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EFFECT OF LEAD TREATMENT ON THE STIPE AND LEAFLET ANATOMY OF *Pteris vittata* AND *Pityrogramma calomelanos* (PTERIDACEAE: FERN)

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ABSTRACT

The effect of various concentrations of Lead on stipe and leaflet anatomy of *Pteris vittata* and *Pityrogramma calomelanos* were investigated. Fernlets were transplanted into 5 kg pots of soil pre-treated with five different concentrations of Lead, adequately watered and arranged in a complete randomized design in the screen house. Pots with no Lead treatment served as control. At twelve weeks after planting, plants were carefully removed, washed and taken to the laboratory. Anatomical sections of leaflets and stipe of each of the treatments were carried out with a sledge microtome (10-15 µm). Microscopic observations of structures were made after staining the sections. In *Pteris vittata*, the thickness of the cuticle of the stipe increased with increasing Lead concentrations but the reverse of this was observed in *Pityrogamma calomelanos.* There was also significant reduction of epidermal and mesophyll cell area of both species with increasing Lead concentrations. It can be concluded from this study that *P. vittata* has a higher tolerance level to Lead than *Pityrogramma calomelanos.*

Key words: Anatomy, Cuticle thickness, Ferns, Lead, Pteridaceae.

INTRODUCTION

The Pteridaceae is a very interesting group of plant family due to the great heterogeneity of its genera and species, coupled with the fact that it is a family with large representation and importance in floristic level (Smith *et al.*, 2008). Among the families of Pteridophyta, the most representative was Pteridaceae family, with about 35 genera and presents a wide geographical distribution (Gracano *et al.*, 2001). The main characteristics of the family include possession of linear marginal sori with false indusium (Rothfels, 2008). *P vittata* have been established as an hyperaccumulator (Ma *et al,* 2001; Oloyede *et al.,* 2012; Akomolafe *et al.*, 2017). *Pteris vittata* and *Pityrogramma calomelanos* are members of the family Pteridaceae. Both were commonly found growing on drainage systems and at bases of water tanks in Southwestern Nigeria. Unlike *Pityrogramma calomelanos*, *P. vittata* were never observed growing in shaded forest (Bamigboye, 2018).

Pteris vittata Linn.

Pteris vittata known as 'Chinese ladder brake fern', is a native of China and also found all over the world, including Nigeria. It is a terrestrial, perennial herb with excellent ornamental value. Its young fronds are used traditionally as an astringent. Benjamin and Manickam (2007) reported that *Pteris vittata* extract is used as demulcent, tonic, in treating hypotensive, as well as an antiviral and antibacterial agent. Its rhizome is sub-erect with pale brown scales; frond is pinnate, dull brown in colour, widely opened, or spreading with dense scales. The leaflet is long, lanceolate, green, simple, unipinnate, hairy with acuminate apex. Terminal pinna is usually the longest among the pinnae. Stipe is short and polished black. The venation is with free veins, which are dichotomously branched. Sori form two rows, linearly arranged on the margins of the abaxial surfaces of the pinnae. Propagation is by the spores and from the rhizomes. Several studies have reported the presence of leucocyanidin, leucodelphinidin, the flavone ester apigenin 7-O-p-hydroxybenzoate and a number of glycosides of apigenin, leutolin, isocutellarein-8-O-methyl-ether, kaempherol and quercetin (Salantina and Prado, 1998; Imperato, 2006). *Pteris vittata* contains potent antimicrobial properties especially against gastrointestinal tract pathogens and the presence of rutin may in part be responsible for added activity (Meenakshi *et al.*, 2008). *Pteris vittata* have been identified as an Arsenic (As) hyper-accumulator (Akomolafe *et al.*, 2017) in having an efficient arsenic accumulation capability as well as an extensive root system (Komar, 1999; Ma *et al.*, 2001).

