



Anatomical changes, osmolytes accumulation and distribution in the native plants growing on Pb-contaminated sites

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Abstract Native plants growing on heavy-metal-contaminated sites are the potential candidates for phytoremediation of contaminated sites due to their tolerance and adaptation to toxic environment. For better application of the technology, in-depth knowledge and understanding of the anatomy and physiology of these plant species are essential. In this study, anatomical changes in roots and leaves of various plant species growing on Pb-contaminated sites as well as osmolytes (proline, PR; glycine betaine, GB; and phenolics, PH) production and distribution in different plant parts were investigated. The soil and plant samples were collected in triplicates from two different Pb-contaminated sites, while control plants were collected from the University of Ibadan. The plants were washed and partitioned into different parts for anatomical and biochemical studies, and soil adhering to the roots of different plant species was collected for the determination of Pb contents. *Sporobolus pyramidalis*, *Cynodon dactylon*, *Imperata cylindrica*, *Eleusine indica*, *Gomphrena celosioides*, *Rhinconspora corymbosa* and *Echinochloa colona* are the plant species common to these contaminated sites. Though with variations based on the site, thick

epidermis and sclerenchyma, pronounced and numerous vascular bundles as well as trichomes were some of the anatomical characteristics of these metallophytes from contaminated sites compared to their counterparts from uncontaminated site. These plants also produced PR, GB and PH, with PH being the highest followed by PR and GB. Accumulation in different parts of the plants also varied depending on the plant species and the osmolyte. They were more in the leaf than other plant parts. Among the plant species, *Sporobolus pyramidalis* had the highest value of proline (3.26 and 2.63 $\mu\text{g/g}$ FW) and glycine betaine (3.44 and 2.23 $\mu\text{g/FW}$) in the leaf and stem, respectively, followed by *Cynodon dactylon* having 2.20 and 0.89 $\mu\text{g/g}$ FW proline and 2.02 and 0.94 $\mu\text{g/g}$ FW glycine betaine. On the Pb accumulation in plant, more lead (Pb) was found in the roots than other plant parts. *Gomphrena celosioides* accumulated the highest Pb (4537.50 and 1081.50 mg/kg) in the root and shoot, respectively, followed by *Eleusine indica* (3988 and 699.00 mg/kg), while the lowest values were recorded for *Echinochloa colona* (36.87 and 5.97 mg/kg). In rhizospheric soils, *G. celosioides* had the highest Pb content (34,405.00 mg/kg) which was higher than other rhizospheric soils. In conclusion, phenolics were produced more in these metallophytes and all the osmolytes were more in leaf than root. Anatomical modifications in response to heavy metal exposure differed between plant species and level of contamination. Lead accumulation in plant also varied depending on plant species.

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Introduction

Exposure to heavy metals (HMs) has been reported to cause different biochemical and anatomical changes in the plants. It causes an increase in the production of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, thereby causing imbalance in cellular metabolism and oxidative stress (Verma and Dubey 2003; Liu et al. 2009; Naz et al. 2015). Lead, most especially, has been reported to induce reactive oxygen species (ROS) production in plant (Verma and Dubey 2003; Reddy et al. 2005; Hussain et al. 2013). These radicals are toxic to plant cell, because of their ability to degrade cell biomolecules (Apel and Hirt 2004; Gopal and Rizvi 2008) and cause lipid peroxidation, membrane damage and enzyme inactivation (Pinto et al. 2003; Zheng et al. 2011).

However, some particular plant species are able to colonize sites that are polluted with HMs despite high level of toxicity. They do not only tolerate these toxic metals but also accumulate these metals in higher concentration in their root and shoot (Adeosun et al. 2017). They are able to achieve this through structural adjustment and their ability to scavenge the toxic ROS through the enhanced production of different metabolites (Singh and Tuteja 2010; Hossain et al. 2012a, b). Several enzymatic and non-enzymatic antioxidant mechanisms are involved in metal detoxification and tolerance (Sharma and Dubey 2009; Hossain et al. 2012a, b). Among the antioxidant compounds that have been reported are phenolics, proline and glycine betaine whose production are induced in plant exposed to environmental stresses like HM toxicity (Smirnoff 2005; Athar et al. 2008). These antioxidant compounds contain different functional groups which are used by the plants to scavenge reactive oxygen species which are produced in the course of metal toxicity. For instance, proline is an important antioxidant reported in heavy metal tolerant plants. Its accumulation in plants under different stress conditions was well documented. It is considered as a potent antioxidant, an osmoprotectant, a metal chelator, a protein

stabilizer, ROS scavenger and potential inhibitor of programmed cell death (PCD) (Ashraf and Foolad 2007; Trovato et al. 2008).

