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Phytoremediation Potentials and Effects of Lead on Growth of *Pteris vittata* L. and *Pityrogramma calomelanos* L. (Pteridaceae: Fern)

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Abstract

Phytoremediation, tolerance and bio-accumulation potentials of lead in the roots and shoots of *Pteris vittata* and *Pityrogramma calomelanos* were investigated to ascertain the effects of lead on thee species' growth and development. Within the experiment, 5 kg of air dried and sieved soil was put inside six plastic pots labelled CT, A, B, C, D and E, each with different concentrations (0, 200, 400, 600, 800 and 1,000 ppm) of lead (II) trioxonitrate (v) salt. The shoots and roots of the plants were air dried, grounded and analysed for lead quantities using x-ray fluorescence before and after the treatments. One-month old healthy fernlets of each species were transplanted into each of the pots, in six replicates. Growth parameters such as leaflet area, number of leaflets and frond height were recorded weekly, for 12 weeks. Quantitative data were analysed for significant difference using analysis of variance for each of the plant species and means were evaluated with Duncan's multiple range test for frond height, leaflet number, leaflet area, roots and shoots biomass. Negative effects (decrease) on the growth parameters were observed in both species. *Pteris vittata* accumulated more lead in all the treatments, in both shoots and roots, compared with *Pityrogramma calomelanos*. The highest bio-accumulation factor in *Pteris vittata* shoot was 2.944 and 0.635 in *Pityrogramma calomelanos.* Transfer factor of *Pteris vittata* was 1.742, while for *Pityrogramma calomelanos* was 0.859 in all the treatments. It could be concluded that *Pteris vittata* is a better bio-accumulator, tolerated more lead and therefore could be used in remediating lead-contaminated soils, better than *Pityrogramma calomelanos.*

Keywords: bioaccumulation; contaminated soil; heavy metals; root; shoot biomass

Introduction

Pteridaceae comprises over 1,000 species found mainly in the tropics a; more notable than their species richness is the remarkable morphological and ecological diversity of the family. This includes obligate epiphytes, aquatics and terrestrial species growing on moist soil and forest floor. One of the best characteristics of this family is the possession of linear sori on the pinnae margins with false indusia.

Pteris vittata L. is an ornamental plant with short, erect rhizome, open, spreading, drooping fronds and long terminal leaflet. The leaflets are linear-lanceolate, green, simple, unipinnate, hairy with acuminate apex, while the stipe is polished black. *Pityrogramma calomelanos* L. is erect, drooping, ornamental, with medicinal properties. Rhizome is short and vertical, crozier is hairy, while stipe is polished dark purple. Leaf is compound bipinnate, while pinnae are arranged in alternate manner. Pinna is found on rachilla with acute to acuminate apex and densely covered with yellow to orange and whitish powdery substances on the abaxial surface.

Lead is one of the major heavy metals and has gained considerable importance as a potent environmental pollutant. It is bluish-grey, with a low melting point of 327.46 °C. Despite regulatory measures adopted in many countries to limit lead input in the environment, it is still one of the most serious global environmental and human hazards. There is significant increase in lead contents of cultivated soils near industrial areas, whereas it is a protoplasmic poison, slow in acting and subtle. Besides natural weathering, the main anthropogenic sources of lead pollution are chimneys of factories that use lead, industries, mining and smelting of lead ores, metal plating and finishing operations, fertilizers, pesticides and additives in pigments, lead containing paints, explosives, disposal of municipal sewage sludge rich in lead and gasoline (Chaney and Ryan, 1994; Eick *et al*., 1999). The major sources of lead pollutant in the environment include exhaust fumes

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from automobiles which contribute substantially to the atmospheric pollution in urban areas. Plants growing near highways are usually exposed to more lead. Lead toxicity inhibits germination of seeds and retards seedling growth; it decreases germination potential, root and shoot length, tolerance index and dry mass of roots and shoots (Mishra and Choudhari, 1998). Verma and Dubey (2003) reported that high concentrations of lead causes decreased germination rate in rice seeds and reduces seedling growth by 13 to 45%. Human exposure to lead causes a variety of health defects especially in children and it is listed as a potential carcinogenic substance by the Environmental Protection Agency and Toxic Release Inventory (WHO, 1993). Inhalation and ingestion are the two routes of contacting lead with the same effects. Too much lead can damage various systems of the body including kidneys, nervous and reproductive systems and it accumulates in the bones. It is highly harmful to the developing brains of foetus, young children and pregnant women. At very high levels, it can cause convulsion, coma and even death. Adults usually experience loss of memory, nausea, insomnia and weakness of the joints when exposed to lead particles.