In addition, *Pteris vittata* has the ability to reduce arsenate (arsenic in soils) to As^{3+} (Ma *et al.,* 2001). It has been proposed that when the plant is exposed to As and under the reducing conditions of plant cells, As^{5+} is readily reduced to As^{3+} in hyper-accumulators and then may be complexed by organic ligands such as thiols to avoid damage to plant cells (Zhang *et al.*, 2002).

Pityrogramma calomelanos (Linn.) Link

Pityrogramma calomelanos Linn. is a native of New Zealand and is commonly called gold back or silverback fern. It grows in the open areas or slightly open or shaded areas under the forest or re-growth forest. It is regarded as an "opportunist" because it rapidly colonizes bare ground (Campos *et al.,* 2018). It is one of the primary colonizers of volcanically devastated areas. It is a tall, erect, bushy plant with ornamental and medicinal values. The rhizome is short, erect, bearing a tuft of fronds, which are lanceolate, fertile and covered with scales. The scales are brown, narrow, 3–6 mm long. Stipes are up to 30 cm long, dark purple, polished, scaly on lower part, glabrous upwards, covered with white powder in young stage. Leaf is bipinnate, pinna is glabrous and densely covered with yellow to orange and whitish powdery substances on the abaxial surface. The leaf is compound bipinnate with 70-100 pinnae arranged in an alternate manner. Pinna is lanceolate, having acute to acuminate apex. The leaf is 17.80 cm long and 4.28 cm wide. Plant decoction is used in treating renal disorders in the Philippines. Tea prepared out of the frond is used as a cure for hypertension, fever and cough (Sajeey *et al.,* 2015). Rhizomes are considered anthelmintic in South Africa. Frond decoction is taken for boils in the mouth and nose. Fronds are also used for asthma, cold and chest congestion (Benjamin and Manickam 2007). *Pityrogramma calomelanos* could be a source of plant-derived natural products with anti-epimastigote activity and low toxicity, representing an interesting alternative to other efforts to combat infectious diseases such Chagas disease (De Souza *et al.,* 2012).

Lead is considered a general protoplasmic poison, which is cumulative, slow acting and subtle. The exposure of Lead on human and animals has toxic effects on kidney and the endocrine system (Assi *et al.*, 2016). Plants growing near highways are usually exposed to more Lead than other localities. Sewage sludge containing large quantities of Lead and other metals is regularly discharged on to field and garden soils due to increasing trends in urbanization (Paivoke, 2002). Lead-affected soils contain Lead in the range of 400-800 mg Kg^{-1} whereas in industrialized areas the level may reach up to 1000 mg $Kg^{-1}$of Lead in the soil (Angelone and Bini, 1992).

Plant roots rapidly respond to the absorbed Pb, through a reduction in growth rate and change in branching pattern. In *Zea mays* seedlings, Obroucheva *et al.* (1998) observed strong growth inhibition of primary root and a shorter branching zone with more compact lateral roots occupying a position much closer to the root tip compared with roots grown in the absence of Lead. It appears that the inhibition of root growth under Lead toxicity is as a result of Lead induced inhibition of cell division in root tips (Fun *et al.*, 2002). When the effect of different concentrations of Lead nitrate was studied on root growth, cell division, chromosome morphology and the nucleolus of root tip cells of onion (*Allium cepa*), a reduction in root growth, mitotic irregularities and chromosome stickiness were observed (Wierbicka, 1994). In corn seedlings, Lead toxicity causes leakage of K^+ from root cells (Malkowski *et al.*, 2002).

This study is aimed at comparing the tolerance of *Pteris vittata* and *Pityrogramma calomelanos* and also to determine the effects of the different concentrations of Lead on the anatomical features of the two plants.

MATERIALS AND METHODS

The study site for this experiment was in the Department of Botany, Obafemi Awolowo University, Ile-Ife (07º 30ʹ N, 04º40ʹ E), Nigeria. Experimental plants were collected from the fern garden and cultivated in the same Department.