The synthesis of proline under abiotic stress has been implicated as a mechanism to alleviate oxidative stress and cytoplasmic acidosis and maintain NADP: NADPH at values compatible with metabolism (Hare and Cress 1997; Hoque et al. 2007; Verbruggen and Hermans 2008). Accumulation of proline in the tissues apart from antioxidative enzymes is involved in pea plants in response to Ni stress (Gajewska and Sklodowska 2005). It has also been found that overexpression of genes for proline biosynthetic pathway enhanced the abiotic stress tolerance in transgenic plants (Tateishi et al. 2005). Similarly, the accumulation of glycine betaine (GB) in plants protects from abiotic stresses through osmoregulation and osmoprotection as well as ROS detoxification (Jitender 2011; Malar et al. 2014). Inducement of GB biosynthetic genes has been reported to stimulate abiotic stress tolerance in transgenic plants (Chen and Murata 2011). Production and distribution of antioxidants in plants, however, vary, depending on the plant species and the level of contamination (Hollander-Czytko et al. 2005; Sharma et al. 2011; Adejumo et al. 2019).

Similarly, changes in the anatomical characteristics have been reported to be part of adaptation strategies employed by most metallophytes for tolerance to heavy-metal-induced stress (Ederli et al. 2004; Al-Saadi et al. 2013). For instance, formation of Casparian strips in the endodermis and exodermis of root after Zn and Cd exposure as well as apoplastic barrier development has been reported to occur in response to toxic metals (Vaculík et al. 2012). Thickness of the outer layers of endodermis and exodermis in the root tissues and the cell walls of the xylem and cortical parenchyma has been reported in response to HM contamination. Suberin lamellae were also found to form earlier in the exodermis than in the endodermis. In some species, secondary thickening of the walls and lignification of cellulose microfibrils in the cell walls are the adaptive mechanisms that help maintain the stability of the root architecture. The changes in anatomical structures of these plants as well as osmolyte production in response to metal contamination, therefore, need to be understood for effective application for phytoremediation.

However, most studies carried out on stress induced osmolyte production focused only on the leaf of the test crop, while fewer reports are available on the concentration of these metabolites in other parts of the plants apart from the leaf. Besides, these reports are usually on model plants like *Arabidopsis spp.*, whereas native plants growing on metal-polluted sites could have served as the best candidates. The study of the behaviour of plants under natural condition will give broad understanding of the strategies being employed for tolerance and how they can be modified for effective phytoextraction and crop tolerance. In our previous studies, we have been able to identify a Pb hyperaccumulator, tolerant and excluder species on these contaminated sites and the variations in their rhizospheric soil properties studied (Adejumo et al. 2015, 2018, 2019). In this study, the anatomical responses and various osmolytes (proline, glycine betaine and phenolics) which are used for tolerance and detoxification of ROS by individual plants sampled from two Pb-contaminated sites were investigated. The distribution of these substances in different plant parts was also assessed.

Materials and methods

The study sites were abandoned auto battery waste dumpsites located in Lalupon and Kumapayi, Ibadan, Nigeria, having Pb concentration ranges from 50,000–67,000 mg/kg. Lalupon is in Lagelu Local Government Area of Oyo State and lies on latitude 7° 28' N and longitude 4° 04' E, while Kumapayi is on latitude 7° 24' N and longitude 4° 00' E in Egbeda Local Government Area of Oyo State. University of Ibadan Campus was used as control/uncontaminated site. The comparative studies were, however, carried out only on the plant species found both on the contaminated site and on university control site. Not all the plant species found on contaminated sites were found in the control site. Soil and plant samplings were carried out by collecting the different plant species growing on these sites in triplicates. The plants were then washed and partitioned into different parts and brought to the laboratory in brown envelopes. The soil adhering to the roots of different plant species was collected separately for the determination of Pb.

Laboratory procedures

Soil analysis

The soil was first air-dried and then sieved. Soil lead (Pb) was determined using the digestion method described by Ogundiran and Osibanjo (2008), and the lead (Pb) content in the extract was determined using an atomic absorption spectrophotometer (VGP 210 BUCK Scientific Model).

Osmolyte determination

The proline content was determined following the procedure of Bates et al. (1973) by homogenizing 0.5 g of the plant material in 3% of aqueous 3, 5-Sulphosalicylic acid, and the homogenate was filtered. Thereafter, 2 ml of the filtrate was taken in a test tube with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. The mixture was heated in boiling water for 1 h after which the tubes were placed in ice bath. Four millilitres of toluene was added to the reaction mixture and stirred well for 30 s, and the toluene layer was separated at room temperature. The red colour intensity was measured at 520 nm using a VIS spectrophotometer. Proline concentration was calculated from a standard curve ranging from 0.0 to 100 µg proline. Proline content was expressed as mg/g FW. Glycine betaine (GB) in leaf tissues was determined following Grieve and Grattan (1983). Fresh plant materials (1.0 g) from each replicate ($r = 4$) were shaken for 5 min in 10 ml of 0.5% toluene solution and filtered. After filtration, 5 ml of 2 N H₂SO₄ was added and allowed to cool down. Thereafter, 0.5 ml of this mixture was taken in a glass tube and 0.2 ml of potassium tri-iodide (KI₃) solution was added. Then, 2.8 ml ice-cooled distilled water and 6 ml of 1, 2-dichloroethane (cooled at 4 °C) were added to the mixture. The upper aqueous layer was discarded, and the absorbance of the remaining organic layer was measured at 365 nm. Total phenolic content (TPC) of sample extract was determined according to Folin–Ciocalteu method (Chen and Murata 2011). Three hundred microlitres of extract was dispensed into test tubes. To this, 1.5 ml of Folin–Ciocalteu reagent (diluted ten times with distilled water) was added, followed by 1.2 ml of Na₂CO₃ solution (7.5w/v). The reaction mixture was stirred, allowed to stand for 30 min at room temperature

before the absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Spectrum Lab 752s, Ningbo, China) against a blank prepared by dispensing 300 µl of distilled water instead of sample extract. These data were used to estimate TPC using a standard calibration curve obtained from various diluted concentrations of gallic acid. The TPC was expressed as gallic acid equivalent (GAE) in mg/g material. The calibration equation was $Y = 0.0645x - 0.0034$ ($R^2 = 0.9997$).

