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Impact of Arsenic Stress on Leaflets and Stipes (Frond Petiole) Anatomy of *Pteris vittata* Linn. and *P. ensiformis* Burm.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FGA and AFO (Supervisor) designed the study, wrote the protocol and wrote the first draft of the manuscript. Author FGA managed the literature searches, analyses of the study, performed the microscopy analysis and author AFO supervised the experimental process and also identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Heavy metals particularly Arsenic has been reported to alter the internal structures of plants in various ways due to higher levels of toxicity. The impacts of varying concentrations (0 mg/Kg, 30 mg/Kg, 90 mg/Kg and 150 mg/Kg) of Arsenic contamination on leaflet and stipe anatomy of *Pteris vittata* and *P. ensiformis* were investigated. The aim was to assess how Arsenic contaminations influence the anatomical properties of these plants. One fernlet was transplanted into each pot containing 5 Kg of soil treated with different concentrations of arsenic. After 12 weeks of planting, the transverse sections of the leaflets and stipes in all the treatments were made, stained and mounted for microscopic observation. The result showed slight changes in the shape and reduction in thicknesses of epidermal and mesophyll cells of *P. vittata* unlike *P. ensiformis* in which the

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anatomical properties changed and reduced drastically as the Arsenic concentration increases. The lethal impact was observed in the 150 mg/Kg treatment of *P. ensiformis* on the fourth week as the plants died. Also, the effect of Arsenic was minimal on the stipe of *P. vittata* unlike *P. ensiformis*. Therefore, this result further affirmed that Arsenic has low negative impact on the internal structures of *P. vittata* compared to *P. ensiformis*.

Keywords: Anatomy; arsenic; fernlet; intoxication; phytoremediation; stipe.

1. INTRODUCTION

Arsenic as a major contaminant of soil and water around the world is obtained from many minerals exploration activities and has been broadly used in chemical industry and in preservation of wood [1]. Several anthropogenic activities have led to drastic increase in the arsenic level in the environment all around the world [2]. It is known to alter photosynthesis in aquatic plants and increase the likelihoods of genetic aberrations which results in birth and developmental modifications in living organisms' especially aquatic life. Also, it can have lethal effect on animals that feed on Arsenic-affected aquatic organisms [3]. The main way humans are opened to arsenic is through consuming food and water, predominantly in areas where the groundwater is contaminated with Arsenic and when in overdose amount, it can cause a lot of diseases even death [4]. In the soil, this metal is dominant in the form of Arsenate and this is most accessible to tolerant plants [5]. Plants may employ two different mechanisms of Arsenic uptake. This includes passive uptake via the cell wall and active uptake via the symplast. This Arsenic that is taken up from the soil is then transported from the roots to the shoot system through the xylem vessels and reallocated between other plant tissues [6].

According to [7], uninterrupted heavy metal pollution seems to be the main challenge of the civilized world which has caused most of the morphological – functional and chromosomal anomalies in plants. Heavy metals such as Arsenic, Chromium, Lead, Zinc, Copper, Manganese, Cadmium, Molybdenum and Nickel induced physiological and biological changes such as growth, total chlorophyll content, sugar and protein contents of *Vigna radiata.* This is because these parameters declined progressively with increasing concentrations of heavy metals compared with the control plants [8]. [9] Also reported that the development and internal structure of *Amaranthus hybridus* were influenced by series of crude oil contaminations. They observed that some of the morphological, physiological and anatomical parameters were progressively reduced with increasing crude oil contamination. The cortical cells of roots and stems which were polygonal in shape also became small and flattened tangentially.

