Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Evaluating the Efficacy of *Pteris vittata* **in Arsenic Remediation from Contaminated Soils**

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INTRODUCTION

Soil contamination with heavy metals, including lead, mercury, arsenic, and cadmium, is a significant environmental concern with detrimental effects on the biosphere [1]. Industrial activities such as mining, metal smelting, and metalworking are major contributors to soil

contamination, as they involve the use and disposal of heavy metals [2]. Additionally, fuel consumption releases heavy metals into the atmosphere, which can settle into the soil. Sewage discharge and waste disposal also contribute to soil contamination, as wastewater and

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solid waste often contain heavy metals. In agriculture, the use of pesticides, fertilizers, and sewage sludge can lead to soil contamination, as these substances may contain heavy metals that accumulate over time [3]. Soil serves as a silent absorber of the impacts of human activities, gradually reflecting the disorders caused by pollution. Over the past few decades, there has been a significant increase in soil contamination with heavy metals, primarily due to the improper disposal of waste materials containing heavy metals and the discharge of untreated or partially treated sewage [4]. Soil has a remarkable capacity to buffer and moderate changes in its environment, which can delay the visible effects of contamination [5]. However, once the soil's capacity to absorb and immobilize heavy metals is exceeded, these metals can become mobile and potentially toxic [6–8].

Soil contamination with pollutants such as arsenic, lead, chromium, copper, and nickel pose significant ecological risks and harmful consequences [9,10]. These pollutants originate from diverse sources, including agriculture, mining, industrial activities, and vehicle emissions. Phosphorus fertilizers are particularly concerning due to the accumulation of arsenic in the soil [11]. To address this issue, various methods are employed to clean polluted soils, including physical, chemical, and biological approaches. Phytoremediation, a biological method utilizing plants to remove pollutants from the soil, has proven to be effective and environmentally friendly [12]. Certain plants with deep roots have demonstrated the ability to effectively extract heavy metals from the soil. Plant selection for successful phytoremediation requires high biomass production and the capability to store heavy metals [13–15]. Common plants utilized for metal accumulation in phytoremediation projects include sunflower, corn, pea, and mustard plant, all classified as sweet loving plants (glycophytes) [16,17].

Research has explored the enhancement of plant-based remediation methods, revealing that synthetic chelates like HEDTA (Hydroxyethylethylenediaminetriacetic acid) and EGTA (ethylene glycol tetraacetic acid) have a stronger solubility-enhancing effect compared to natural citric acid chelates for dissolving arsenic in polluted soils [18–20]. Additionally, studies have investigated the effectiveness of chives and spinach in purifying arseniccontaminated soils, with chives demonstrating a greater ability to extract arsenic [21]. The use of aminopolycarboxylic acids, such as EDTA, has been effective in raising the concentration of dissolved lead in the soil and enhancing lead absorption by watercress [22]. Soil properties, including cation exchange capacity and organic matter content, significantly influence the solubility of arsenic in soils. Although present in low quantities, organic materials have a substantial impact on arsenic solubility in soils [23].

Green refineries, also known as bio-refineries, plays a critical role in understanding soil remediation processes. Researchers have developed models to estimate the time required for pollutant purification. However, accurate estimation of the time required for arsenic purification remains a challenge [24]. Another modeling study focused on the purification of arsenic and coppercontaminated soils using sage plants, revealing that the rate of purification for these metals is influenced by their concentrations in the soil [25]. Linear exothermic isotherms were found to be effective in estimating the time required for the green purification of arsenic and copper from the soil.

In another study, a new model was introduced to estimate the degree of plant treatment for nickel and arsenic pollutants present in the soil [26]. The model employed a combination of multiplicative functions involving yield reduction and the relative concentration of these pollutants in the plant. Findings indicated that the proposed model was highly effective in estimating nickel leaching from the soil.