Anatomical study

Anatomical studies were carried out at the Department of Botany, University of Ibadan, Nigeria. Roots of different plant species were cut, and a transverse section of it was made using a table rotary microtome (Spencer 820 model), while the leaves were cut from the basal portion close to the leaf sheath together with its transverse section. Cuttings were made at 15 micromes. Thereafter, the sectioned roots and leaves were stored in 70% ethanol before the best cuts were selected and taken for staining. The sections were successfully stained with safranin O and then counterstained with methylene blue. The sections were then examined using a Fischer light microscope under different magnifications ($\times 40$, $\times 100$ and $\times 400$), and for capturing of photomicrographs, a Sony digital camera mounted on the microscope was used at $\times 40$ magnification.

Statistical analysis

The data obtained were analysed statistically using excel and SPSS (17.0 version), and the means were separated using DMRT.

Results

Metallophytes distribution and lead accumulation in different plant parts

The plants were not uniformly distributed in all the sites used for the study. In Lalupon, plant samples collected were: *Gomphrena celosioides*, *Sporobolus pyramidalis*, *Imperata cylindrica*, *Cynodon dactylon*, *Eleusine indica*, *Rhynchospora corymbosa* and *Echinochloa colona*. In Kumapayi, *Gomphrena*

celosioides, *Sporobolus pyramidalis*, *Imperata cylindrica*, *Cynodon dactylon*, *Sida acuta* and *Chromolaena odorata* were found, while *Gomphrena celosioides*, *Sporobolus pyramidalis*, *Imperata cylindrica*, *Cynodon dactylon*, *Sida acuta* and *Chromolaena odorata* were collected from control site. The *G. celosioides*, *S. pyramidalis*, *I. cylindrica* and *C. dactylon* were the common plant species colonizing these contaminated sites and also in abundance. *Eleusine indica*, *Rhynchospora corymbosa* and *Echinochloa colona* were only found in Lalupon site, while *S. acuta* and *C. odorata* were found in Kumapayi (Table 1). In all the plant species, more lead (Pb) was found in the roots than in other parts of the plants. In plant species collected from Lalupon site, *Gomphrena celosioides* had the highest value (4537.50 mg/kg) in the root, while the lowest was found in *Echinochloa colona* (36.87 mg/kg). The rhizospheric soil of *Gomphrena celosioides* also had the highest value of Pb (34,405.00 mg/kg), which was significantly $p \geq 0.05$ higher than other rhizospheric soils (Table 2). Among the metallophytes collected from Kumapayi site, more lead (Pb) was found in the root of *Sporobolus pyramidalis* (2596.00 mg/kg) and was significantly $p \geq 0.05$ higher than other values obtained for the other metallophytes, while the lowest was found in *Sida acuta* 101.50 mg/kg. The rhizospheric soils of *Gomphrena celosioides*, however, contained the highest Pb contents of 46,653.00 mg/kg, which was significantly ($p \geq 0.05$) higher than other values obtained in other rhizospheric soils including that of

Table 1 Plant samples collected from experimental sites

Plant samples	Location		
	Lalupon	Kumapayi	Control
<i>Gomphrena celosioides</i>	+	+	+
<i>Sporobolus pyramidalis</i>	+	+	+
<i>Imperata cylindrica</i>	+	+	+
<i>Cynodon dactylon</i>	+	+	+
<i>Eleusine indica</i>	+	–	–
<i>Rhynchospora corymbosa</i>	+	–	–
<i>Echinochloa colona</i>	+	–	–
<i>Sida acuta</i>	–	+	+
<i>Chromolaena odorata</i>	–	+	+

(+) present (collected)

(–) absent (not collected)

Table 2 Lead (mg/kg) accumulation in different parts of the plant samples collected from Lalupon contaminated site and their rhizospheric soils

Plant species	Leaf	Stem	Root	Flower	Soil
<i>Cynodon dactylon</i>	485.00d	1147.00a	3600.50c		31,251.00a
<i>Imperata cylindrica</i>	321.00e	250.00d	902.50f		2578.00c
<i>Eleusine indica</i>	699.00c	952.50ab	3988.00b		33,152.00a
<i>Gomphrena celosioides</i>	1081.50a	462.00c	4537.50a	457.5	34,405.00a
<i>Sporobolus pyramidalis</i>	737.50b	786.00b	2593.00e		10,659.00b
<i>Rhinconspora corymbosa</i>	379.50de	96.50e	3258.50d		3968.00c
<i>Echinochloa colona</i>	5.97f	17.65f	36.87 g		14,077.00b