Pteris vittata L. is popularly known as 'Chinese ladder brake fern' because it is native to China. The name 'ladder' was derived from the step ladder-like look of the fronds and *Pteris ensiformis* Burm, FL. is commonly known as 'silver lace fern' (for the lovely lacelike textures of its fronds) or slender brake fern [10]. *P. vittata* and few other members of the genus *Pteris* except *P. ensiformis* have been reported as hyperaccumulators of Arsenic around the world. However, there is paucity of information on the impact of Arsenic stress on the leaflet and stipe anatomical properties of these plants. Hence the present study aimed at investigating the impact of arsenic stress on leaflets and stipes anatomy of *Pteris vittata* and *P. ensiformis.*

2. MATERIALS AND METHODS

2.1 Soil Preparation and Treatments

The soil used for this study was air-dried and sieved using 2 mm mesh gauze to remove debris. The physical properties of the soil (which are determining factors in metal bioavailability to plants) were determined using standard method [11]. The chemical properties (i.e. elemental concentrations) of the soil and the parent plants were also determined using X-ray fluorescence in order to have the background knowledge of Arsenic contents of the soil and the plants. Four plastic pots of size 24 cm x 21 cm were filled with 5 kg of the soil each and were thoroughly saturated with water. After this, each of the pots labeled CT, A, B and C were treated with different levels of concentration of arsenic in the form of Sodium arsenate ($Na_2HAsO_4.7H_2O$) i.e. 0 mgAs/Kg, 30 mgAs/Kg, 90 mgAs/Kg and 150 mgAs/Kg respectively. Sodium arsenate was added in solid form and was thoroughly mixed with the soil. The pot labeled CT served as the control. This is a completely random experimental design of 4 x 2 factorial arrangements. The factors are four soil arsenic concentrations and two species of *Pteris* (*P. vittata* and *P. ensiformis*) making a total of 8 treatments. Each treatment was replicated thrice and left for two days for equilibration.

2.2 Fernlet Transplanting

Healthy, one month old fernlets of both *P. vittata* and *P. ensiformis* of not more than three fronds were transplanted into each of the pots mixed with varying quantities of Arsenic. The experiment was left for twelve weeks after transplanting in a green house where the environmental conditions necessary for growth were controlled. The temperature of the green house was 27 ± 3℃ with light intensity, ca 1000 lux, 16 hr/d. Each pot was kept moist with 10 ml of distilled water every 2 days. After 12 weeks, the plants were harvested and analyzed for Arsenic contents in both roots and shoots using x-ray fluorescence.

2.3 Anatomical Studies

Transverse sections of the leaflets and stipes of the fronds in each treatment of the two plants were made at 8 µm using a rotary microtome. These were hereafter placed inside few drops of 1% Safranin O for 15 minutes, rinsed in 3 changes of distilled water. They were then counterstained in 1% solution of Alcian blue for 3 to 5 minutes, rinsed exhaustively in water and dehydrated through sequences of ethanol: 50%, 70%, 80%, 90% and 100%. These were mounted in 25% glycerine for microscopic examination. The mean cell area (in μm^2) of parenchyma (PCA) in stipe ground tissue and mesophyll of leaflet midrib was measured by counting the number of cells in accurately specified areas in the cross sections. Photographs of cross sections were imported into the computer, and mean cell areas were measured directly on the monitor from other randomly chosen areas of the parenchyma tissues using the Picasa 3.9.139 version program. The percentage reduction in the PCA of treatments was calculated using this formula:

$$\% \text{ reduction} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \quad \text{X } 100$$

2.4 Statistical Analysis

Data were analyzed for least significance differences with one way ANOVA using the SPSS 17 package.

3. RESULTS AND DISCUSSION

3.1 Background Soil Condition and Plant Metal Concentrations after 12 Weeks of Planting

The physical and chemical properties of the soil used for the experiment is shown in Tables 1 and 2 respectively. The results after 12 weeks of treatment showed that *P. vittata* hyperaccumulated up to 64132 ppm Arsenic in the root and 65747 ppm in the frond in all the treatments while *P. ensiformis* hyperaccumulated up to 15662 ppm in the root and 15120 ppm in the frond and these data were extracted from a work done previously [12]. *Pteris vittata* grew well in all the treatments of Arsenic (Plate 1). The plants in the treatment C of *P. ensiformis* died at exactly fourth week after planting as they could not withstand the high concentrations of Arsenic (Plate 2).