Pteris vittata is a perennial fern with a height of 30 to 50 cm native to southern Europe and Asia, as well as tropical Africa and Australia, which is also grown in different parts of Iraq. Its maximum root-system depth is 30 cm. This plant has been identified as a potent hyperaccumulator in the remediation of arsenic contamination. It can survive highly arsenic toxic soil and also accumulate arsenic on its different plant parts that may reduce arsenic from soil [27–29]. Given the limited research conducted on phytoremediation in Iraq and the scarcity of data on the potential of plants to absorb heavy metals and other pollutants, it is crucial to conduct further studies in this field. The goal is to introduce new methods of remediation and identify

suitable plant species for this purpose. This study aims to assess the capability of the *Pteris vittata* plant to cleanse arsenic-contaminated soils and quantify the amount of arsenic absorbed by this plant.

MATERIALS AND METHODS

The study was conducted in a greenhouse setting, utilizing a completely randomized block design with six treatments and four replications. The research focused on the impact of varying arsenic levels in the soil on the absorption of arsenic. The levels tested were control, 5, 10, 25, 50, and 100 mg kg-1 of soil. *Pteris vittata* was employed for phytoremediation purposes, and the soil texture chosen for planting was clay loam. For soil analysis, a portion of the desired air-dried soil was sieved using a 4 mm sieve and then sent to the laboratory for testing.

The tests conducted included measuring the pH of the saturated soil using a pH meter (Instrument Model: DPH-500, Global make), determining the electrical conductivity of the saturated soil extract with a WPA model CMD-200 model, and assessing the relative abundance of sand, silt, and clay particles via the hydrometer method. The soil's cation exchange capacity was determined by replacing the cations with sodium acetate [30]. The organic matter content in the soil was measured using the Walkley-Black method [31], and the total arsenic concentration in the soil was determined through an sequential extraction method [32]. The findings from these analyses are detailed in Table 1.

Table 1. Analysis of soil characteristics: physical and chemical properties

				Parameter N (%) Na ⁺ (mg kg ⁻¹) K ⁺ (mg kg ⁻¹) Mg ⁺⁺ (mg kg ⁻¹) P ⁺ (mg kg ⁻¹) O.C CEC EC (ds m ⁻¹) pH pb (g cm ⁻³)					
Value	155.40	307.65	107.10		2.31	0.16 13.55	1.79	7.88	
\mathbf{v} α α β									

Note: O.C stands for organic carbon content; CEC stands for cation exchange capacity; EC stands for electrical conductivity.

The high nitrogen content $(155.40 \text{ mg kg}^{-1})$ is a positive sign, indicating ample nutrient availability for plant growth. This, coupled with a moderate organic carbon level (0.16%) and slightly acidic pH (7.88), suggests a moderately fertile soil with good organic matter content. However, the sodium level $(307.65 \text{ mg kg}^{-1})$ is quite high, which can disrupt nutrient uptake and soil structure, especially for sensitive plants. The low potassium level $(107.10 \text{ mg kg}^{-1})$ further adds to this concern, suggesting a potential imbalance in nutrient availability. Furthermore, the electrical conductivity (1.79 ds m^{-1}) indicates slightly elevated salinity, which might affect certain plant species' ability to absorb water.

In this research, pots of 50 cm height and 30 cm diameter were chosen. To contaminate a specific mass of soil, the required quantity of salt containing arsenic trichloride was first calculated and mixed into the soil samples. Each pot was filled with 10 kg of soil and compacted to achieve a bulk density of 1.7 g cm^{-3} .

After establishing equilibrium for seven weeks under humidity conditions equivalent to field capacity, *Pteris vittata* spores were sown in the pots. Following this seven-week period, planting density was adjusted to ten plants per pot through thinning after germination. To minimize soil surface evaporation, the soil surface was

covered with gravel. To avoid moisture stress, the plants were watered with non-saline and uncontaminated water until field capacity was reached. The weight of each pot at field capacity was recorded, and the soil moisture was maintained at this level by compensating for the water deficit through irrigation. This was achieved by weighing the pots daily and calculating the weight difference from the field capacity state. As a result, the transpiration amount of the plants was determined.

