NJB, Volume 37 (1), 83 - 95, June, 2024

IMPACTS OF CADMIUM TREATMENTS ON LEAFLET AND STIPE ANATOMY OF PTERIS VITTATA LINN. AND PTERIS ENSIFORMIS BURM. F. (PTERIDACEAE: FERNS)

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Received 4th June, 2024; accepted 30th June, 2024

ABSTRACT

Impacts of cadmium contamination in soil at different concentration levels on leaflet and stipe anatomy of *Pteris vittata* L. and *Pteris ensiformis* Burm. F. were investigated. Fernlets of *P. vittata* and *P. ensiformis* collected from ferns garden at the Natural History Museum and identified at IFE Herbarium both in Obafemi Awolowo University, Ile-Ife, Nigeria were transplanted into planting pots containing 5 kg of soil pre-treated with cadmium at four concentration levels with the control untreated. The experiment was laid out in a completely randomised block design. The plants were nursed for twelve weeks with adequate watering, after which they were carefully removed and washed. Anatomical sections of the leaflets and stipes were carried out according to standard procedures. After proper staining, microscopic observations of internal structures were carried out on the samples. The major changes observed in the anatomical structures were changes in the thickness of the cuticles of both *P. vittata* and *P. ensiformis* as well as the distortion of some parenchyma cells, as the cadmium contamination levels increased in both species. The study concluded that cadmium treatment majorly impacted the cuticle thickness compared to other anatomical features; the negative effect was more pronounced in *P. ensiformis*.

Keywords: Anatomical; bioaccumulation factor; cadmium; cuticle; fernlets; pteridacaeae; stipes

https://dx.doi.org/10.4314/njbot.v37i1.7

INTRODUCTION

Pteridophytes consist of large and extremely diverse groups of plants composed of fossils as well as living plants having several characteristics in common. They are characterised by gametophyte (free-living, short-lived, fragile, the dependent haploid phase) and sporophyte (the dominant diploid phase) generations (Chapman, 2009). The genus *Pteris* L. has over 300 species of ferns in the subfamily Pteridoidae in the family Pteridaceae. Species of this genus are mostly found on land or growing on rocks. Rhizomes are usually erect or creeping and branched with pale-brown to black scales. The fronds are scaly at the base, their leaflet margins are entire and their leaflets are clustered (Lellinger, 1985). *Pteris vittata*, although grows readily in the wild, is sometimes cultivated. It is grown in gardens for its attractive appearance, and useful in pollution control schemes (Oloyede, 2012). It is well known as an arsenic hyper-accumulator plant. The Chinese brake fern (*P. vittata*) thrives in tropical and sub-tropical environments and has a great ability to hyper-accumulate high levels of arsenic (Ma *et al*., 2001). *P. vittata* may, therefore, be quite helpful in phyto-extraction in certain areas. *P. vittata* has also demonstrated the ability to lower the amount of lead in contaminated soil (Yusuf *et al*., 2018). *Pteris ensiformis* Burm F. is also known as thin brake fern or silver lace fern. It is an ornamental, herbaceous, perennial terrestrial plant. The rhizome is strong, erect and drought-resistant. The frond is pinnatifid and glabrous, with a central silver-white stipe (Oloyede, 2012). Unlike *P. vittata*, the efficacy of *P. ensiformis* in hyper-accumulating heavy metal pollutants has not been widely reported (Oloyede *et al*., 2013).

