021).

PTERIDOPHYTES AS A POTENT PHYTOREMEDIATOR

Pteridophyta is an independent plant division under kingdom plantae, originated in earth approximately 400 million years ago. Pteridophytes are cosmopolitan in distribution, and are found in diverse habitat ranges, including rocky crevices, moist forest, shaded terrestrial or aquatic environments (Qian et al., 2022). Habit of pteridophytes ranges from small herb to long tree ferns. Pteridophytes are often known as vascular cryptogams due its well-developed vascular system and absence of seeds. Vascular tissues are devoid of vessels. They can serve as bioindicators of environmental contamination and ecological stability of a particular region. Because of their unique potential of uptake and accumulation of metals and metalloids certain pteridophytes can be effectively used as phytoremediator although they are not used as food or fodder. They exhibit the following characteristic traits and adaptive strategies that help them to remove toxic soil contaminants like As, as an effective phytoremediation agent: (a) Short life cycle that allows repeated harvesting and replanting, ensuring faster, continuous removal of soil As compared to plants with long life cycle; (b) Faster multiplication rate and biomass accumulation in shorter time period that accelerates As removal from soil involving more tissue to absorb and store As; (c) low maintenance because of thriving naturally without the need for extra care or maintenance; (d) Self-sustaining features combined with additional bonus of removing As from environment; (e) Potential of phytoremediation via phytoextraction, phytostabilisation, phytoaccumulation and phytovolatilisation.

1. Adaptive strategies for fighting As-stress

1. Strong antioxidative defense system

Arsenic triggers reactive oxygen species (ROS) generation in excessive level, which

results in oxidative damage of cell membranes, proteins, and nucleic acids, if not efficiently detoxified. There are several enzymatic (e.g. CAT, SOD, APX, GPX, GR) and non-enzymatic (e.g. ascorbate, glutathione, carotenoids, flavonoids, and phenolic compounds) role players of antioxidative defense in pteridophytes to overcome or mitigate oxidative stress.

2. Efficient transport and detoxification system

Pteridophytes utilize various transporter system for receiving As from environment based on concentration and available form [As(V), As (III) or organic form]. Accumulated As gets efficiently translocated from root to above-ground part and becomes compartmentalized in cell vacuole after detoxification.

3. Adaptation against herbivores

Pteridophytes become unappealing to grazers because they produce defensive secondary metabolites compounds like phenolics and alkaloids. They are used effectively to perform phytoremediation of toxic metal(loid)s.

4. Adaptation at molecular level

Well known As hyper-accumulator *Pteris vittata* possesses the ACR3 gene, responsible for production of a protein, responsible for transfer of arsenous acid into vacuoles. This gene is absent in angiosperms, helps the Pteridophytes safely store arsenic within its cells.

2. As accumulation by pteridophytes

Certain Pteridophytes have evolved to take, tolerate, compartmentalize and store As from environment into their root, rhizome, frond without suffering from its toxic effects. Continuous search for new As-hyperaccumulating pteridophyte species is going on. A comprehensive table (Table 1) illustrates an overview of As accumulating pteridophytes.

Table 1: List of As-accumulating pteridophytes used for environmental remediation

Plant species	As treatment	Duration	As in plant biomass (dry wt.)	References
<i>Pteris vittata</i>	As contaminated soil (400 mg kg ⁻¹)	6 weeks	6,805 mg kg ⁻¹ in frond	(Ma et al., 2001)
	As spiked soil (1500 mg kg ⁻¹)	6 weeks	22,630 mg kg ⁻¹ in frond	
<i>Pteris cretica parkerii</i>	As spiked soil (100 mg kg ⁻¹)	2 months	2493 mg kg ⁻¹ in frond	(Meharg, 2003)
<i>Equisetum hyemale</i>	As spiked soil (100 mg kg ⁻¹)	2 months	86.2 mg kg ⁻¹ in frond	
<i>Selaginella caulesens</i>	As spiked soil (100 mg kg ⁻¹)	2 months	26.3 mg kg ⁻¹ in frond	

Table Cont...