Pteris vittata has been identified as an arsenic hyperaccumulator with efficient arsenic accumulation potentials (Komar, 1999; Ma *et al*., 2001; Oloyede *et al*., 2013). *Pityrogramma calomelanos* is compared with *Pteris vittata* in its ability to accumulate lead in its roots and shoots biomass, tolerance, bio-concentration potentials, transportation of lead and used along as metal selective species, in its remediating ability.

The aim of the present study was to investigate the phytoremediation and bio-accumulation potentials, transportation of lead from roots to the shoots and effects of lead on the growth and developments of these taxa.

Materials and Methods

Experimental plants were collected from the fern garden in the Department of Botany, Obafemi Awolowo University, Ile-Ife (07°30' N, 04° 40' E), Nigeria and were identified at IFE herbarium.

Physical and chemical properties of the soil and the plants used

The soil samples used for the study were collected from the 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), while soil chemical properties were determined using x-ray fluorescence. For each of the plant species, six pots were used and labelled as control (CT), A, B, C, D and E. These pots were filled with 5 kg of soil and saturated with water. Except for the control pot, they were treated with different levels of lead concentrations in the form of lead (II) trioxonitrate (v) salt [Pb $(NO_3)_2$] (0, 200, 400, 600, 800 and 1,000 ppm, respectively) in a completely randomized design in six replicates, and left for two days for even mixture and equilibration. The chemical properties of the two plant species were determined using x-ray fluorescence before and after the treatments.

Healthy fernlets of both *Pteris vittata* and *Pityrogramma calomelanos* having two leaflets, were transplanted into each pot treated with varying quantities of lead (Pb) and were monitored for 12 weeks after transplanting for further studies.

The soil was watered to make the environment conducive to the plants. Frond height (plant growth) in each of the treatments was monitored and recorded weekly throughout the experimental period. The length and width of the selected terminal leaflets and frond length were carefully measured using a meter rule and the number of leaflets per each treatment was recorded. The leaflet area was calculated using the formula:

Leaflet area (LA) = Length × Width × Correction factor (cm^2) (Osei-Yeboah *et al*., 1983). The correction factor for *Pteris vittata* is 2.325, while for *Pityrogramma calomelanos* is 0.305.

Results

Physical and chemical characteristics of the soil and lead concentration in the plants used

The elemental analysis of the soil and the parent plants used in the study were deficient in lead before the experiment, as *Pteris vittata* had 0.050 ppm of lead in the shoot (frond) and 0.075 ppm in the root, while *Pityrogramma calomelanos* had 0.025 ppm in the shoot and 0.010 ppm in the root, respectively. The texture of the soil was smooth, with a good content of humus; pH was 6.06 indicating a slightly acidic soil condition. The organic carbon content of the soil was 5.3 ppm, Calcium 31.0 ppm, Magnesium 2,322.7 ppm, Potassium 2,611 ppm, Nitrogen 3.20 ppm, Phosphorous 10.4 ppm, with no lead detected.

Growth measurement and analysis of Pteris vittata
Frond height

The frond height of *P. vittata* grown in different levels of Pd concentrations are shown in Fig. 1. There was a significant increase in frond height in all the treatments ($p < 0.05$), but there was no significant difference ($p < 0.05$) in week one and two. At the end of week 12, frond height of control plant was the highest, with mean value of 28.3 cm, while treatment E had the lowest mean value of 12.8 cm and the others were of intermediate values (Fig. 1). Treatments B and C showed no significant difference from each other ($p < 0.05$). Increase in Pb concentrations had little negative effects on the frond height of *P. vittata*.

Number of leaflets

Fig. 2 shows the number of leaflets in *P. vittata* grown in different levels of Pb concentrations. At week one, there was no significant difference ($p < 0.05$) in the number of leaflets in all the treatments when compared with control. However, there was significant increase ($p < 0.05$) from week 2 to 12. The number of leaflets in the control plant became the highest, with mean value of 23.43 and treatment E had the lowest result,with mean value of 12.69, while others were of intermediate values (Fig. 2). This shows that as the concentration of lead in the soil increased, the number of leaflets decreased significantly across the weeks.