Means in the same column followed by the same letters are not significantly different according to Duncan multiple range test ($P < 0.05$)

Table 3 Lead (mg/kg) accumulation and distribution in different parts of plant samples collected from Kumapayi contaminated site and their rhizospheric soils

Plant species	Leaf	Stem	Root	Flower	Soil
<i>Cynodon dactylon</i>	376.50a	310.00b	1768.00c		5627.00c
<i>Imperata cylindrica</i>	36.50e	9.50e	1902.00bc		9706.00b
<i>Sida acuta</i>	182.50c	1117.00a	101.50e		3638.00d
<i>Gomphrena celosioides</i>	313.00b	31.50d	2067.50b	252.00	46,653.00a
<i>Sporobolus pyramidalis</i>	162.00c	207.00c	2596.00a		1727.00e
<i>Chromolaena odorata</i>	74.00d	201.50c	806.00d		3462.00d

Means in the same column followed by the same letters are not significantly different according to Duncan multiple range test ($P < 0.05$)

Lalupon site (Table 3). From the control site, though lower than what was recorded from the contaminated site, the highest lead (Pb) content was found in the root of *Cynodon dactylon* (83.00 mg/kg) which was significantly $P < 0.05$ higher than the values obtained in all other species collected from control site. The highest value for the rhizospheric soils was also found in *Cynodon dactylon* soil for the control site with a value of 501.00 mg/kg, which was significantly ($P < 0.05$) higher than other values obtained in the rest of the rhizospheric soils (Table 4).

Proline, phenolic and glycine betaine contents in different parts of the plants collected from contaminated and uncontaminated sites

Generally, the proline, glycine betaine and phenolic contents produced in the leaf were more than those in other parts of the plant. The root also has the osmolytes

but in very small quantity. Across the various metallophytes found in Kumapayi and Lalupon, more osmolytes (proline and glycine betaine) were produced in the leaf and stem. This was also the same for the phenolic compound, with more being produced in the leaf and stem than in the roots. However, in Lalupon site, *Gomphrena celosioides* had the highest value of proline produced in the leaf and stem followed by that of *Imperata cylindrica* and *C. dactylon*. The flower of *Gomphrena celosioides* also produced proline but lower than that of other plant parts. Compared to other metallophytes collected from Kumapayi site, *C. odorata* had the highest proline in the leaf and stem followed by that of *Sida acuta*. Conversely, proline was also present in the leaf of all the plant species collected from control site but lower in concentration (Table 5).

Sporobolus pyramidalis had the highest value for glycine betaine produced in the leaf and stem among

Table 4 Lead (mg/kg) accumulation and distribution in different parts of plant samples collected from Control site and their rhizospheric soils

Name of the plant	Leaf	Stem	Root	Flower	Soil
<i>Cynodon dactylon</i>	37.00c	76.50a	83.00a		501a
<i>Imperata cylindrica</i>	30.50d	44.50b	42.00c		41c
<i>Sida acuta</i>	57.00b	49.00b	59.00b		47c
<i>Gomphrena celosioides</i>	25.00e	33.00c	21.50e	16.00	22d
<i>Sporobolus pyramidalis</i>	50.50b	30.50c	42.00c		110b
<i>Chromolaena odorata</i>	91.00a	13.00d	29.00d		29d

Means in the same column followed by the same letters are not significantly different according to Duncan multiple range test ($P < 0.05$)

Table 5 Proline contents in different parts of the plant samples collected from contaminated and uncontaminated sites

Plant species	Lalupon			Kumapayi			Control		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
<i>Cynodon dactylon</i>	3.25ab	2.21b	1.31b	2.00c	1.92ab	0.32f	2.20b	0.89b	0.11d
<i>Imperata cylindrica</i>	3.87a	1.48c	2.81b	2.82a	1.50d	0.99b	1.58c	0.19d	0.35b
<i>Eleusine indica</i>	0.98e	0.59b	0.69a	–	–	–	–	–	–
<i>Gomphrena celosioides</i>	3.96a	2.55b	2.55c	3.18a	2.10a	0.73c	1.43d	0.20c	0.04e
<i>Sporobolus pyramidalis</i>	2.94b	3.94a	2.17a	3.62a	2.63a	0.30b	2.72b	1.73b	0.40e
<i>Rhinconspora corymbosa</i>	1.80d	0.44e	0.69d	–	–	–	–	–	–
<i>Echinochloa colona</i>	3.02a	1.67c	1.36a	–	–	–	–	–	–
<i>Chromolaena odorata</i>	–	–	–	3.87a	2.96b	2.31b	1.09	0.26c	0.27c
<i>Sida acuta</i>	–	–	–	3.87a	2.46b	2.17a	1.80c	0.19d	0.35b