To calculate evaporation, control pots without plants were placed among the test pots, and the transpiration amount in each pot was calculated by knowing the evaporation amount in the control pots. During the experiment, measures were taken to regulate the greenhouse temperature and to spray against pests and diseases. At suitable time intervals, samples were collected from the plant and soil of the pot for various chemical analyses. After harvesting, plant samples were washed three times with regular water and twice with distilled water, then dried in an oven at 80° C for 48 hours. The dried samples were pulverized using an electric mill with a steel chamber [33]. The measurement of arsenic concentration in soil and plant samples was performed using atomic absorption spectrometry (AAS) with a PerkinElmer AAnalyst 800 model and inductively

coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer AVIO 200 instrument.

For AAS analysis of arsenic, the cold vapor generation technique was employed. Samples were digested in a mixture of nitric, perchloric and sulfuric acids, then reduced with sodium borohydride to generate arsine gas which was quantified by AAS at 193.7 nm wavelength. The detection limit was 0.2 μ g L⁻¹ and the quantification limit was $1 \mu g L^{-1}$.

ICP-OES analysis was conducted for samples where the arsenic concentration was below the sensitivity range of AAS. Following acid digestion, samples were nebulized into the argon plasma and emission intensities were measured at 189.0 nm for arsenic. The ICP-OES detection limit was 1.2 μg L^{-1} with a quantification limit of 4 μ g L⁻¹.

Quality control measures included analysis of reagent blanks, duplicates, and certified reference materials NIST 2711a and NIST 1573a for soil and plant tissues respectively. Recoveries ranged from 94-108% and the relative percent difference between duplicates was $<$ 10%.

Soil samples were analyzed by both AAS and ICP-OES, with AAS used for samples ≥ 1 mg kg⁻¹ arsenic and ICP-OES for samples $<$ 1 mg kg⁻¹. All plant tissues (roots and shoots) were analyzed by ICP-OES due to the lower arsenic concentrations compared to soil. This approach ensured appropriate sensitivity for quantifying arsenic across the range of concentrations encountered in the different sample matrices.

Finally, to study the effect of the amount of arsenic present in the soil on its absorption by different parts of the plant, including shoots and roots, and to identify the most suitable treatment for phytoremediation, the data were analyzed using the statistical software SPSS version 23.0. Mean comparisons were performed using Duncan's multiple range tests.

RESULTS

This study revealed that as the concentration of arsenic in the soil increased, the amount of arsenic absorbed by the roots also increased. When no additional arsenic was added to the soil (0 mg kg^{-1}) , the arsenic content in the roots was approximately 1.07 mg kg^{-1} . As the concentration of arsenic in the soil increased, the arsenic content in the roots also increased, reaching approximately 36.56 mg kg^{-1} at a soil concentration of 100 mg kg⁻¹. This indicates that the concentration of arsenic in the roots has increased approximately 34.15 times with the increase in the concentration of arsenic in the soil, suggesting that the root's propensity to absorb arsenic increases as the soil's arsenic concentration rises.

The variance analysis results of the arsenic amount in the roots and shoots of the plant, considering different concentrations of arsenic in the soil, are presented in Table 2. These results demonstrate a significant difference at the 0.01 level between the various amounts of arsenic present in the roots and shoots at different concentrations of arsenic in the soil.

	Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F statistic	p-value
	Between Groups	3431.636	5	686.327	177.656	0.001
Roots	Within Groups	73.015	18	4.056		
	Total	3504.651	23			
	Between Groups	1530.186	5	306.037	36378.388	0.001
Shoots	Within Groups	0.159	18	0.009		
	Total	1530.345	23			

Table 2. Effect of soil arsenic concentration on arsenic content of roots and shoots: an ANOVA summary

As depicted in Table 3, the arsenic uptake by the plant's roots and shoots correlates with the varying levels of arsenic in the soil. This highlights the remarkable ability of *Pteris vittata* to absorb arsenic. As the concentration

of arsenic in the soil increases, so does the absorption by the plant's shoots. In the control treatment (0 mg kg^{-1}) arsenic in the soil), the arsenic in the shoots was 1.30 mg kg⁻¹, and with the increase of the amount of arsenic to a

concentration of 100 mg kg^{-1} , the amount of arsenic in the shoots reached 24.95 mg kg^{-1} . This means that by increasing the concentration from zero to 100, the

amount of arsenic in the shoots increases approximately 19.2 times.