Plant species	As treatment	Duration	As in plant biomass (dry wt.)	References
<i>Adiantum capillus veneris</i>	As spiked soil (600 mg kg ⁻¹)	6 months	~2000 mg kg ⁻¹ in frond ~260 mg kg ⁻¹ in root	(Singh <i>et al.</i> , 2010)
<i>Nephrolepis exaltata</i>	As spiked soil (200 mg kg ⁻¹)	20 weeks	~4000 mg kg ⁻¹ in whole plant	(Slonecker <i>et al.</i> , 2009)
<i>Pityrogramma calomelanos</i>	As spiked hydroponic solution (10 µg g ⁻¹)	6 weeks	110 µg g ⁻¹ in frond	(Wongkongkatep <i>et al.</i> , 2003)
	As spiked hydroponic solution (10 µg g ⁻¹)	6 weeks	4616 µg g ⁻¹ in frond	

Chinese brake fern (*Pteris vittata* L.) the first known As hyper-accumulator, can accumulate 15,861 ppm of As in its above-ground parts, while growing in As-spiked soil (1,500 mg kg⁻¹) only after two weeks (Ma *et al.*, 2001). Eleven other species of Pteridaceae family have been

reported as As-hyperaccumulators (H.B. Wang *et al.*, 2007). Aquatic plants *Myriophyllum propinquum* and *Callitriche stagnalis* have been reported as As accumulator, with concentration exceeding 1g kg⁻¹ dry weight (Favas *et al.*, 2016).