Soil Preparation

The soil sample used for this study was randomly collected from the top soil, 0 -20 cm, behind

Department of Botany, Obafemi Awolowo University, Ile-Ife. The soil was air-dried for a week, and sieved using 2 mm mesh gauze to remove debris. The physical properties of the soil were determined using standard method (Gee and Bauder, 1986). X-ray fluorescence was used to determine the background knowledge of the chemical properties of Lead in the soil and in the parent plants. Sixty plastic pots (24 cm×21cm) perforated at the base to allow aeration and drainage were filled with 5 kg of the soil. Plastic trays were placed under each pot for the collection of excess water to prevent loss of pollutants. For each of the plant species, five pots were labelled as control (CT) and five pots each of the treatments as A, B, C, D and E. These pots were thoroughly saturated with water and arranged in a completely randomized design. The pots labelled as A, B, C, D and E were treated with different levels of Lead concentrations in the form of Lead (II) trioxonitrate (v) salt [Pb $(NO_3)_2$], that is, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm respectively in five replicates.

Plantlets Transplanting

This was carried out after two-day of equilibration of the prepared soil. The fernlets used for this study were obtained from the vegetative propagation of the parent plants. Healthy fernlets of both *Pteris vittata* and *Pityrogramma calomelanos* having two leaflets were transplanted into each of the pots and were treated with varying quantities of Lead. This experiment was monitored for twelve weeks after transplanting.

Anatomical Studies

Transverse sections of the stipes of the fronds in each treatment were made at 10-15 µm thickness using a rotary microtome. The sections were stained with 1% aqueous solution of Safranin O for 15 minutes, washed in distilled water in Petri dishes to remove excess stain and counterstained in 1% solution of Alcian blue for three to five minutes. They were washed thoroughly in water and dehydrated through series of ethyl alcohol: 50 %, 70 %, 80 %, 90 % and 100 % and were later mounted in 25 % glycerine for microscopic examinations.

Statistical Analysis: Data were analysed for least significant differences with one-way ANOVA using SAS version 9.2.

RESULTS AND DISCUSSION

The results of the effect of the various Lead concentrations on the anatomical features of the stipe of the two plant species are summarized in tables 1 and 2 while those of the leaflet sections are on tables 3 and 4. The result showed that the thickness of stipe in *P. vittata* increased with increasing Lead concentration but decreased in *Pityrogramma calomelanos*. In the leaflet anatomy of both plant species there was also significant reduction of cell areas as Lead concentrations treatment increased.

Table 1: Effect of Various Treatment of Lead on the Stipe Anatomy of *P. vittata*

Important Stipe Anatomical Characters					
Treatment	Cuticle thickness	Epidermis	Sclerenchyma cells	Parenchyma cells	Vascular bundles
Control	12.24±1.2µm	Single layered, no intercellular	3-6 layers of polygonal cells	Circular, oval-polygonal cells	U-shaped with hippocampus xylem
A	13.41±1.25µm	Single layered, no intercellular spaces	3-6 layers of polygonal cells	Circular, oval-polygonal cells	U-shaped with hippocampus xylem
B	15.32±1.98µm	Single layered, no intercellular spaces	3-6 layers of polygonal cells	Circular, oval-polygonal cells	U-shaped with hippocampus xylem
C	16.21±1.29µm	Single layered, no intercellular spaces	3-6 layers of polygonal cells	Circular, oval-polygonal cells	U-shaped with hippocampus xylem
D	17.98±2,0µm	Single layered, no intercellular spaces	3-6 layers of polygonal cells	Circular, oval-polygonal cells	U-shaped with hippocampus xylem
E	20.63±2.65µm	Single layered, no intercellular spaces	3-6 layers of polygonal cells	Circular, oval-polygonal cells	U-shaped with hippocampus xylem

Legend: -Control, A-200 ppm, B-400 ppm, C-600 ppm, D-800 ppm, E-1000 ppm

Table 2: Effect of Various Treatment of Lead on the Stipe Anatomy of *Pityrogramma calomelanos*