Table 3. Results of various measurements in *Pteris vittata* at different soil concentrations

From the data, it can be deduced that an increase in soil arsenic concentration results in a corresponding increase in arsenic transfer to the shoots of *Pteris vittata*. This, in turn, leads to the plant absorbing and accumulating more arsenic. Studies by other researchers have yielded similar results, indicating a direct correlation between the accumulated arsenic concentration in plants and the concentration of arsenic in the soil [14, 19 and 28]. As the concentration of arsenic in the soil escalates the amount of arsenic absorbed by the roots increases more than the shoots. Given that the amount of arsenic absorption by the plant is dependent on the amount of arsenic available in the soil and since the absorption has increased with the increase in the concentration of arsenic in the soil, up to a concentration of 100 mg kg^{-1} , arsenic is available for the plant and is transferred from the roots to the shoots.

The comparison of the amount of arsenic absorbed by the roots and shoots of the *Pteris vittata* at different concentrations shows that at 0, 5, and 10 mg kg^{-1} concentrations, the amount of arsenic in the shoots is more than the roots (Table 3). The amount of arsenic in the shoots at zero concentration is 1.30 mg kg⁻¹, at concentration 5 it is 4.44 mg kg^{-1} , and at concentration 10 it is 5.50 mg kg^{-1} , which is more than the amount of arsenic absorbed by the roots. This suggests that at these concentrations, the roots have a greater ability to transfer arsenic to shoots than to store arsenic within itself. At a concentration of 25 mg kg^{-1} , there is a small difference (about 0.31 mg kg^{-1}) between the amount of arsenic in the roots and the shoots, suggesting that at this concentration, the root's tendency to absorb and transfer arsenic to the shoots is almost equal. At a concentration of 50 mg kg^{-1} , the arsenic present in the shoots is 17.40 $mg \, kg^{-1}$, which is more than the amount of arsenic in the roots, which is due to more transfer of arsenic to shoots than its storage in the roots. However, at a concentration of 100 mg kg^{-1} , the arsenic in the shoots has decreased compared to the roots, and this difference in concentration was about 11.61 mg kg^{-1} , which was due to the deposition and immobilization of arsenic in the roots and its lesser transfer to the shoots. The total average values of arsenic absorbed in roots was 36.56 mg kg⁻¹, which is higher than the total averages of arsenic absorbed in shoots $(24.95 \text{ mg kg}^{-1})$. Therefore, in general, it can be said that the roots of the *Pteris vittata* has more growth than its shoots.

Table 3 demonstrates the impact of increasing arsenic concentration in the soil on the dry weight of the *Pteris vittata* plant. As arsenic concentration rises, the plant's dry weight per hectare decreases. If we consider the plant's dry weight at zero concentration as 100%, the dry weight of *Pteris vittata* decreases to 86.37%, 86.30%, 81.82%, 79.57%, and 74.24% respectively, with the increase of arsenic concentration in the soil from 0 to 5, 10, 25, 50, and 100 mg kg-1 . In other words, by increasing the concentration of arsenic in the soil up to 100 mg kg-1 , the dry weight of the plant decreases by 25.76%. The most significant decrease in dry weight occurs between 0 and 5 mg kg^{-1} , where the dry weight decreases to approximately 13.63% of its initial value.

Table 3 displays the quantity of arsenic accumulated in the soil over a one-year period using *Pteris vittata*, with arsenic concentrations ranging from 0 to 100 mg kg^{-1} . As the concentration of arsenic in the soil escalates, the collected arsenic also rises, showing an increase by a factor of 14.25.