Leaflet area

The leaflet area of *P. vittata* at different levels of Pb concentration is shown in Fig. 3. There was no significant difference ($p < 0.05$) in the leaflet area from week 1 to 4 in all the treatments when compared with control. In treatments A, B and C, there was no significant difference in the pinna area in week 5 ($p < 0.05$). Similar result was observed in treatments D and E from week 6 to 8. At the end of week 12, the leaflet area of treatment A showed the highest value of 20.93 cm^2, while treatments D and E showed the lowest values of 6.88 cm^2 and 6.98 cm^2 and were not significantly different from one another. This implies that as Pb content increased with time, while there were drastic reduction or decrease [significant difference ($p < 0.05$)] in the pinna area.

Growth measurement and analysis of Pityrogramma calomelanos

There was slow growth and necrosis of the leaflets in the treatments D and E from week 9 to week 12.

Frond height

The results of frond heights of *P. calomelanos* grown in different levels of Pb concentrations are shown in Fig. 4. There was no significant different ($p < 0.05$) in the frond height in week one in all the treatments. However, in week two, the frond height of control, treatment A and B were significantly different ($p < 0.05$) from other treatments. Also, in week three, all treatments were significantly different from one another, except for control and treatment A, which were not significantly different. For weeks four to twelve, all treatments were significantly different from one another, while control had the highest mean value of 39.5 cm, treatment E had the lowest mean value of 18.1 cm and others were of intermediate values (Fig. 4). This shows that increase in Pd concentrations led to drastic decrease in frond height ($p < 0.05$).

Number of leaflets

There was an increase in the number of leaflets of *P. calomelanos* grown in soil at different levels of Pb concentrations across the experiment as shown in Fig. 5. At week one, no significant difference ($p < 0.05$) was noted within all treatments when compared with control. At week two, all treatments were significantly different from one another and three groups were identified: group 1 consisted of control and treatment A, group 2 consisted of treatments B and C, while group 3 consisted of treatment D and E. There was significant difference ($p < 0.05$) in all the treatments from weeks 3 to 12, except treatments D and E that showed no significant difference in weeks 10, 11 and 12 (Fig. 5). At week 12, control had the highest mean value of 54, after which the value started decreasing from A (51) to D and E with the same mean value of 35. This shows that as the concentration of Pb increases, leaflet numbers decreases significantly ($p < 0.05$).

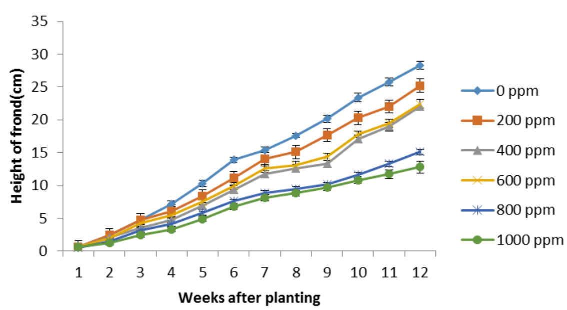

Fig.1. Effect of different treatments of lead on the frond height of *Pteris vittata*

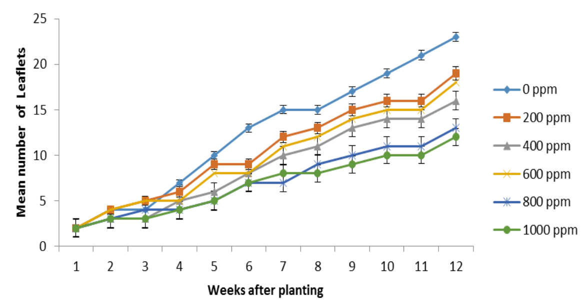

Fig. 2. Effect of different treatments of lead on the number of leaflets of *Pteris vittata*

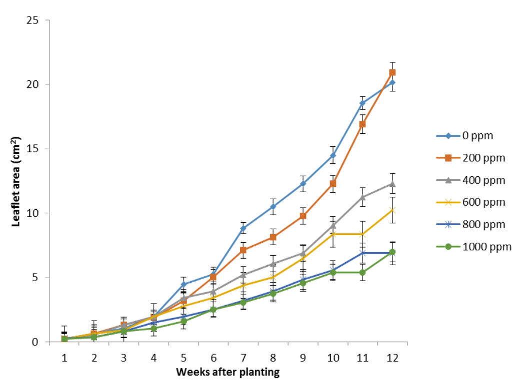

Fig. 3. Effect of different treatments of lead on the leaflet area of *Pteris vittata*