Treatment	Important Stipe Anatomical Characters				
	Cuticle thickness	Epidermis	Sclerenchyma cells	Parenchyma cells	Vascular bundles
Control	10.21±1.76µm	Single layered	No sclerenchyma layer	Circular - oval cell, no intercellular space	2 distinct, oval shaped vascular bundle
A	9.34±1.71µm	Single layered	No sclerenchyma Layer	Circular to oval cell, no intercellular airspace	2 distinct, oval shaped vascular bundles
B	8.54±1.69µm	Single layered	No sclerenchyma layer	Circular - oval cell, no intercellular space	2 distinct, oval shaped vascular bundle
C	7.01±1,72±m	Single layered	No sclerenchyma layer	Circular - oval cell, no intercellular space	2 distinct, oval shaped vascular bundle
D	5.57±1.68µm	Single layered	No sclerenchyma layer	Circular - oval cell, no intercellular space	2 distinct, oval shaped vascular bundle
E	4.43±1.42µm	Single layered	No sclerenchyma layer	Circular - oval cell, no intercellular space	2 distinct, oval shaped vascular bundle

Legend: Control, A-200 ppm, B-400 ppm, C-600 ppm, D-800 ppm, E-1000 ppm

Table 3: Effect of Various Treatments of Lead on the Leaflet Anatomy of *vittata*

Leaflet structure	Control	A	B	C	D	E
Mean value of the Upper epidermis	20.23±2.4µm	20.60±2.2µm	22.20±2.45µm	24.12±3.12µm	24.2 ±2.9µm	26.00±3.1µm
Mean value of the Lower epidermis	18.53±2.1µm	17.93±1.9µm	19.96±2.4µm	21.91±2.62µm	21.89±2.1µm	23.49. ± 5.3µm
Mean value of the Palisade Mesophyll	89.34±4.52µm	86.20±3.9µm	82.40±3.52µm	80.36±4.30µm	79.61±2.43µm	78.94±2.56µm
Mean value of the Spongy mesophyll	72.14± 3.51µm	64.21±3.51 µm	61.42±5.0µm	57.20±3.72µm	55.61±2.76µm	52.94±4.29µm

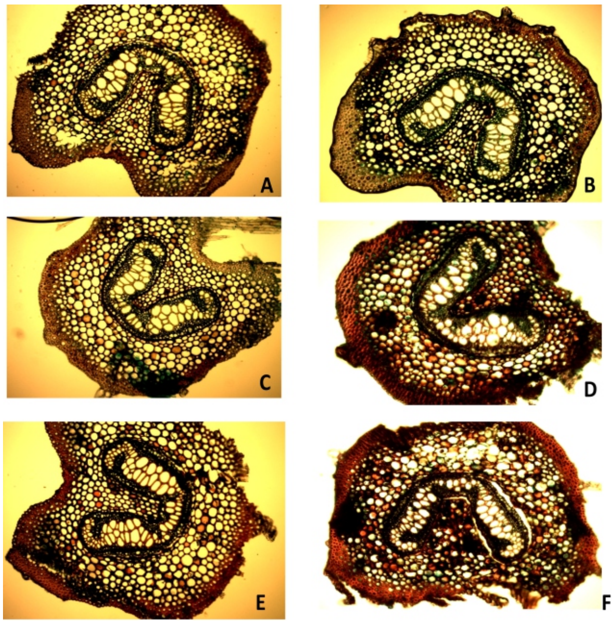
Legend: Control, A-200 ppm, B-400 ppm, C-600 ppm, D-800 ppm, E-1000 ppm

Table 4: Effect of Various Treatment of Lead on the Leaflet Anatomy of *Pityrogramma calomelanos*