Table 1. The physical properties of the soil used for the experiment

Properties	Values
P^H	6.4
% sand	75.32
% silt	15.64
% clay	9.04

Table 2. Background metal concentrations (ppm) in the soil and the two species before transplanting

Elements	Soil	*P. vittata*		*P. ensiformis*	
		Fronds	Roots	Fronds	Roots
K	12336±215	14978±155	4836±88	9842±127	3334±73
Ca	16819±304	2034±48	3455±62	4787±73	2695±55
Ti	2833±81	ND	455±12	89±5	374±11
Mn	545±15	54±2	171±4	44±2	99±3
Fe	15030±94	246±4	1996±11	348±5	1820±11
Cu	29±1	10±0	7±0	9±0	6±0
Zn	137±5	21±1	19±1	30±1	28±1
As	ND	ND	ND	ND	ND

Values represent mean ± standard deviation. ND – not detected

Plate 1. Showing 12 weeks old *Pteris vittata* growing on different concentrations of arsenic

A represents the control treatment (0 mg As/Kg) of P. vittata after 12 weeks after planting
B represents 30 mg As/Kg treatment of P. vittata after 12 weeks after planting
C represents 90 mg As/Kg treatment of P. vittata after 12 weeks after planting
D represents 150 mg As/Kg treatment of P. vittata after 12 weeks after planting

Plate 2. Showing 12 weeks old *P. ensiformis* growing on different concentrations of arsenic

A represents the control treatment (0 mg As/Kg) of P. ensiformis after 12 weeks after planting
B represents 30 mg As/Kg treatment of P. ensiformis after 12 weeks after planting
C represents 90 mg As/Kg treatment of P. ensiformis after 12 weeks after planting
D represents 150 mg As/Kg treatment of P. ensiformis after 12 weeks after planting

3.2 Impact of Arsenic on Anatomy of Leaflet

There were slight changes in the leaflet anatomy of *P. vittata* unlike that of the *P. ensiformis* which was greatly affected by the different concentrations of Arsenic contamination (Tables 3 and 4). The thickness of cuticle, palisade and spongy mesophyll cells were slightly reduced in *P. vittata* as the Arsenic contamination increased. No epidermal hair was observed in the leaflets of both plants except in the control of *P. ensiformis*. The amphicribal-type vascular bundles of the leaflets of both plants were not affected by Arsenic treatments. The progressive reduction in the thickness of cuticle, palisade and spongy mesophyll as well as little distortion in shape and arrangement of epidermal and mesophyll cells of treatment A to C of *P. vittata* could be attributed to a little phytotoxic effect of arsenic on *P. vittata.* Although this is minimal when compared with *P. ensiformis* which was greatly affected by the Arsenic contamination as there was high progressive reduction from control to treatment B in the thickness of cuticle and the mesophyll cells. This agrees with earlier reports that the anatomical properties such as cuticle thickness, stomata index and mesophyll cell thickness decreased as the level of pollution increased [9,13]. A similar trend on the influence of industrial discharges on the internal structures of *Abelmoschus esculentus* (okra) was observed. It was discovered that the differences in the anatomical properties might have resulted from the effect exerted by different chemical species present in the various effluents which had affected the chloroplast [14]. These anatomical changes are direct consequences of the changes in metabolic activities taken place in plants under the influence of heavy metals [7].

3.3 Impact of Arsenic on Anatomy of Stipe

Also, little reductions were observed in the thickness of cuticle of stipe of both plants (Table 5 and 6). The vascular bundles of the stipes of both plants were U-shaped with Hippocampus xylem. The open sides were oriented towards the adaxial sides of the stipes whereas the abaxial portions of the strands were consistently convex and followed the contours of the stipe. The collenchyma cells of both plants are of the lamellar type. The shapes of the parenchyma cells range from circular to polygonal. There was not much negative effect of Arsenic on the anatomy of stipe of *P. vittata* except that the thickness of the cuticle slightly reduced from control to treatment C. However, the effect of Arsenic is more pronounced in the stipe of *P. ensiformis* as the Arsenic concentration increased. The pronounced effect of Arsenic in the stipe of *P. ensiformis* as the Arsenic concentration increased unlike in *P. vittata* is comparable with the one reported by [7] on a proportional increase in reduction of thickness of walls in all tissues of *Nicotiana tabacum* with increasing concentration of heavy metals.