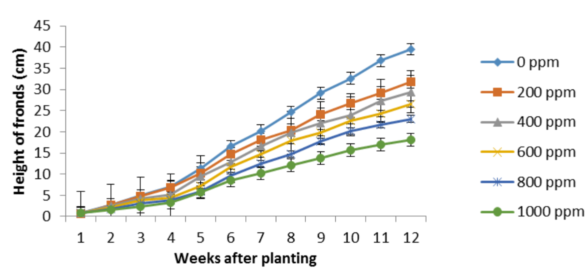

Fig. 4. Effect of different treatments of lead on the frond height of *Pityrogramma calomelanos*

Leaflet area

The leaflet area of *P. calomelanos* at different levels of Pb concentrations is shown in Fig. 6. There was no significant difference ($p < 0.05$) in the leaflet area from week one to three in all the treatments when compared with control. There was little difference in the leaflet area in weeks four and five. However, distinct significant difference ($p < 0.05$) was observed in all the treatments from week six to twelve. At week 12, the leaflet area of control showed the highest mean value of 34.6 cm^2, then the value started decreasing from treatment A (27.44 cm^2) to E with the least mean value of 6.91cm^2 (Fig. 6). This shows that increase in the concentration of Pb results in decrease in the leaflet area of *P. calomelanos*.

Effect of lead on plant biomass of P. vittata

All the treatments at the end of the 12th week resulted in higher shoot biomass than the root biomass. The shoot biomass decreased with increasing concentration levels of Pd, with treatment E showing the the least (5.20 g) shoot biomass.

However, shoot biomass in treatments A and C were higher than the control. Similar trend was observed for the root biomass in the control plant and treatment E having 5.16 g and 2.16 g, respectively (Table 1).

Effect of lead on plant biomass of P. calomelannos

All the treatments at the end of the 12th week had higher shoot biomass than root biomass. The shoot biomass decreased with increasing concentration levels of Pd, control has the highest (9.51 g) and treatment E the least (3.75 g) root biomass. The root biomass also decreased as the concentrations of Pb increased, with control having the highest value (7.64 g) and treatment E the lowest value (2.50 g) respectively (Table 2).

Bioaccumulation and transfer factor

Bioaccumulation factor is defined as the ratio of metal (Pb) concentration in the plant to that in the soil and it is used to measure the effectiveness of a plant in concentrating Pb into its biomass. The bioaccumulation factor for the shoots of the two species increased with increase in Pb concentrations (Table 3). Shoot and root bioacumulation factor within treatment D of *P. calomelanos* decreased as Pb concentration increased (Table 4). The lowest bioaccumulation factor in the shoot and root of *P. vittata* were 1.691 and 1.165 respectively, while that of *P. calomelanos* were 0.445 and 0.570, respectively.

The highest transfer factor in the shoot and root of *P. vittata* were 2.944 and 1.690, while for *P. calomelanos* were 0.635 and 0.740, respectively.

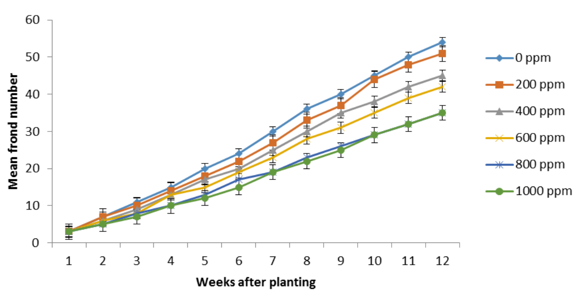

Fig. 5. Effect of different treatments of lead on the number of leaflets of *Pityrogramma calomelanos*

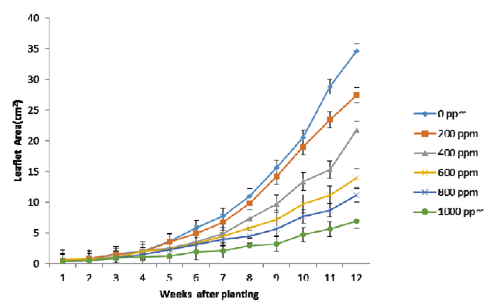

Fig. 6. Effect of different treatments of lead on the leaflet area of *Pityrogramma calomelanos*

Table 1. Plant biomass (g) of *Pteris vittata* after growing for 12 weeks in lead contaminated soil