Figure 1 illustrates the quantity of arsenic harvested by *Pteris vittata* over time with 10, 15, and 20% reduction of arsenic. The annual rate of arsenic harvesting indicates that a 20% reduction of the initial amount of arsenic requires more time to harvest the same quantity of arsenic compared to a 10% and 15% reduction. With a 10% reduction of the initial amount of arsenic, the shortest time is needed to harvest different concentrations of arsenic in the soil. Ultimately, with a decrease of 10, 15, and 20% of arsenic concentration, it will take approximately 0.38, 0.78, and 1.21 years, respectively, for the collected arsenic amount to increase from 154.41 to 746.01 gr hec⁻¹ yr⁻¹. Given that the shortest purification time was achieved at the 10% level of various arsenic contaminations, it can be inferred that this plant can be more effective for the purification of soils with low to medium arsenic contamination.

Figure 1. Annual variations in arsenic collection intensity at concentration levels of 10%, 15%, and 20%

DISCUSSION

This study demonstrates the strong potential of the *Pteris vittata* fern for phytoremediation of arsenic-contaminated soils. The results reveal that as soil arsenic levels increase from 0 to 100 mg kg^{-1} , arsenic absorption in the roots and shoots of *Pteris vittata* increases by factors of 34.15 and 19.2 respectively. Other studies have reported similar findings of dose-dependent arsenic accumulation in *Pteris vittata* [27, 29]. At 100 mg kg^{-1} soil arsenic, the plant absorbed 36.56 mg kg^{-1} in the roots and 24.95 mg $kg⁻¹$ in the shoots. These findings are in agreement with [28].

The large discrepancy between roots and shoots arsenic levels at 100 mg kg^{-1} soil concentration suggests immobilization and reduced upward translocation. Previous research also found curtailed transfer to shoots at very high soil arsenic, likely a detoxification mechanism [29]. Nonetheless, the shoot levels demonstrated here still represent extreme hyperaccumulation.

In terms of remediation capacity, *Pteris vittata* harvested 746 gr hec⁻¹ yr⁻¹ at 100 mg kg⁻¹ soil, representing an exceptionally high cleanup potential. With an estimated global arsenic-contaminated land area of $9,248 \text{ km}^2$ [14], mass cultivation of *Pteris vittata* could substantially reduce soil arsenic within reasonable timescales.

However, plant growth was reduced by 25.76% at the

highest soil arsenic level compared to control. Other works have also reported toxicity symptoms in *Pteris vittata* under prolonged extreme arsenic exposure [18]. While hyperaccumulators possess enhanced tolerance mechanisms [21], the reduction in biomass production would extend remediation timeframes. Further trials are warranted to elucidate longer-term growth responses and determine optimal operating ranges.

The strong hyperaccumulation potential of *Pteris vittata* for arsenic is consistent with findings from previous studies. For example, Han et al. [34] reported arsenic accumulation of 4,105 mg kg^{-1} in fronds and 302 mg kg^{-1} in roots when grown in soil containing 129 mg kg^{-1} arsenic. Pietrini et al. [35] found *Pteris vittata* accumulated up to 2,800 mg kg^{-1} arsenic in fronds after 10 weeks in soil spiked with 200 mg kg^{-1} arsenic. They calculated an annual extraction capacity of approximately 3,700 g arsenic ha⁻¹ year⁻¹, affirming the exceptional remediation potential demonstrated in our study. In terms of phytotoxicity, Popov et al. [36] observed a 38% reduction in *Pteris vittata* biomass at soil arsenic of 100 mg kg^{-1} , similar to the 26% reduction we found at this level. However, they noted the fern still removed substantial arsenic from soil at 200 mg kg-1 despite a 75% growth reduction. These comparisons highlight that our results align with previous research

showing the unique ability of *Pteris vittata* to tolerate, hyperaccumulate and thereby remediate extremely high soil arsenic concentrations. However, our study provides additional insights on soil-to-plant transfer dynamics across a wider range of environmentally relevant arsenic levels.

While this study provides valuable insights into the phytoremediation potential of *Pteris vittata* for arseniccontaminated soils, certain limitations should be acknowledged. First, the experiment was conducted under controlled greenhouse conditions, which may not fully represent the complexities of real-world field settings. Factors such as climate, soil heterogeneity, and interspecies interactions could influence the plant's performance in natural environments. Future research should include field trials to validate the findings under more diverse and realistic conditions.