Means in the same column followed by the same letters are not significantly different according to Duncan multiple range test ($P < 0.05$)

all the metallophytes collected from Lalupon and Kumapayi sites. This was followed by that of *C. dactylon* and *I. cylindrica* in Lalupon and Kumapayi sites, respectively. *Sida acuta* had the highest value of glycine betaine produced in the leaf among all the metallophytes collected from the control site. The value was significantly ($p \geq 0.05$) higher than values obtained for other metallophytes collected from control site (Table 6). The same trend was observed for phenolics, and more phenolics were produced in the leaf compared to other plant parts except for *Eleusine indica* on Lalupon site. The amount in the stem of this plant was more than that of the leaf. On Kumapayi site, *Imperata cylindrica* had the highest value for phenolic compound produced in the leaf followed by that of *S. pyramidalis* and *S. acuta*, while the lowest was obtained in *C. dactylon*. Among the plant species

collected from the control site, *Sida acuta* had the highest phenolic compound produced in the leaf which was significantly ($p \geq 0.05$) higher than values obtained for other plant species. This was followed by that of *G. celosioides*, *C. odorata* and *I. cylindrica*. The lowest was found in *S. pyramidalis*. The distribution also followed the pattern concentration in the leaf > stem > root except in *S. pyramidalis* where the value in stem was more than that of the leaf and root. However, the value obtained in the stem of *I. cylindrica* was the highest. The flower of *Gomphrena celosioides* also produced GB and phenolic compound which were more than that of proline (Table 7).

Table 6 Glycine betaine in different parts of the plant samples collected from contaminated and uncontaminated sites

Plant species	Lalupon			Kumapayi			Control		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
<i>Cynodon dactylon</i>	2.02	0.94	0.12	1.64c	0.15c	0.19c	0.22b	0.26d	0.65d
<i>Imperata cylindrica</i>	1.50	0.13	0.43	2.59a	0.23b	0.14c	0.28a	0.02e	0.29d
<i>Eleusine indica</i>	0.96	0.69	0.72	–	–	–	–	–	–
<i>Gomphrena celosioides</i>	1.43	0.24	0.10	1.57c	0.27b	0.10d	0.39a	1.04c	0.62e
<i>Sporobolus pyramidalis</i>	3.44a	2.23	0.32	2.95a	1.26a	1.03a	0.20b	0.13a	0.26c
<i>Rhinconspora corymbosa</i>	1.09	0.26	0.24	–	–	–	–	–	–
<i>Echinochloa colona</i>	1.80	0.19	0.07	–	–	–	–	–	–
<i>Chromolaena odorata</i>	–	–	–	2.33b	0.24b	0.31b	0.85a	0.42b	0.06a
<i>Sida acuta</i>	–	–	–	1.04d	0.26b	0.16c	0.90a	0.15c	0.25b

Means in the same column followed by the same letters are not significantly different according to Duncan multiple range test ($P < 0.05$)

Table 7 Phenolic contents in different parts of the plant samples collected from contaminated and uncontaminated sites

Plant species	Lalupon			Kumapayi			Control		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
<i>Cynodon dactylon</i>	2.81b	2.63c	2.82b	1.97d	1.17d	0.13d	2.09c	2.14b	1.11b
<i>Imperata cylindrica</i>	3.63a	2.83c	1.86c	3.36a	2.19b	1.09b	3.91a	2.73a	1.91a
<i>Eleusine indica</i>	2.68c	2.72c	1.13e						
<i>Gomphrena celosioides</i>	3.90a	3.65a	1.11e	2.23c	1.41c	1.28a	3.63b	1.85c	0.86c
<i>Sporobolus pyramidalis</i>	3.05b	1.45	1.38d	3.15ab	1.15d	0.45c	1.68d	2.12b	0.65c
<i>Rhinconspora corymbosa</i>	3.96a	3.42a	3.31a						
<i>Echinochloa colona</i>	3.85a	3.23b	1.38d						
<i>Chromolaena odorata</i>				2.33c	0.24e	0.31c	3.99a	1.21d	1.87a
<i>Sida acuta</i>				3.09b	2.52a	0.90b	3.99a	0.27e	0.62c

Means in the same column followed by the same letters are not significantly different according to Duncan multiple range test ($P < 0.05$)

Leaf anatomy of the metallophytes collected from contaminated sites and control site

Though their internal tissues were found to be intact, the leaf anatomy of the metallophytes from the two contaminated sites revealed that these plants were severely affected by high Pb concentrations on the sites. The plants from heavy-metal-contaminated sites showed serious effect of lead contamination more than that of control site. There was a marked variation in the anatomy of the leaf collected from contaminated sites and the control site. For instance, plants collected from lead-contaminated site showed numerous trichomes that are well developed and pronounced when compared to that of control site. The parenchyma cells within them were no more intact but already cutoff.

The leaf of plants from contaminated sites also has numerous and well developed vascular bundles as well as thick epidermis compared to that of non-contaminated site. This was more pronounced in the *S. pyramidalis* and *G. celosioides* (Figs. 1, 2). Similarly, variations were observed in the different metallophytes based on the plant species and the sampling site (Fig. 3).