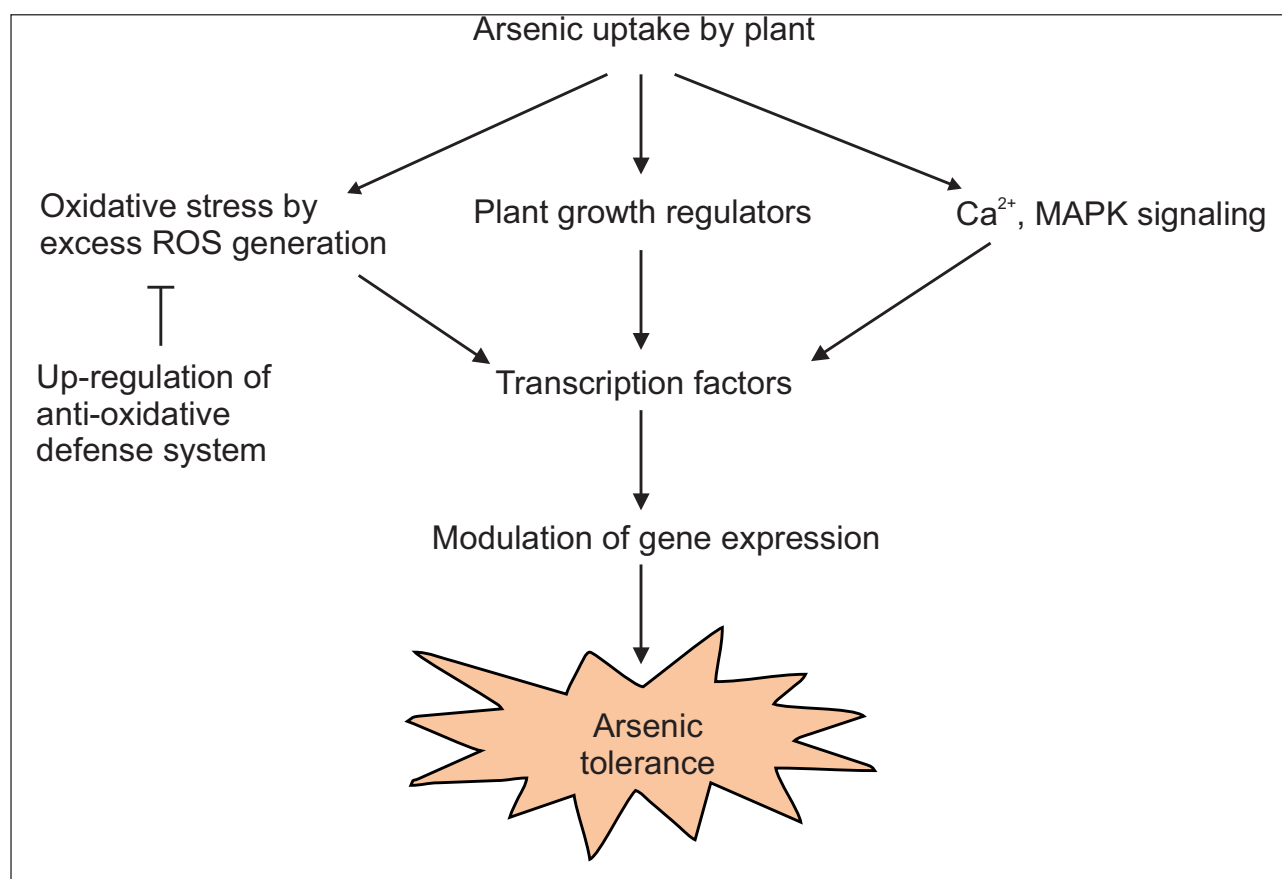


Figure 1: Transcription factors contribute significantly to As-stress tolerance by regulating the expression of stress-responsive genes

1. Arsenate uptake

Major fraction of As in soil remains in the form of As(V) under aerobic environments. Since As(V) (arsenate, AsO₄³⁻) is a structural and chemical analogue of inorganic phosphate (P_i; PO₄³⁻), it takes entry into root via Pi transporter through members of the Pht1 subfamily within the larger phosphate transporter (Pht) superfamily (Shi *et al.*, 2019).

Molecular studies have identified several Pht1 phosphate transporters involved in arsenate (AsV) uptake in *Pteris vittata*, particularly PvPht1;3, PvPht1;4, and PvPht1;6, which play key roles in facilitating the entry of arsenate into root cells (Y. Cao *et al.*, 2019). In hydroponic system with increased phosphate concentration As(V) uptake decreases in *P. vittata* while As(V) uptake increases under phosphorus-deficient

conditions (Fu *et al.*, 2017). But conflicting results came from some studies which showed that external amendment of phosphorus in soil increased As accumulation in *Pteris vittata*, primarily due to phosphorus-mediated release of As(V) in soil particles, thereby enhancing its bioavailability. Recent genomic studies revealed that PvPht1;3 functions as high affinity AsV transporter, facilitates As(V) absorption, translocation, accumulation in shoot when expressed heterologously in tobacco (Y. Cao *et al.*, 2019; Sun *et al.*, 2022). This is stronger As transport from soil to root mediated by PvPht1;4 (X. Li *et al.*, 2020). In anaerobic (mainly in flooded condition) As(III) in rhizospheric soil, is taken up by plants root through the aquaglyceroporin channels in the form of neutral As(OH)³ (Han *et al.*, 2017). A tonoplast intrinsic aquaporin protein PvTIP4;1 in *P. vittata* root is upregulated in the presence of As(III) which in turn helps in As(III) accumulation (Z. He *et al.*, 2016). Two inositol transporter AtINT2 or AtINT4 are involved in As(III) loading in phloem and transport to seed of *Arabidopsis thaliana* (Duan *et al.*, 2015). As(III) uptake in higher plants is mediated by nodulin 26-like intrinsic proteins (NIPs) subfamily (e.g. PsPvH in *Pteris vittata* known as under aquaporins) (Fox *et al.*, 2017). As(III) is transported through OsLsi1 (also known as OsNIP;1) transporter in rice root along with soluble silicic acid (Bakhat *et al.*, 2017; Moulick *et al.*, 2023). Methylated As species in soil like monomethylarsonic acid [MMA(V)] and dimethylarsinic acid [DMA(V)] get entry into the *P. vittata* root through P_i transporter but less efficiently unlike As(V). While the trivalent form of aforesaid As species are more effectively enter cells via aquaglyceroporins.