Cadmium concentrations in the ground have risen considerably as a result of un-regulated and poor waste disposal practices. According to Wahid and Khaliq (2015) and Mahar *et al*. (2016), high Cd concentrations can be harmful to soil organisms, easily migrate through vegetative cover and make their way into the food chain. According to Vázquez *et al*. (1992); Mohamed *et al*. (2012) and Khan *et al*. (2015), Cd is highly cell-damaging, causing oedema, mitochondrial deterioration which results in necrosis, chlorosis and vein reddening. It also slows root and shoot growth and lowers nutrient uptake. It affects the plant's physiological and metabolic processes (Chaffei *et al*., 2004). According to Belkhadi *et al*. (2010), Cd increased the generation of reactive oxygen species at the cellular level, causing cell membrane damage. Ci *et al*. (2009) reported that Cd alters the ratio of free amino acids to total soluble sugar in both roots and shoots. According to Khan *et al*. (2015), Cd has substantial adverse impacts on planted calorie content along with development rates, and more than 90 per cent of crops thriving in soil contaminated with Cd are nutrient-deficient.

Soil contamination with heavy metals is a pressing global issue. While there have been a series of works on the phyto-remediation potentials of ferns, much emphasis is placed on the morphological aspect. To bridge this gap, this study was aimed at determining the effects of cadmium at different contamination levels on anatomical features of *P. vittata* and *P. ensiformis*, and to evaluate and compare the tolerance levels of the two plants.

MATERIALS AND METHODS

Study Site

Experimental plants were collected from the Natural History Museum and identified at IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. The study was conducted in the Department of Botany, Obafemi Awolowo University, Ile-Ife (07^0 30^I N, 04^0 40^I E), Nigeria.

Soil Preparation and Treatments

The topsoil used for this experiment was randomly sampled and collected from the Botanical Garden at Obafemi Awolowo University, Ile-Ife, Nigeria. The soil was air-dried for 7 days and sieved with 2 mm mesh gauge to get rid of debris. Analysis of the physical properties of the soil was done using standard procedures to determine the metal bioavailability to plants. X-ray fluorescence was used to determine the chemical properties of the soil and the parent plants (before planting) for background knowledge of Cd contents of the soil and the two plant species studied (Tables 1 and 2).

Experimental Layout

The experiment was a completely randomised 2 x 5 factorial arrangement. Sixty plastic pots (15 cm x 18 cm), perforated at the base to allow aeration and drainage of excess water, were filled with 2 kg of the soil each. Plastic trays were placed under each pot for the collection of excess water to prevent the loss of pollutants. The plants were grouped into five treatments each, labeled CT, A, B, C and D. All the pots except the CT were treated with different levels of Cd concentrations in the form of cadmium (II) chloride ($CdCl_2$) i.e 50 ppm, 100 ppm, 150 ppm and 200 ppm, respectively and each treatment for each plant species was replicated six times.

Table 1: Chemical properties of the soil used for the study before the experiment

Heavy metals content	Value (ppm)
Organic Carbon	5.200
Magnesium	2327.100
Potassium	2610.000
Copper	0.042
Manganese	0.051
Zinc	0.081
Iron	0.109
Cobalt	0.035
Cadmium	0.021

Table 2: Cadmium concentration level (ppm) in the plants before transplanting

	Pteris vittata		*Pteris ensiformis*	
Plant part	Frond	Root	Frond	Root
Cadmium	0.004	0.006	0.003	0.004

Fernlet Transplanting

Healthy fernlets with three fronds of *P. vittata* and *P. ensiformis* obtained from vegetatively propagated parent plants of three fronds were transplanted after two days of soil treatment to ensure the mixtures had attained equilibrium. The experiment was monitored for 12 weeks after transplanting for further studies and

50ml of water was used to water the soil daily to have good and favourable environmental conditions for the plants.

Anatomical Studies

The transverse sections of the leaflets and stipes of each treatment were made using a microtome at a thickness of 18 µm. The sections were first stained for 15 minutes with a 1% aqueous solution of Safranin O, washed three times with distilled water to remove excess stain, and then counterstained for three to five minutes with a 1% solution of Alcian blue. The sections were mounted in 25% glycerine after being carefully cleansed in water and dehydrated using a sequence of ethyl alcohol concentrations of 50%, 70%, 80%, 90% and 100% for microscopic examination. Microscopic observation of the sections of the stipes and leaflets of both plants was done using an Olympus Light microscope. An ocular micrometer that was inserted into the eyepiece of the microscope was used to measure the thickness of the cuticle. The thickness measurements were multiplied by the ocular constant with respect to the power under which they were taken for conversion to micrometer measurement. Photomicrographs of stipes and leaflets of both plants were made.