Root anatomy of the metallophytes collected from contaminated sites and control site

Compared to the control plants, the transverse section of the root of the metallophytes from lead-contaminated sites revealed that they all have well developed and numerous vascular bundles, large air spaces

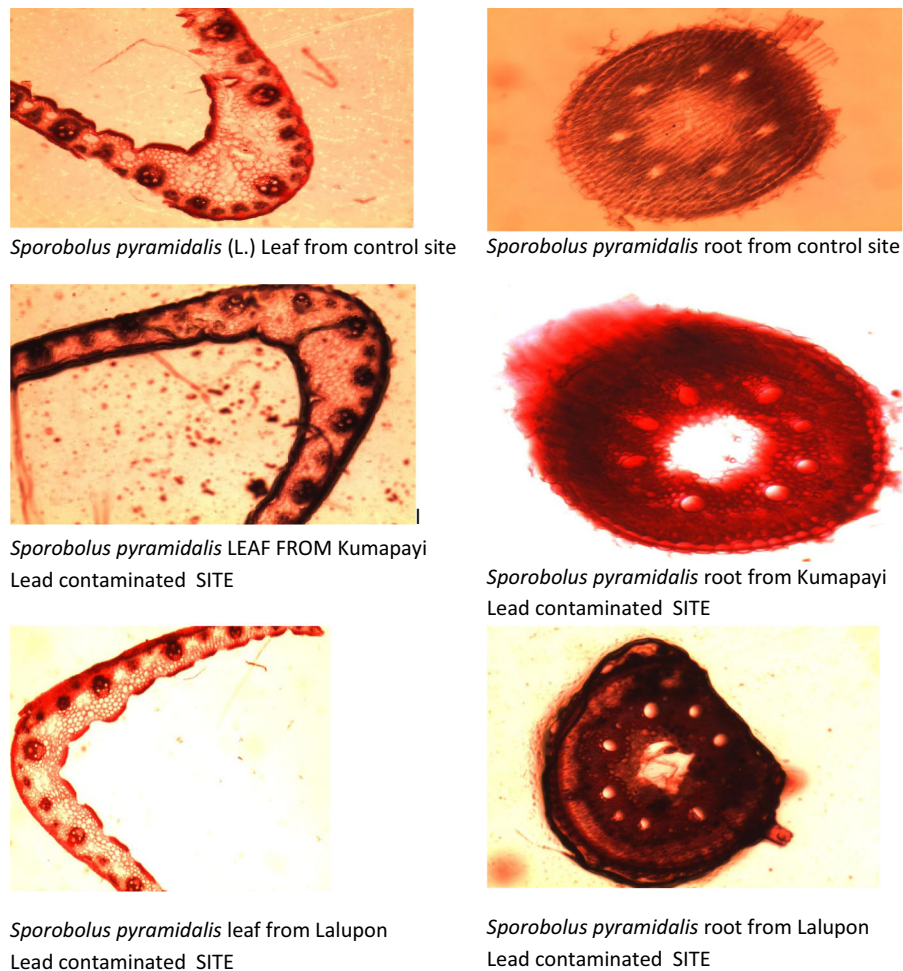


Fig. 1 Transverse sections of the leaves of *Sporobolus pyramidalis* from control site, Kumapayi and Lalupon lead-contaminated sites. Magnification $\times 40$

within their internal tissues and thickened sclerenchyma and epidermal cells. Microscopy observations also revealed that apart from well-developed and numerous vascular bundles, large lacunae were also found within their internal tissues. The parenchyma cells have collapsed from the cortical region. They formed very thick epidermal cells, and the cortical cells were found to be empty compared to that of the control site (Figs. 1, 2 and 3).

Discussion

Vegetation distribution and abundance were generally low on these contaminated sites due to high Pb concentrations in the soils which could be responsible

for the poor plant growth on these sites. The high Pb concentration according to Vanik et al. (2005) and Adejumo et al. (2011) has detrimental effects on the plant's physiological processes, thereby causing reduction in plant growth and development. Higher concentration of lead (Pb) was found in the rhizospheric soil collected from Kumapayi compared to that of Lalupon site as revealed in this study but, shoot and root of plants collected from Lalupon had higher concentration of Pb than those collected from Kumapayi site. The lower value recorded for Lalupon soil could therefore be due to uptake by plants which consequently might have reduced the soil Pb concentration. Generally, more Pb was found in the roots of these metallophytes than in other plant parts, i.e. stems and leaf. This agrees with the previous report (Kabata-