2. Arsenate to arsenite reduction and efflux mechanism

Unlike hyperaccumulator fern *P. vittata*, other As non-hyperaccumulator pteridophytes rapidly convert As(V) to As(III). As(III) is chelated through cytosolic thiols specially by phytochelatins, *metallothionein* proteins or effluxed to the external environment. However, in As overloading condition roots of *P. vittata* exhibit significant As(III) efflux, which serves as an As detoxification strategy, otherwise As(III) would be readily loaded in xylem for upward translocation to shoot (Han *et al.*, 2016).

The key enzyme, ACR2 arsenate reductase regulates As(V) to As(III) conversion in plants. OsACR2, AtACR2, HIACR2, PvACR2

are the arsenic reductase enzyme found in rice, *Arabidopsis thaliana*, *Holcus lanatus*, and *Pteris vittata* respectively. However, recent research indicates that ACR2 is not solely responsible for As(V) to As(III) conversion in *Arabidopsis thaliana* while a new arsenate reductase enzyme named High Arsenic Content 1 (HAC1) or Arsenate Reductase QTL1 (ARQ1) has been reported as operating vital role in arsenate reduction in plants (F. Zhao *et al.*, 2023). PvHAC1 and PvHAC2 are two novel genes identified in *P. vittata* which predominantly expressed in rhizomes ad frond respectively, but not in root. The distinct expression profiles of HACs in *P. vittata*, along with other evidences, indicate that arsenate reduction may primarily takes place in the rhizomes that facilitates rapid xylem loading of arsenite and subsequent translocation into fronds (X. Li *et al.*, 2020).

3. As translocation

Accumulated As is efficiently translocated to above ground parts of hyperaccumulating pteridophytes. Non-hyperaccumulators with translocation factor <1 are not be able to translocate As in above ground parts with maximum As concentration in root. The preferential translocation of arsenite from the roots to the fronds results in a markedly higher proportion of arsenite in the fronds than in the roots of *P. vittata* (F. Zhao *et al.*, 2023).

ACR3 (Arsenic Compounds Resistance 3) gene has been identified recently in yeast with no similarities with ACR2 gene (Y.S. Chen *et al.*, 2015). ACR3 is found in bacteria, moss, ferns, fungi but absent in angiosperms. Four ACR3 genes (PvACR3, PvACR3;1, PvACR3;2, and PvACR3;3) are identified in *P. vittata*. In hydroponic system treated with 5 µM As(III) or As(V). PvACR3 transgenic *Arabidopsis* sp. has exhibited 40 to 75-fold higher As translocation factors with 25 to 34 fold higher As concentration in shoot.

More recently, (H. Yan *et al.*, 2022) a new plasma membrane protein named PvAsE1, a member of the Solute Carrier Family 13 (SLC13)-like family has been identified. PvAsE1 functions as a plasma membrane As(III) efflux transporter that facilitates As(III) xylem loading from root parenchyma cells, assisting in its long-distance translocation.

4. As detoxification mechanisms

As non-hyperaccumulators with TF<1 protect their photosynthetic machinery by

preventing efficient translocation of As from root to frond (Y. Chen *et al.*, 2013). However, As hyperaccumulators like *P. vittata* employs a cascade of As detoxification mechanisms to cope up with excess As in its aerial parts. One of the key mechanisms is to sequester As(III) in cellular vacuoles of fronds that facilitates detoxification, storage, and hyperaccumulation of As. Significant As(III) sequestration occurs in *P. vittata* mediated by PvACR3;1 and PvACR3;3 located in the tonoplast (J. X. Chen *et al.*, 2021; C. Wang *et al.*, 2018).