Treatment	Shoot	Root
Control	8.11c	5.16a
A (200 ppm)	8.14b	4.64b
B (400 ppm)	7.00d	3.66e
C (600 ppm)	8.23a	4.51c
D (800 ppm)	6.36e	4.47d
E (1,000 ppm)	5.20f	2.16f

Mean with different letters within the same column are significantly different ($p < 0.05$)

Table 2. Plant biomass (g) of *Pityrogramma calomelannos* after growing for 12 weeks in lead contaminated soil

Treatment	Shoot	Root
Control	9.51a	7.64a
A (200 ppm)	7.47c	6.81b
B (400 ppm)	7.93b	4.52c
C (600 ppm)	5.12d	4.28d
D (800 ppm)	4.62e	3.39e
E (1,000 ppm)	3.75f	2.50f

Mean with different letters within the same column are significantly different ($p < 0.05$)

Table 3. Lead bioaccumulation and transfer factors in *Pteris vittata* after growing for 12 weeks in lead contaminated soils

Treatment	Transfer Factor	Bioaccumulation Factor	
		Fronds	Roots
Control	0	0	0
200 ppm	1.451	1.691	1.165
400 ppm	1.444	1.866	1.292
600 ppm	1.712	2.470	1.442
800 ppm	1.695	2.541	1.499
1,000 ppm	1.742	2.944	1.690

Table 4. Lead bioaccumulation and transfer factor in *Pityrogramma calomelanos* after growing for 12 weeks in lead contaminated soils

Treatment	Transfer Factor	Bioaccumulation Factor	
		Fronds	Roots
Control	0	0	0
200 ppm	0.745	0.454	0.609
400 ppm	0.807	0.460	0.570
600 ppm	0.828	0.603	0.728
800 ppm	0.768	0.445	0.579
1,000 ppm	0.859	0.635	0.740

Discussion

According to the Environmental Protection Agency, Pb is the most common heavy metal contaminant in the environment (Islam *et al*., 2007). Plants undergo significant morphological and metabolic changes in response to metal stress. Visible symptoms of metal toxicity in plant are the expression of metal-induced changes at structural levels (Singh and Sinha, 2004). The effects of heavy metals such as Pb, Cd and Al on plants include decrease in leaf numbers and leaf size, decrease in shoot biomass, inhibition of root elongation, chlorosis and necrosis of leaves leading to decrease photosynthetic activity and reduced stomata aperture (Özyiğit and Akinci, 2009; Rai *et al*., 2010). Plant biomass is a good indicator of growth performance of plants in the presence of heavy metals.

In the present study, at different levels of Pb concentrations, there was a significant difference in the growth of both species studied with different treatments which may be attributed to excessive accumulation of Pb in these plants. The fresh and dry weights of both roots and shoots of the two species increased with increased levels of Pb concentrations. Nicholls and Mal (2003) reported similar results of complete and rapid death of shoots of *Lythrium salicaria* treated with Pd and Cu at high concentrations. The shoot biomass of *Pteris vittata* in treatment A and C was higher than the control. This corroborates with the findings of Fayiga *et al*. (2004) who reported an increase in the shoot biomass of *P. vittata* grown in a soil contaminated with arsenic and some heavy metals (Cu, Zn and Pb).

It was reported that an increase in concentration of Pb induced significant growth inhibition in two varieties of maize (*Zea mays* L.). The results in the current study showed inverse relationship between soil Pb concentrations and *Pityrogramma calomelanos* plant biomass. Similarly, Omosun *et al*. (2008) reported significant reduction in dry and fresh weights of *Amaranthus hybridus* as crude oil concentrations increased. Verma and Dubey (2003), Strubińskai and Hanaka (2011) and Miao *et al*. (2012) also reported that Pb inhibited plant growth by affecting the biochemical and metabolic processes which are linked with normal growth and developments. Kopyra and Gwóźdź (2003) reported that heavy metals such as Pb reduce plant growth and development.