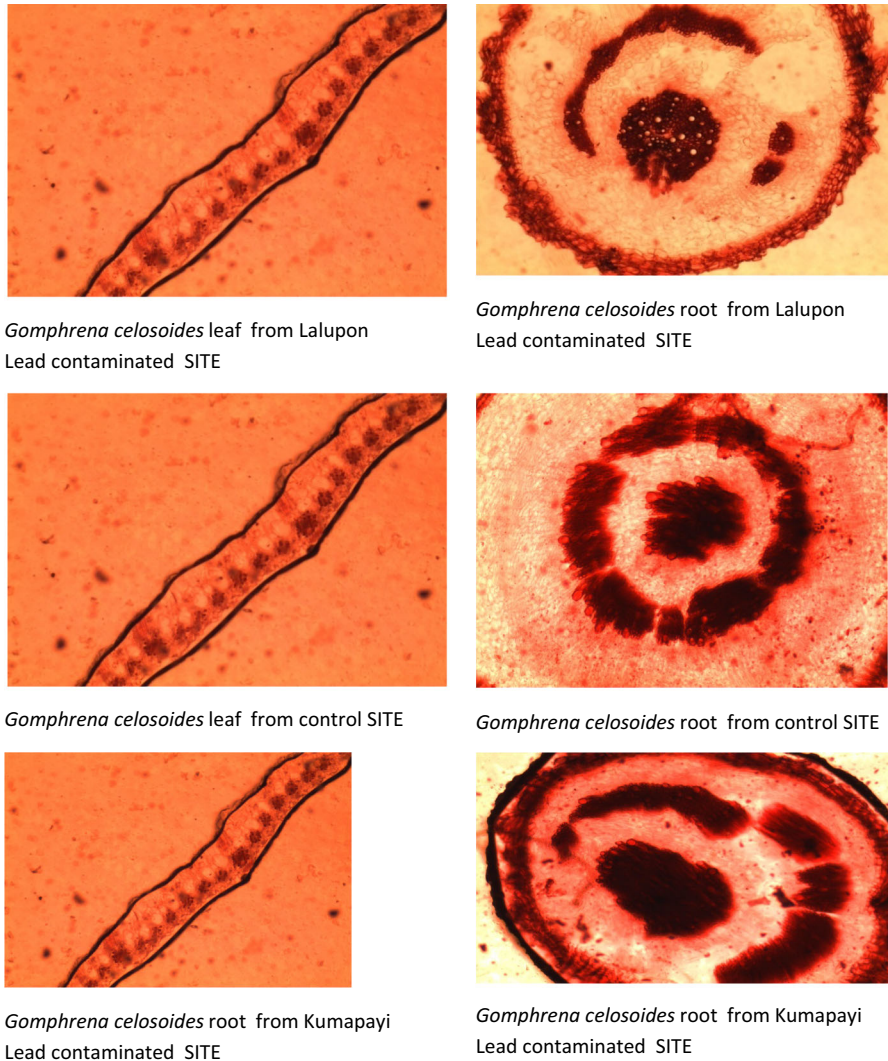


Fig. 2 The transverse section of the leaf and root of *Gomphrena celosoides* at control site, Kumapayi and Lalupon lead-contaminated sites. Magnification $\times 40$

Pendias and Pendias 2001; Ogundiran and Osibanjo 2008). The results also revealed that the heavy metals concentration in the plants varied with the plant species and the levels of the metals in the soil. This is because of variation in the accumulating ability and tolerance mechanisms being displayed by individual plant species. Similarly, strong correlation has also been found with regard to heavy metal uptake by plant and the soil metal concentration (Adejumo et al. 2018). Though, in small quantity and within the background level, there were also traces of lead in the rhizospheric soil of the control site used for this study probably because of the natural occurrence of heavy

metals in soil. This accounts for the Pb accumulation in the roots, stems and leaf of the control plants which in turn induced osmolyte production.

The high concentration of osmolytes found in the shoot of all the metallophytes corroborates previous reports that osmolytes are produced in the delicate parts of the plants where important physiological processes are being carried out (Verbruggen et al. 2009; Pena et al. 2011; Adejumo et al. 2015; Hossain et al. 2012a, b). These metabolites or osmolytes serve as osmoregulators to protect the plants against oxidative stress. The root also have the osmolytes but in very small quantity. However, depending on plant