Leaftlet structure	Control	A	B	C	D	E
Mean value of the Upper epidermis	20.46±2.3µm	18.24±1.8µm	17.50±2.12µm	15.12±1.12µm	12.1±1.0µm	11.34±1.8µm
Mean value of the Lower epidermis	18.11±1.8µm	15.9±1.6µm	6.00±2.2µm	12.9±1.9µm	10.89±2.1µm	9.21±0.8µm
Mean value of the Palisade Mesophyll	84.10±3.0µm	81.12±3.13µm	76.92±4.3µm	75.41±3.44µm	71.24±3.47µm	62.10`±3.29
Mean value of the Spongy mesophyll	79.11±4.1µm	68.25±2.20µm	63.31±3.24µm	61.24±2.55µm	55.38±2.1µm	50.61±2.44µm

Legend: -Control, A -200 ppm, B -400 ppm, C -600 ppm, D -800 ppm, E -1000 ppm

Structurally, plants from polluted sites present important modifications to their tissues. Some of the structural modifications include reduction in thickness of palisade parenchyma, spongy mesophyll, epidermis and cuticles (Ekpemerechi *et al.,* 2014). From this study, stipe of *Pteris vittata* showed a significant increase in thickness of the cuticle as the Lead concentrations increased. This is contrary to the finding of Omosun *et al.* (2008) and Svetlana *et al.* (2010) who reported that anatomical properties such as cuticle thickness, stomata index decreases as the level of pollution increased. Increase in the thickness of the cuticles observed may be an adaptation to prevent entrance of other pollutants through the leaves since leaves are the first tissue exposed to pollutants among all other plant tissues. However, the progressive decrease in the cuticle thickness of *Pityrogramma calomelanos* from the control to treatment E is similar to the findings of Omosun *et al.* (2008) and Ekpemerechi *et al.* (2014). Ekpemerechi *et al.* (2014) also suggested that the response of plants to changes in environmental conditions whether anthropogenic or otherwise is species specific. The specificity expressed by plant species point to the fact that species could be considered to be susceptible, threatened or otherwise when exposed to pollutants. The reduction in the epidermal and mesophyll layers of both *Pteris vittata* and *Pityrogramma calomelanos* across each treatment agrees with Jahan and Iqbal (1992), who reported a significant reduction in cuticle, hypodermis, palisade and spongy parenchyma cells in polluted environment as compared to non-polluted area. Uaboi - Egbenni *et al.* (2009) reported similar trend on the effect of industrial effluents on the anatomical structures of *Abelmoscus esculentum.* Similarly, Akomolafe *et al.* (2017) reported more pronounced negative impact on the internal structure of *P. ensiformis* than in *P. vitata.*



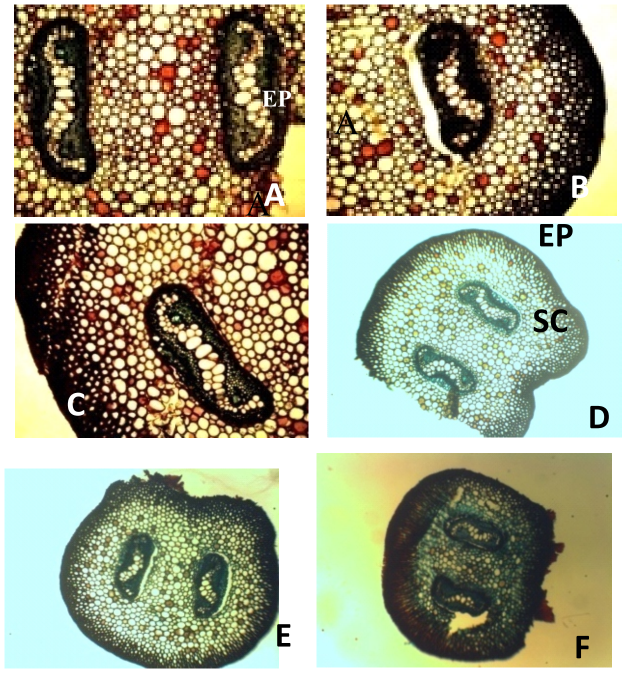
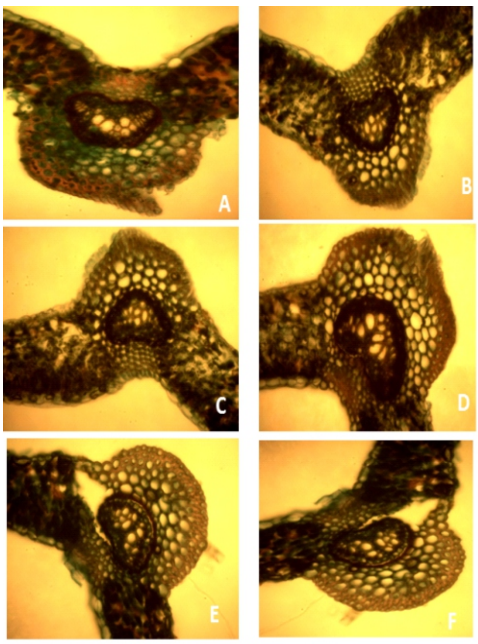