Statistical Analysis

Descriptive and inferential analysis were used.

RESULTS

Background Soil Condition and Plant Cadmium Concentration before Transplanting

The chemical analysis of the soil and Cd analysis of the experimental plants are shown in Tables 1 and 2, respectively. A pH of 6.12 was recorded for the topsoil, which indicated a slightly acidic condition. The results revealed that they were both extremely low in Cd content and could not in any way have interfered with the treatments.

Impacts of Cadmium Treatment on Stipe and Leaflet Anatomy of *Pteris vittata* and *Pteris ensiformis*

The results of the effect of Cd contamination on the stipe anatomy of the two species are presented in Tables 3 and 4 while the effect of Cd on the leaflet anatomy of the two plant species is presented in Tables 5 and 6, respectively. Figures 1a and 1b showed the results of stipe anatomical features of *P. vittata* and *P. ensiformis*. The results of transverse sections of *P. vittata* and *P. ensiformis* are shown in Figures 2a and 2b, respectively.

Table 3: Effect of cadmium contamination on stipe anatomy of *P. vittata*

	Important stipe anatomical character				
Treatment	Cuticle outline	Cuticle thickness	Cortex	Epidermis	Vascular bundles
Control	Thin, gently undulating and not striated	13.20 ± 1.2 µm	It is occupied by 5-10 polygonal-shaped parenchyma cells	1-3 layers of polygonal-shaped, elongated and cylindrical epidermal cells	One cortical bundle, more-or-less U-shaped conjoint and concentric amphicribral
A	Thin, undulating and not striated	14.31 ± 1.2 µm	It is occupied by slightly distorted 5-10 polygonal-shaped parenchyma cells	1-3 layers of polygonal-shaped elongated and cylindrical epidermal cells	One cortical bundle, more-or-less U-shaped conjoint and concentric amphicribral
B	Thin, undulating and not striated	14.92 ± 2.40 µm	It is occupied by slightly distorted 5-10 polygonal-shaped parenchyma cells	1-3 layers of polygonal-shaped elongated and cylindrical epidermal cells	One cortical bundle, more-or-less U-shaped conjoint and concentric amphicribral
C	Thin, undulating and not striated	15.30 ± 1.78 µm	It is occupied by slightly distorted 5-10 polygonal-shaped parenchyma cells	1-3 layers of polygonal-shaped elongated and cylindrical epidermal cells	One cortical bundle, more-or-less U-shaped conjoint and concentric amphicribral
D	Thin, undulating and not striated	16.24 ± 1.29 µm	It is occupied by slightly distorted 5-10 polygonal-shaped parenchyma cells	1-3 layers of polygonal-shaped elongated and cylindrical epidermal cells	One cortical bundle, more-or-less U-shaped conjoint and concentric amphicribral

Legend: Control- 0 ppm, A- 50 ppm, B- 100 ppm, C- 150 ppm, D- 200 ppm

Table 4: Effect of cadmium contamination on stipe anatomy of *P. ensiformis*

	Important stipe anatomical characters				
Treatment	Cuticle outline	Cuticle thickness	Cortex	Epidermis	Vascular bundles
Control	Thin, gently undulating, not striated	10.50 ± 2.6 µm	It consists of distorted 5-10 layers of polygonal-shaped parenchyma cells	1-2 layers of elongated cylindrical shaped epidermal cells. The abaxial and adaxial surfaces of the stipe are concave	U-shaped, one corticular bundle, conjoint and concentric amphicribral
A	Thin, gently undulating, not striated	10.45 ± 1.71 µm	It consists of distorted 5-10 layers of polygonal-shaped parenchyma cells	1-2 layers of elongated cylindrical shaped epidermal cells. The abaxial and adaxial surfaces of the stipe are concave	U-shaped, one corticular bundle is conjoint and concentric amphicribral
B	Thin, gently undulating, not striated	9.24 ± 1.61 µm	It consists of distorted 5-10 layers of polygonal-shaped parenchyma cells.	1-2 layers of elongated cylindrical shaped epidermal cells. The abaxial and adaxial surfaces of the stipe are concave	U-shaped, one corticular bundle, conjoint and concentric amphicribral.
C	-	-	-	-	-
D	-	-	-	-	-