Accumulated As in plants creates oxidative burst through the generation of reactive oxygen species (ROS). Excess ROS formation disrupts normal cellular and metabolic process ultimately cell death. Plants along

with pteridophytes have evolved several antioxidative defense system that helps in maintaining cellular optimal titer of ROS. Enzymatic [catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX)] and non-enzymatic antioxidants [Ascorbate, Carotenoids, glutathione (GSH, GSSG), phytochelatin (PC)] play the key role in cellular antioxidative defense system. In As hyperaccumulators enzymatic and non-enzymatic antioxidants are playing more vigorously than that of non-hyperaccumulators (Praveen & Pandey, 2020). However, studies have shown that once the fronds reach their maximum As accumulation capacity, they undergo senescence and eventually abscise from the plant (Tu & Ma, 2002).

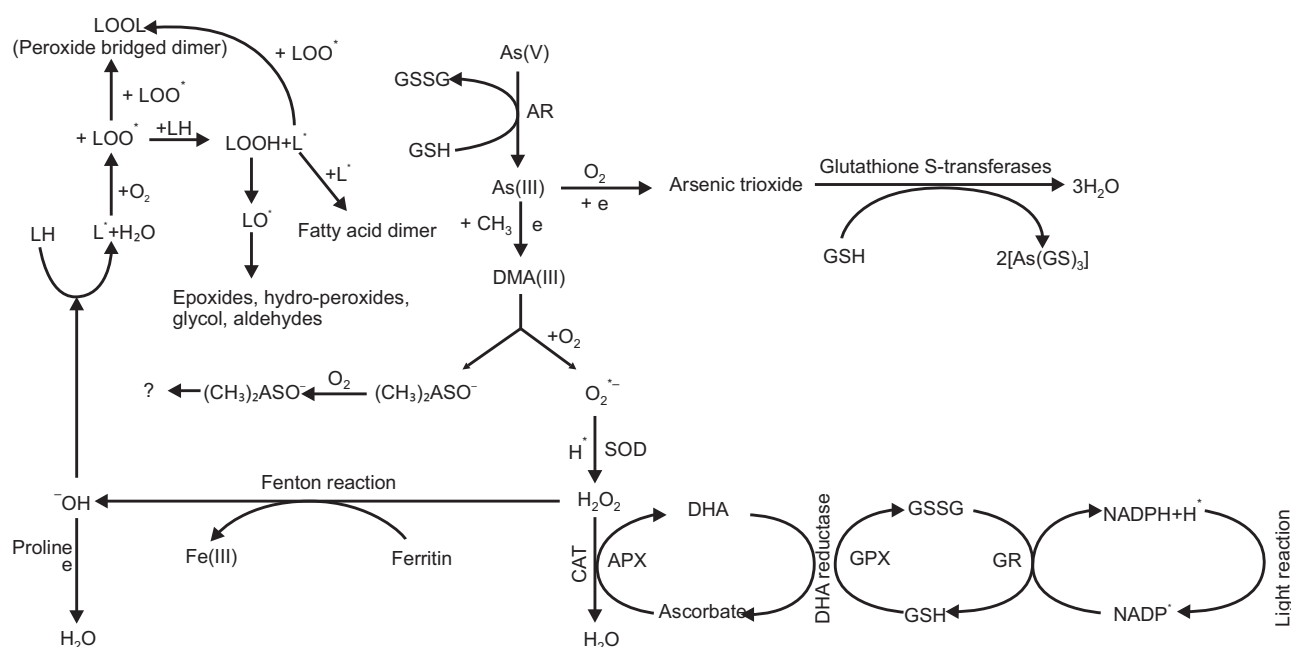


Figure 2: Arsenic detoxification pathway via cellular antioxidative defense system in pteridophytes, APX - ascorbate peroxidase, CAT - catalase, DHA - dehydroascorbate, DMA - dimethylarsenate, GPX - glutathione peroxidase, GR - glutathione reductase, GSH - reduced glutathione, GSSG - oxidized glutathione, LH - lipid, L* - lipid radical, LO* - lipid alkoxy radical, LOO* - lipid peroxy radical, LOOL - lipid peroxide, LOOH - lipid hydroperoxide, NADP - oxidized nicotinamide adenine dinucleotide phosphate, NADPH + H⁺ - reduced nicotinamide adenine dinucleotide phosphate, SOD - super oxide dismutase.