The absorption of Pb by *Pteris vittata* and *Pityrogramma calomelanos* had negative effects on plants' growth and development. This is because there was a reduction in the frond height, leaflet numbers and leaflet area (Figs. 1-6). This decrease did not result into death of any of the two plant species, even when slow growth and necrosis were observed in *Pityrogramma calomelanos* in treatment D and E. Omosun *et al*. (2008) also reported a progressive decrease in the height, number of leaves and leaf area of *Amaranthus hybridus* when treated with different crude oil concentrations. In contrast, Ogbo *et al*. (2009) reported highest leaf area in the highest crude oil contaminated soil when investigating the phytoremediation potential of *Paspalum scrobiculatum*. Even so, in the hereby study, *Pteris vittata* showed a decrease in the growth parameters measured as Pb concentration increased, thus it was more tolerant to Pb toxicity and stress than *Pityrogramma calomelanos*. Many fern species such as *Blechnum orientale* and *Sesbania drummondii* have been identified to absorb and accumulate toxic metals such as As, Hg, Cu, Zn and Pb in their fronds (Ma *et al*., 2001).

The present study investigated phytoremediation potentials, tolerance ability of Pb, its transport within *Pteris vittata* and *Pityrogramma calomelanos* in Pb contaminated soil. From the results obtained, it was noted that *P. vittata* accumulated more Pb in all the treatments in the fronds compared with *P. calomelanos*. Furthermore, there was an increase in Pb accumulation by the two species with increased Pb concentrations. These trend agrees with the results of Oloyede *et al*. (2013) who reported that *Pteris vittata* accumulated more arsenic in its fronds than *Pteris ensiformis*. In contrast, Chen *et al*. (2009) reported that *Pteris vittata* accumulated less mercury in its fronds than

Nephrolepis exaltata. *Pteris vittata* accumulated more Pb in its roots than *Piytrogramma calomelanos* and this is in agreement with Zhang *et al*. (2002) who reported that *Pteris vittata* accumulated more arsenic in its root than *Pteris cretica*.

One of the characteristics of hyperaccumulator is the ability to accumulate high quantity of metal(s) in shoot biomass (Mrittunjai *et al*., 2006). Plants' ability to accumulate metals absorbed from the contaminated soils can be estimated using the bioaccumulation factor (BCF). More Pb molecules were accumulated in the shoot than in the roots in *Pteris vittata* in all the treatments (Table 3). This is in agreement with Xu *et al*. (2009) who reported higher bioaccumulation factor in *Pteris vittata* than *Pityrogramma calomelanos* grown in arsenic contaminated soils. Ma *et al*. (2001) and Zhao *et al*. (2002) stated that *Pteris vittata* possessed bioaccumulation factor greater than 10 for a relatively short time in spiked soils with arsenic. Wei and Chen (2006) reported BCF from 0.06 - 7.40 for *Pteris vittata* growing at a mining site and suggested that soil properties played a significant role in the arsenic accumulation of this fern species.

More Pd was accumulated in the roots of *Pityrogramma calomelanos* than in its fronds in all the treatments (Table 4). Nabeel *et al*. (2011) reported that bioaccumulation factor of *Pityrogramma calomelanos* is greater in the root than those of *Pteris vittata* when grown in the soil contaminated with arsenic. In *Pteris vittata*, the bioaccumulation factor is greater than 1, but was less in *Pityrogramma calomelanos* in all the treatments (Tables 3 - 4).

The ability of a plant to absorb and translocate metals from the roots to the shoots is measured by transfer factor (TF). The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable plant part i.e. shoots. The difference between BCF and TF is the ability of plants to absorb heavy metals from the soil and translocating them to the shoots. Plants exhibiting transfer factor and most importantly bioaccumulation factor less than 1 are unsuitable for phytoextraction (Fitz and Wenzel, 2002). The transfer factor in *Pteris vittata* was greater than 1, but was less in *Pityrogramma calomelanos* in all the treatments (Table 4) and this agrees with the findings of Fayiga *et al*. (2005). The TF of less than 1 indicates that *Pityrogramma calomelanos* is not effective in transferring Pb from its roots to the shoots.

Conclusions

It can be noted that *Pityrogramma calomelanos* was not a good phytoremediator of Pb since its BCF and TF were less than 1 in the current experiment. There were profound negative effects on the morphology and vegetative growth parameters, which were significantly depreciated, especially at higher doses, leading to slow growth and necrosis of the leaflets observed in treatments D and E. *Pteris vittata* had BCF and TF for Pb more than 1, while its morphological features and vegetative growth were not significantly depreciated even at higher doses of Pb. Hence it is therefore not metal selective in its phytoremediaton ability. *Pteris vittata* has a better phytoremediation potential and better ability to accumulate more molecules of Pb compared with *Pityrogramma calomelano*.

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