3.4 Impact of Arsenic on Parenchyma Cell Area of Leaflet and Stipe

There were significant differences ($P < 0.05$) in the parenchyma cells area of the leaflet mesophyll and stipe ground tissue of both plants (Table 7 and 8). The percentage reductions in the parenchyma cells area of both plants increased progressively as the Arsenic treatments increased (Fig. 1).

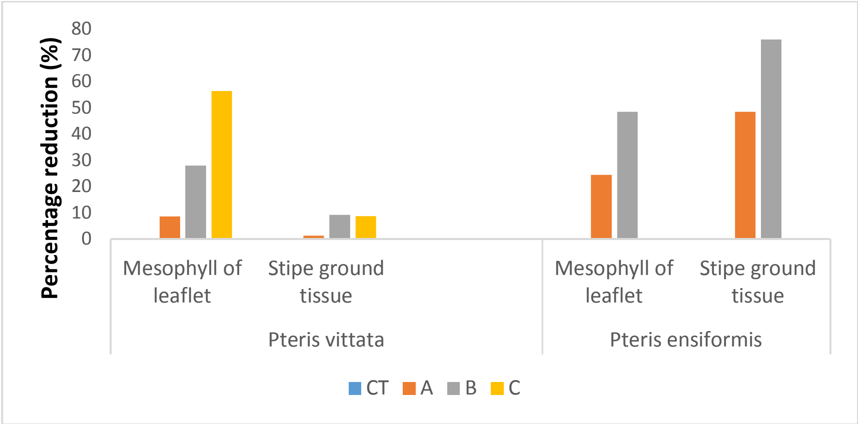

Fig. 1. The percentage reduction in the Parenchyma cells area of leaf mesophyll and stipe ground tissues *Pteris vittata* and *P. ensiformis*

Table 3. Effect of different treatments of arsenic on the leaflet anatomy of *Pteris vittata*

Treatment	Cuticle thickness	Upper epidermis	Palisade mesophyll	Spongy mesophyll	Vascular bundle	Lower epidermis
Control	ranges from 24.0 - 24.3 µm	single layered and rectangular in shape	72.40 - 72.55 µm thick, elongated, closely packed and double layered	117.5 – 119.7 µm thick, circular to irregular in shape and loosely packed with intercellular spaces.	concave in shape and it is amphicribal type	single layered
A	ranges from 21.2 – 21.5 µm	single layered and rectangular in shape	60.5 – 61.5 µm thick, elongated, closely packed and double layered	98.65 – 98.90 µm thick, circular to irregular in shape and loosely packed with intercellular spaces.	concave in shape and it is amphicribal type	single layered
B	ranges from 18.70 – 18.95 µm	single layered and rectangular in shape	62.20 – 62.65 µm thick) are loosely packed and double layered	90.5 – 91.5 µm thick) cells are irregular and loosely packed with intercellular air spaces	concave in shape and it is amphicribal type	single-layered
C	13.50 – 13. 95 µm thick	single-layered and irregular in shape	52.20 – 52.65 µm thick, double layered and more loosely packed	77.25 – 77.75 µm thick, more irregular and loosely packed with more intercellular spaces	concave in shape and it is amphicribal type	single-layered

Table 4. Effect of different treatments of arsenic on the leaflet anatomy of *Pteris ensiformis*

Treatment	Cuticle thickness	Upper epidermis	Palisade mesophyll	Spongy mesophyll	Vascular bundle	Lower epidermis
Control	ranges from 36.20 – 36.35 µm	single layered and rectangular in shape	77.25 – 77.65 µm thick, elongated, more closely packed and double layered	96.15 – 96.55 µm thick, circular to irregular in shape and loosely packed with intercellular spaces.	concave in shape and it is amphicribal type	single layered
A	ranges from 26.15 – 26. 45 µm	single layered and irregular in shape	58.55 – 58.85 µm thick, elongated, scattered and double layered	82.6 – 83.85 µm thick, circular to irregular in shape and loosely packed with intercellular spaces.	concave in shape and it is amphicribal type	single layered
B	ranges from 21.2 – 21.43 µm	single layered and is distorted in shape	48.55 – 48.85 µm thick, elongated, scattered and double layered	77.3 – 77.8 µm thick, cells are irregular and loosely packed with intercellular air spaces	concave in shape and it is amphicribal type	single-layered