ENHANCEMENT OF PHYTOREMEDIATION OF AS

Enhancement of As phytoremediation helps to improve the efficiency of As removal from contaminated soils, which may be achieved by following methods.

1. Microbes-assisted phytoremediation

Rhizospheric microbial communities particularly bacteria and fungi enhance the potential of As sequestration and mitigation of As-induced oxidative stress in plants. Rhizospheric

microorganisms help to convert As into less toxic forms through redox reactions and immobilize it within their biomass which modify As bioavailability and enhance uptake by root (C. Yang *et al.*, 2022). The As transformation processes employed by rhizospheric microorganisms include As(V) reduction, As(III) oxidation, methylation of inorganic As into various n-methylated arsenic compounds. Plant growth-promoting rhizobacteria (PGPR) enhance plant growth and arsenic tolerance by producing siderophores that complexed

with As, phytohormones that promote plant growth, and enzymes that improve As-induced stress resistance. Recent studies have shown that inoculation of *Alcaligenes* sp. enhances As bioavailability and improves the overall remediation efficiency of the fern *P. vittata* by promoting As(V) reduction. Inoculating five potent As reducing bacterial strains, namely *Rhodococcus* sp. TS1, *Delftia* sp. TS41, *Delftia* sp. TS33, *Comamonas* sp. TS37, and *Streptomyces lividans* PSQ22 improved As accumulation in *P. vittata* by 53%, As uptake by 44%, and reduced As leaching in soil by 29 to 71% (Q. Yang et al., 2012).

2. Nano-phytoremediation

Nano-phytoremediation is a relatively new emerging branch of phytoremediation. In this technique engineered nanomaterials are used to improve As phytoremediation along with plant growth, stress tolerance, and other contaminants uptake from soil and water. The application of nanoparticles (NPs) such as TiO₂, ZnO, Au, and Si have been reported to alleviate metal toxicity significantly in *Oryza sativa* (Shukla et al., 2023; Wu et al., 2021), *Glycine max* (Y. Li et al., 2020), *Zea mays* (Lian et al., 2020). As remediation can be enhanced in *Isatis cappadocica* by application of salicylic acid-based NPs (Souri et al., 2017) whereas As stabilization is improved in *Helianthus annuus* by Zn nanoparticles (Vítková et al., 2018). Nano-TiO₂ application (4g L⁻¹) in *Vigna radiata* alleviates As toxicity (Katiyar et al., 2020) while at lower dose (1g L⁻¹) reduces As bioaccumulation in rice by 40 – 90% (Wu et al., 2021). Application of ZnO nanoparticles in rice helps to mitigate As-toxicity through up regulation of phytochelatin production and vacuolar sequestration of AsIII-PC complex (Shukla et al., 2023; S. Yan et al., 2021).

3. Biotechnological approach for enhancement of As phytoremediation

Transgenic As tolerant plants that can accumulate or detoxify As from environment have been developed by genetic engineering (Gunarathne et al., 2019; Lwanga et al., 2025). These genetically modified plants are designed for higher As uptake, efficient translocation from roots to fronds, transformation of As into less toxic forms, and improved arsenic tolerance (S. Thakur et al., 2019). In pteridophytes the uptake and transport of As(V) and As(III) are facilitated mainly by P_i transporters (PHTs)

and membrane intrinsic proteins (MIPs), respectively. Employing new transgenic approach where MIPs and PHTs genes are overexpressed could bring an enhanced As uptake and translocation, resulting in greater As accumulation in the engineered plants (Preetha et al., 2023).

A transgenic *Arabidopsis thaliana* has been developed by expressing PvACR3 gene which encodes an As(III) antiporter, under the control of the CaMV promoter, which results in 7.5 fold increase in As accumulation over the wild type. This transgenic *A. thaliana* has tolerance to 80 µm arsenite and 1200 µm arsenate which are lethal dose for wild type *A. thaliana* (Y. Chen et al., 2013).