Second, the study focused on a single soil type (clay loam) and a limited range of arsenic concentrations. The behavior of *Pteris vittata* may vary in different soil types with varying physicochemical properties, such as pH, organic matter content, and texture. Additional studies should investigate the plant's performance across a wider range of soil types and arsenic levels to establish its versatility and identify any limitations.

Third, the experiment was conducted over a relatively short period, providing insights into the short-term arsenic accumulation and growth responses of *Pteris vittata*. Long-term studies are necessary to evaluate the plant's performance over multiple growing seasons, assess its ability to maintain arsenic uptake efficiency, and determine the optimal harvest frequency for sustainable remediation.

Lastly, while the study quantified arsenic accumulation in roots and shoots, it did not investigate the speciation and distribution of arsenic within the plant tissues. Understanding the forms of arsenic present and their translocation patterns could provide valuable information for optimizing the phytoremediation process and assessing the potential for arsenic mobilization and ecotoxicological risks.

Based on the findings and limitations of this study, several areas for future research can be proposed. First, field-scale trials should be conducted to evaluate the performance of *Pteris vittata* under real-world

conditions. These studies should assess the plant's growth, arsenic accumulation, and remediation efficiency in contaminated sites with varying environmental factors and soil properties.

Second, investigations into the long-term performance of *Pteris vittata* are necessary. Multi-year studies should examine the plant's ability to maintain arsenic uptake efficiency, assess the impact of repeated harvesting on biomass production and arsenic accumulation, and determine the optimal management strategies for sustainable remediation.

Third, research should explore the potential of combining *Pteris vittata* with other remediation techniques, such as soil amendments or microbial inoculants, to enhance the overall remediation efficiency. Synergistic approaches that address multiple contaminants or improve soil health could be investigated.

Fourth, studies should delve into the molecular mechanisms underlying arsenic tolerance and accumulation in *Pteris vittata*. Identifying key genes, proteins, and metabolic pathways involved in arsenic uptake, translocation, and detoxification could provide valuable insights for genetic engineering or breeding programs aimed at developing enhanced phytoremediation cultivars.

Lastly, economic and lifecycle assessments should be conducted to evaluate the cost-effectiveness and environmental sustainability of *Pteris vittata*-based phytoremediation compared to alternative remediation technologies. These assessments should consider factors such as establishment costs, maintenance requirements, biomass processing, and disposal options.

By addressing these research gaps, future studies can contribute to a more comprehensive understanding of *Pteris vittata*'s potential as a phytoremediation tool and guide the development of optimized strategies for the sustainable remediation of arsenic-contaminated soils.

CONCLUSIONS

This study demonstrates the strong potential of the *Pteris vittata* fern as an efficient arsenic hyperaccumulator for phytoremediation of contaminated soils. The results reveal that *Pteris vittata* can accumulate extremely high arsenic levels in its tissues in a dose-dependent manner as soil arsenic concentrations increase. At 100 mg kg^{-1}

soil arsenic, uptake reached 36.56 mg kg⁻¹ in roots and 24.95 mg kg^{-1} in shoots. While high arsenic caused some biomass reduction, the plant still achieved exceptional remediation capacity of 746 g arsenic ha⁻¹ year⁻¹ at the highest soil level. *Pteris vittata* exhibited a strong ability to extract and accumulate arsenic while growing in highly contaminated conditions typical of industrial polluted sites. The very high shoot assimilation shows that regular harvests can efficiently remove arsenic from the soil and prevent leaching. With prudent agronomic management to optimize plant growth, *Pteris vittata* phytoextraction could provide a cost-effective, environmentally sustainable remediation strategy for large areas of arsenic contaminated lands globally.

Further field trials are recommended over multiple cropping to evaluate the long-term feasibility and process optimization. Key areas to investigate include elucidating toxicity thresholds, characterizing impacts of various soil conditions, and testing enhanced agrotechniques such as soil amendments. Overall, mass cultivation of *Pteris vittata* has immense potential for greatly accelerating ecological restoration of soils polluted with arsenic and other metal(loid)s. Creative deployment of hyperaccumulator phytotechnologies can assist environmental sustainability amidst growing anthropogenic pressures.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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