Table 5. Effect of different treatments of arsenic on the stipe anatomy of *Pteris vittata*

Treatment	Cuticle thickness	Epidermis	Collenchyma cells	Parenchyma cells	Vascular bundle
Control	13.50 – 13.95 µm thick	single layered and compactly arranged with no intercellular air spaces	three to four layered and of the lamellar type	ranged from circular to polygonal in shape and were 8 to 12 layered	U-shaped with Hippocampus xylem
A	11.10 – 11.45 µm thick	single layered and compactly arranged with no intercellular spaces	three to four layered and of the lamellar type	circular to polygonal with few intercellular spaces and are 8 to 11 layered	U-shaped with Hippocampus xylem
B	8.65 – 8.95 µm thick	single layered and compactly arranged with no intercellular spaces	three to four layered and of the lamellar type	8 to 12 layers of cells with more intercellular spaces	U-shaped with Hippocampus xylem
C	5.9 - 6.5 µm thick	single layered and compactly arranged with no intercellular spaces	two to three layered and of the lamellar type	8 to 10 layers of cells	U-shaped with Hippocampus xylem

Table 6. Effect of different treatments of arsenic on the stipe anatomy of *Pteris ensiformis*

Treatment	Cuticle thickness	Epidermis	Collenchyma cells	Parenchyma cells	Vascular bundle
Control	13.45 – 13.98 µm thick	single layered and compactly arranged with no intercellular air spaces	three to four layered and of the lamellar type	circular to polygonal in shape and were 8 to 11 layered	V-shaped with Hippocampus xylem
A	10.30 – 10.75 µm thick	single layered and compactly arranged with no intercellular spaces	three to four layered and of the lamellar type	circular to polygonal in shape and were 7 to 10 layered	V-shaped with Hippocampus xylem
B	5.56 – 5.75 µm thick	single layered and less compactly arranged with intercellular spaces	three to four layered and of the lamellar type	circular to polygonal in shape and were 4 to 5 layered	V-shaped with Hippocampus xylem
C	-	-	-	-	-

Table 7. The Parenchyma cell area (PCA) of *Pteris vittata* as affected by different treatments of arsenic

	CT	A	B	C
Mesophyll of leaf	87.5±1.04	80±2.04	63±1.08	38.1±1. 25
Stipe ground tissue	404.5±2.39	399±3.34	367±7.21	369±4.21

Value represents mean ± SE and are significantly different at P < 0.05

Table 8. The Parenchyma cell area (PCA) of *Pteris ensiformis* as affected by different treatments of arsenic

	CT	A	B	C
Mesophyll of leaf	102.25±1.11	77.25±0.85	52.75±1.11	-
Stipe ground tissue	390.50±1.55	201.50±1.55	93.75±1.75	-

Value represents mean ± SE and are significantly different at P < 0.05

The significant reduction in the parenchyma cell area of the leaflet mesophyll and stipe ground tissue of these two plants is similar to earlier work on *Sorghum bicolor* which was reported to have significant reduction in the thickness of mesophyll parenchyma of the leaf, root diameter and mid-rib total thickness of when treated with combination of Copper and Cadmium [15].

4. CONCLUSION

This study has showed that more effects of Arsenic were observed on the leaflet anatomical properties of both plants than in the stipes. However, the effects were more pronounced in the anatomy of *P. ensiformis* and the lethal effect showed on treatment C. Therefore, Arsenic has low negative impact on the internal structures of *P. vittata.* This further affirms that *P. vittata* can be used for the restoration of Arsenic contaminated sites as it showed great potentials by being able to withstand high levels of Arsenic with minimal effect on its anatomy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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