Legend: Control- 0 ppm, A- 50 ppm, B- 100 ppm, C- 150 ppm, D- 200 ppm
- Plants in treatments C and D died before the end of the experimental period

Table 5: Effects of cadmium contaminations on leaflet anatomy of *P. vittata*

Important stipe anatomical characters						
Treatment	Cuticle outline	Cuticle thickness	Cortex	Epidermis	Vascular bundles	Mesophyll
Control	Thin, gently undulating and not striated, not prominent when present	24.15 ± 2.4 µm	It is occupied by polygonal-shaped parenchyma cells.	Uniseriate, circular, oval, short cylindrical to irregular shape on both surfaces. Epidermal cells are not distinguishable from the cortex, the cells of the epidermal layer are uniseriate, elongated, cylindrical and protruding to the cortex	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers. Occupied by parenchyma cells whose shape ranges from circular, oval, polygonal to irregular
A	Thin, gently undulating and not striated, not prominent on both surfaces	24.20 ± 2.1 µm	It is occupied by polygonal-shaped parenchyma cells	Uniseriate, circular, oval, cylindrical to irregular shape on both surfaces. Epidermal cells are not distinguishable from the cortex, the cells of the epidermal layer are uniseriate, elongated, cylindrical and protruding to the cortex	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers
B	Thin, gently undulating in some portions and straight in some other portions and not striated, not prominent on both surfaces	26.5 ± 2.5 µm	It is occupied by polygonal-shaped parenchyma cells	Uniseriate, circular, oval, cylindrical to irregular shape on both surfaces. Epidermal cells are not distinguishable from the cortex, the cells of the epidermal layer are uniseriate, elongated, cylindrical and protruding to the cortex	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers
C	Thin, gently undulating in some portions and straight in some other portions and not striated, not prominent on both surfaces	28.12 ± 3.2 µm	It is occupied by polygonal-shaped parenchyma cells	Uniseriate, circular, oval, cylindrical to irregular shape on both surfaces. Epidermal cells are not distinguishable from the cortex, the cells of the epidermal layer are uniseriate, elongated, cylindrical and protruding to the cortex	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers
D	Thin, gently undulating in some portions and straight in other portions and not striated, not prominent on both surfaces	28.24 ± 2.6 µm	It is occupied by polygonal-shaped parenchyma cells	Uniseriate, circular, oval, cylindrical to irregular shape. Epidermal cells are not distinguishable from the cortex, the cells of the epidermal layer are uniseriate, elongated, cylindrical and protruding to the cortex	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers

Legend: Control- 0 ppm, A- 50 ppm, B- 100 ppm, C- 150 ppm, D- 200 ppm

Table 6: Effects of Cadmium Contaminations on Leaflet Anatomy of *P. ensiformis*

	Important Stipe Anatomical Characters					
Treatment	Cuticle outline	Cuticle thickness	Cortex	Epidermis	Vascular bundles	Mesophyll
Control	Thin, gently undulating, not striated, not prominent on both surfaces	40.21 ± 1.8 µm	It is occupied by polygonal-shaped parenchyma cells	Uniseriate, circular, oval, cylindrical to irregular shape on both surfaces	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers
A	Thin, gently undulating and not striated, not prominent on both surfaces	31.15 ± 3.3 µm	It is occupied by polygonal-shaped parenchymal cells	Uniseriate, circular, oval, cylindrical to irregular shape on both surfaces	One cortical bundle that is conjoint and concentric amphicribral	Not differentiated into palisade and spongy mesophyll layers
B	Thin, gently undulating and not striated, not prominent on both surfaces	26.20 ± 4.2 µm	It is occupied by polygonal-shaped parenchyma cells	Uniseriate, circular, oval, cylindrical to irregular shape on both surfaces	One cortical bundle that is conjoint and concentric amphicribral	Not distinguishable into palisade and spongy mesophyll layer
C	-	-	-	-	-	-
D	-	-	-	-	-	-

Legend: Control- 0 ppm, A- 50 ppm, B- 100 ppm, C- 150 ppm, D- 200 ppm
- Plants in Treatments C and D died before the end of the experimental period