Two transgenic rice varieties Nipponbare and Kasalath with gene OsPT8 mutation exhibit 33%–57% reduction in As(V) uptake than wild type (P. Wang et al., 2016). Mutations in the As transporters OsPht1:8 and Lsi 1/2 in rice has been found to significantly lower As uptake in the plants, consequently decreasing its accumulation in the rice grains (Y. Chen et al., 2017).

4. Amendments used to increase As phytoremediation efficiency

As phytoremediation efficiency of pteridophytes can be enhanced through application of phosphate, As-chelating agents, biochar etc. These amendments help to modify As bioavailability, uptake, transport, and also promote plant growth thereby increasing overall As remediation potential.

The application of plant growth regulators such as indole-3-acetic acid (IAA) has been found to enhance As uptake and growth in *Pteris cretica* var. *nervosa*, IAA treatment increased antioxidant enzyme activities, particularly superoxide dismutase (SOD) and peroxidase (POD), which suggest improved antioxidative activity (S. He et al., 2017).

The application of 20 mM calcium acetate improved drought tolerance and increased shoot As concentration by 165% and total As uptake by 55.1% in *P. vittata* (G. Wang et al., 2023). CaCO₃ treatment increased As accumulation in *Pteris vittata* fronds by 39% (up to 1460 mg kg⁻¹) compared to the CaCl₂ + As treatment, while reducing MDA by 16% (Y. F. Yang et al., 2025).

Application of 2.5 mmol kg⁻¹ oxalic acid (OA) significantly enhanced *P. vittata* growth and arsenic accumulation in multi-metal(loid) contaminated soil, increasing plant As uptake by 44.2% compared to the control after 20 days (Liang *et al.*, 2019).

In P limiting condition As accumulation is increased in pteridophytes whereas high P concentrations lower As absorption due to competitive inhibition at the root surface.

P. vittata grown in insoluble 2mM Ca-phytate or phosphate rock with 50 µM As in hydroponic system increased As accumulation (275–384% higher in fronds) whereas higher soluble P in medium reduce As accumulation capacity. An increase in phosphate concentration in the nutrient solution significantly reduced arsenic accumulation in both roots and shoots, indicating that phosphate competes with arsenate for uptake sites in *Pteris vittata* (Hu *et al.*, 2025).

Application of rice straw derived biochar (1.5% W/W) in As contaminated soil increased labile As in the soil, therefore accumulation reaching up to 350 mg kg⁻¹ in *P. vittata*, which is two times higher than control (Z. Y. Liu *et al.*, 2023).

Application of the chelating agent EDTA (ethylenediaminetetraacetic acid) resulted in a two-fold increase in shoot As accumulation in hydroponically grown *Pityrogramma calomelanos* after 6 months, exposure to 10 mg L⁻¹ As (disodium hydrogen arsenate). The shoot As concentration increased from 4,616 µg⁻¹ in the absence of EDTA to 7,290 µg⁻¹ following EDTA treatment (Wongkongkatap *et al.*, 2003).

CONCLUSIONS

It is well established that pteridophytes particularly *Pteris vittata* and related ferns play a crucial role as natural scavengers of soil As, which is attributed to their significant ability to uptake, translocate, and sequester As in above ground tissues. Physiological, molecular and environmental factors interplay and govern the efficiency of phytoremediation. In contrast to other plant groups pteridophytes are less economically important, herbaceous, fast-growing and generally non-edible, making them more suitable candidates for phytoremediation of As-contaminated soils. Their strong antioxidative defense helps reduce As-induced oxidative stress

by scavenging reactive oxygen species and maintaining cellular redox balance. However, further detailed investigations and pilot-scale studies are essential to optimize and validate the sustainable use of pteridophytes for large-scale arsenic bioremediation in affected environments. In the future, more research should focus on elucidating how *P. vittata* benefits from As to bridge the existing knowledge gap, while simultaneously exploring and identifying new As-hyperaccumulating pteridophyte species for improved phytoremediation potential with global acceptance.

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