Figure 1a **Figure 1b**

Mag x400

Figure 1a: The Transverse Sections of the Stipe of *Pteris vittata* with Varying Lead Treatment Concentrations.
Figure 1b: The Transverse Sections of the Stipe of *Pityrogramma calomelanos* with Varying Lead Treatment Concentrations
Legend: A- Control, B- 200 ppm, C-400 ppm, D-600 ppm, E-800 ppm, F-1000 ppm
EP- Epidermis, SC- Sclerenchyma, VB-Vascular Bundle.



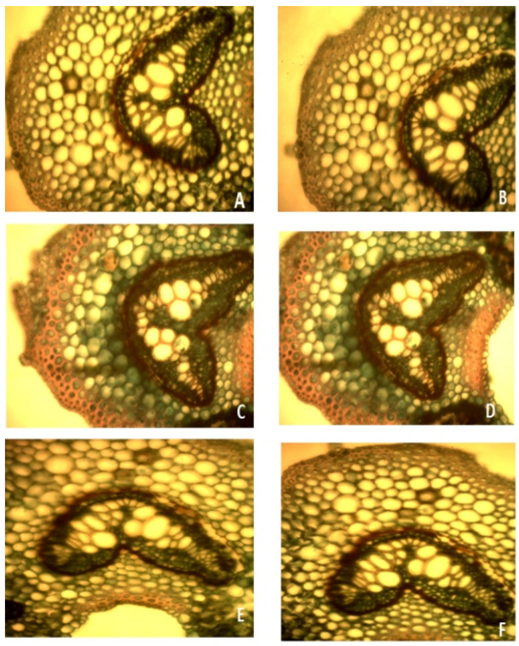


Figure 2a **Figure 2b**

Mag. x400

Figure 2a: The Transverse Sections of the Leaflet of *Pteris vittata* with Varying Lead Treatment Concentrations
Figure 2b: The Transverse Sections of the Leaflet of *Pityrogramma calomelanos* with Varying Lead Treatment Concentrations
Legend: A- Control, B-200 ppm, C-400 ppm, D-600 ppm, E-800 ppm, F-1000 ppm
EP- Epidermis, SC- Sclerenchyma, VB-Vascular Bundle.

Thus, the observed reduction of some anatomical features in the plants used for this study may be attributed to the toxic effect of lead on the plant species. Such anatomical changes are direct consequences of the change in metabolic activities taken place within plants under the influence of heavy metals (Cvetanovska *et al*., 2010). In the leaflet anatomy, the effect was minimal in *Pteris vittata* when compared to *Pityrogramma calomelanos* which showed more reduction in mesophyll cells from control to treatment E. There was no negative effect of Lead on the anatomy of the stipe of *Pteris vittata* in that the cuticle thickness increased across the treatment. Nevertheless, in *Pityrogramma calomelanos* the effect was pronounced in the stipe as cuticle thickness decreased progressively with increase in the Lead concentration.

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