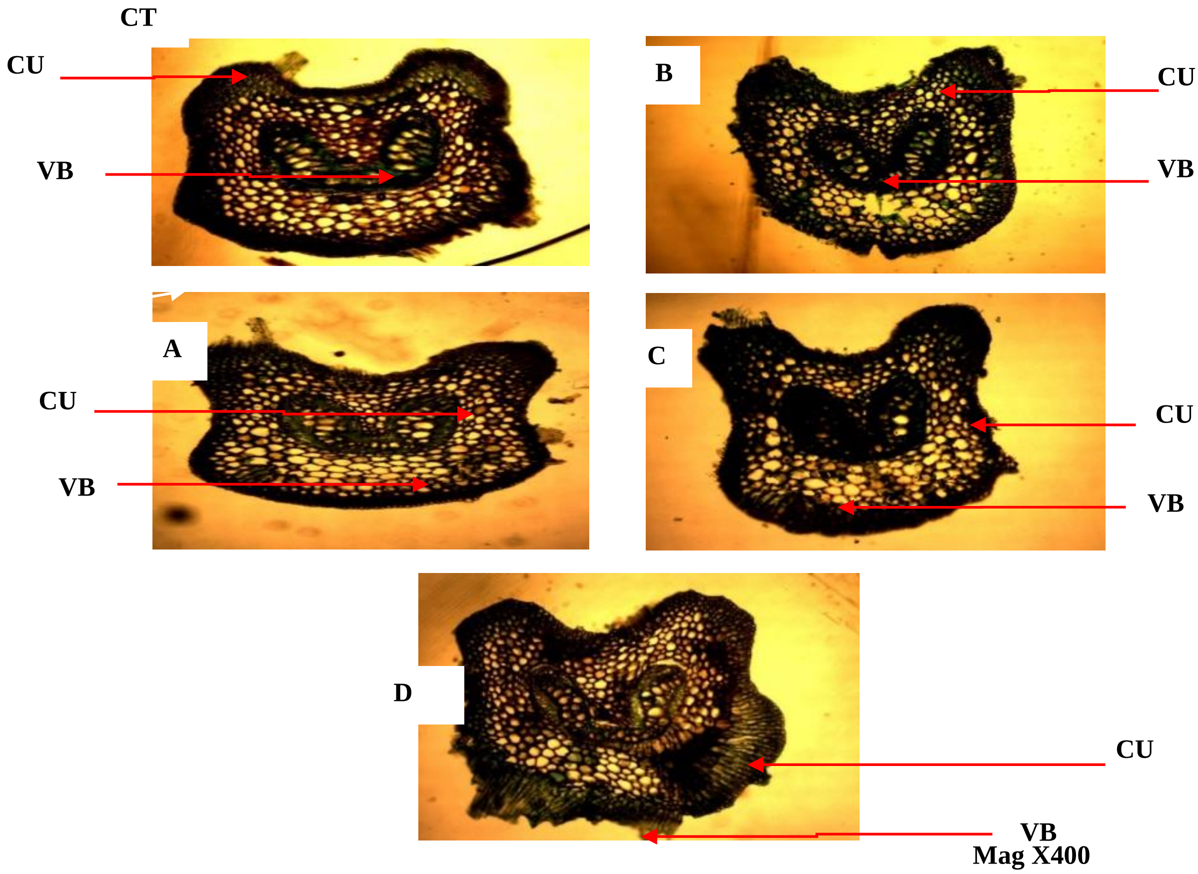

Fig. 1a: Transverse sections of the stipes of *Pteris vittata* with different levels of cadmium treatments.
Legend: CT- Control, A- 50 ppm, B- 100 ppm, C-150 ppm, D- 200 ppm, CU- Cuticle, VB- Vascular bundle

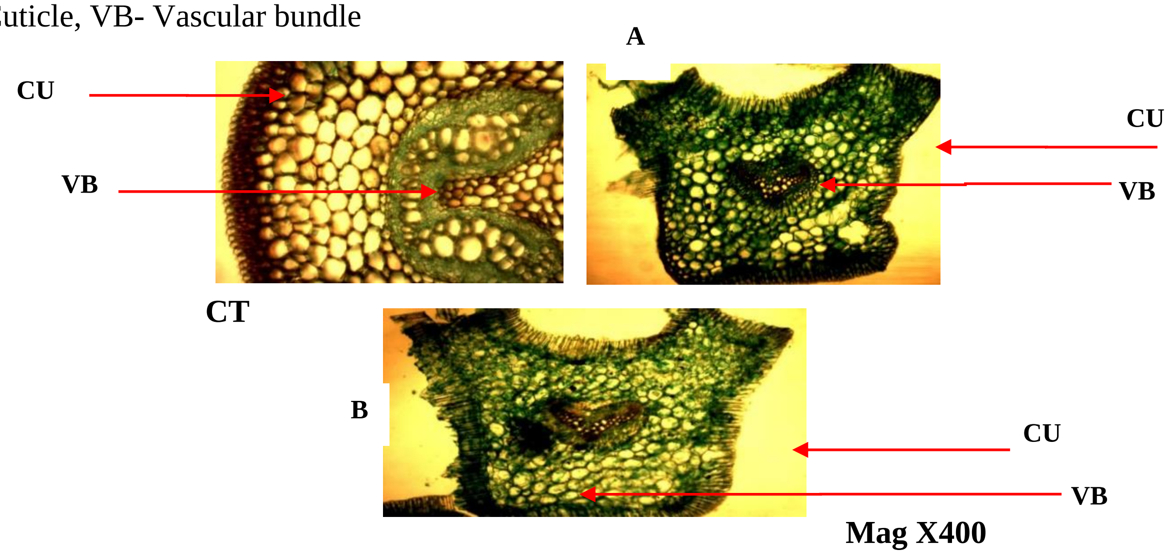

Fig. 1b: Transverse sections of the stipes of *Pteris ensiformis* with different levels of cadmium
Legend: CT- Control, A- 50 ppm, B- 100 ppm, CU- Cuticle, VB- Vascular bundle

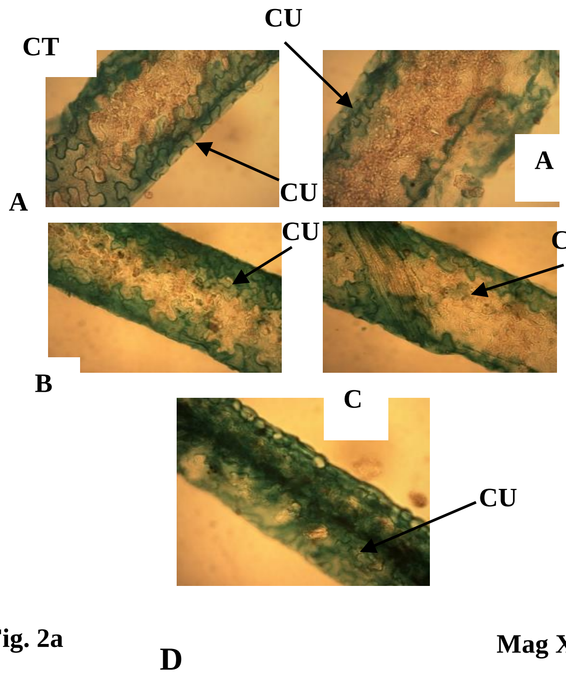

Fig. 2a

Mag X400

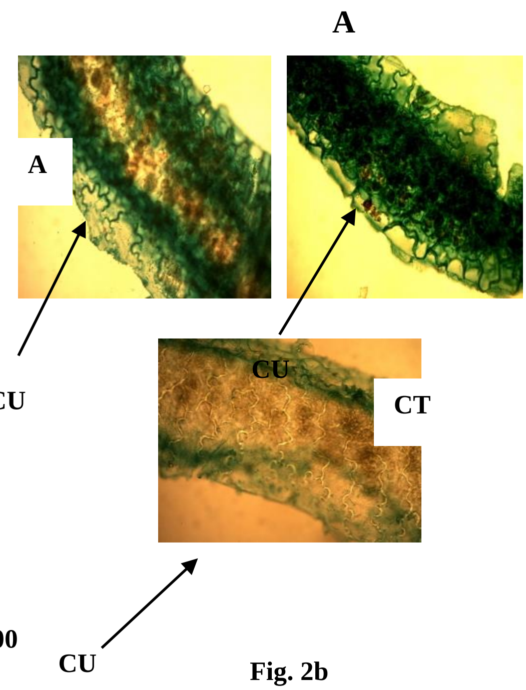

Fig. 2b

Fig. 2a: Transverse sections of the leaflets of *Pteris vittata* at different levels of cadmium treatment
Fig. 2b: Transverse sections of the leaflets of *Pteris ensiformis* at different levels of cadmium treatment

Legend: CT- Control, A- 50 ppm, B- 100 ppm, C-150 ppm, D- 200 ppm, CU- Cuticle.

DISCUSSION

Cadmium contamination causes some modifications in plant tissues, majorly in the thickness of palisade parenchyma, epidermis and cuticle. This study observed increased cuticle thickness in *P. vittata* which is in line with the report of Uaboi-Egbenni *et al*. (2009), who observed similar changes in the leaves of *Abelmoschus esculentus* due to industrial effluents. There was decreased thickness in *P. ensiformis*, along with distortion in parenchyma cells, sizes and arrangement as Cd levels increased. These findings are similar to those of Omosun *et al*. (2008) and Svetlana *et al*. (2010), who reported decreased cuticle thickness and cellular pore index with increased pollution. The increased cuticle thickness in *P. vittata* may be an adaptation to prevent pollutant entry through the root. On the other hand, *P. ensiformis* showed reduced cuticle thickness with higher contamination in line with the reports of Omosun *et al*. (2008), Svetlana *et al*. (2010) and Ekpemerechi *et al*. (2014). This suggests species-specific responses to environmental changes. Cvetanovska *et al*. (2010) linked the reduced cuticle thickness in *P. ensiformis* to the phytotoxic impacts of arsenic, indicating that these anatomical changes reflect the immediate effects of heavy metal contamination on plant metabolic processes. The impact was minimal in *P. vittata* but more pronounced in the stipes and leaflets of *P. ensiformis*.

CONCLUSION

This study has shown that the effects of cadmium contamination on ferns can be species-specific. The findings showed that Cd treatments majorly impacted the cuticle thickness compared to other anatomical features, with a more pronounced negative effect in *P. ensiformis*. The observed variation in the responses of the plants to Cd treatment suggests that different species have distinct mechanisms for adapting to heavy metal contamination.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Botany and the Management of the Natural History Museum, Obafemi Awolowo University, Ile-Ife, Nigeria for providing the necessary facilities and enabling environment for this study.

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