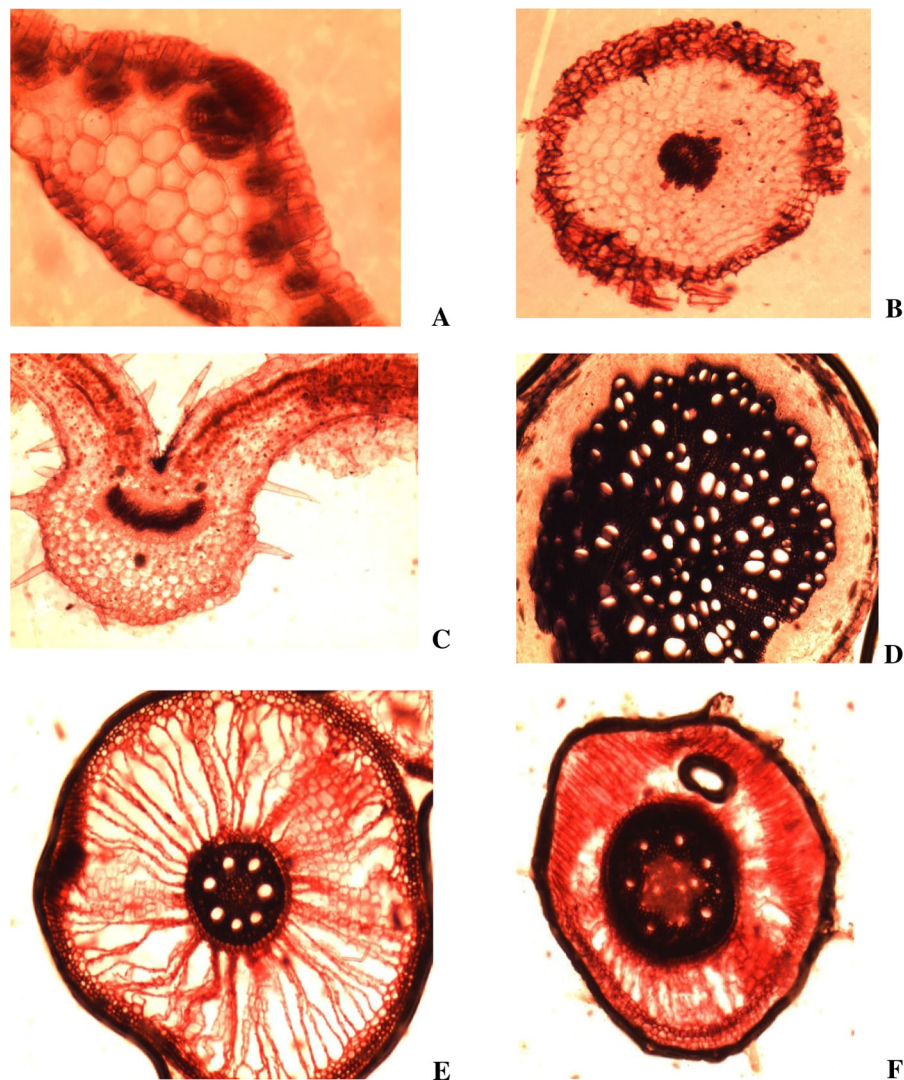


Fig. 3 A and B show the transverse section of leaf and root of *Echinochloa colona* from Lalupon Pb-contaminated sites. C and D show the leaf and root of *Imperata cylindrical* from Lalupon,

E and F show transverse section of root of *Cynodon dactylon* from Kumapayi and Lalupon lead-contaminated sites. Magnification $\times 40$

species, variations were recorded in the accumulation of these osmolytes. As reported above, *Gomphrena celosioides* and *Sporobolus pyramidalis* produced more osmolytes (proline and glycine betaine) in both sites considered for this study. These variations showed that different plants respond differently to environmental stress depending on the plant species, genetic composition and the level of stress. The roots, stems and leaf of plants collected from the control site also produced osmolytes and antioxidants which could be response to the presence of Pb in the soil or other environmental stresses. However, presence of

antioxidants in these plant species and the significant differences observed in their values showed that plants from the contaminated environment like any other plant exposed to different environmental stresses also use antioxidation as a survival mechanism (Rausser 1990; Prasad 1999; Harinder et al. 2007; Sharma and Dubey 2009; Verbruggen et al. 2009; Pena et al. 2011; Zheng et al. 2011; Venkatachalam et al. 2017).

Heavy metal contamination easily affects the anatomy of the stressed plant due to the toxic effects of these metals. The anatomical structure of the plants has been reported to change in response to heavy metal

contamination (Voigt et al. 2006; Li et al. 2009). They disrupt the external and internal tissues of the plants. In this study, the anatomy of the roots and leaves of the different plant species collected from Pb-contaminated sites corroborated these. It was clear that Pb severely affected the arrangement of their internal tissues compared to the control plants. However, the alterations may occur in plants exposed to abiotic stress to protect the plant, and ensure their adaptation to abiotic stress. In some cases, the alterations are probably due to the deposition of lignin or suberin in the exodermis and endodermis layers of the cell. These protect the plants against desiccation, thereby preventing cell death. It also restricts the intake of these toxic elements by the plant root. This disparity in the anatomical structures was in line with the observation of Pena-Valdivia et al. (2005). Similarly, in the Pb-contaminated plants, the number of trichomes in the epidermal cells was more, compared to the control plants. Increased trichome density under environmental stresses may also be a mechanism to enhance tolerance to stress (Aguirre-Medina et al. 2002). They also showed well developed, abundant and thickened vascular bundles when compared to those of the control sites. The parenchyma cells were found to be collapsing, and cortex was almost emptied. In vascular plants, secondary wall thickening plays a fundamental role in providing mechanical strength to support the plant body (Ye 2002). Secondary cell wall thickening and lignification are controlled to a significant extent by individual xylem elements which are regulated by environmental conditions (Gindl et al. 2000). The thickening and lignification might, however, be relative to the amount of lead accumulation as was previously reported (Rascio et al. 2008; Li et al. 2009). This study also confirms the differences in lead tolerance capacity of different plant species as reflected in the pattern of root and leaf anatomical structure. For example, it was observed that root anatomical structure varied in the different plant species and on different sites. The epidermis of root at Kumapayi site began to break off, the edge of the cortex was damaged, and there were large air spaces within their root cells. However, the air space and thickened epidermal cells were minimal in Lalupon lead-contaminated sites followed by the control plants. These symptoms in root correspond to previous findings of Panou-Filotheou and Bosabalidis (2004) and Gratao et al. (2009). This observation might be

connected with the variations in soil Pb concentration. Higher Pb concentration was recorded for Kumapayi soil compared to that of Lalupon. In addition, there were black spots in cortex and in parenchyma cells exhibiting deep staining in parenchyma cell and thickened cell wall. Although the roots of the plants from the lead-contaminated sites were found to generally have abundance of lacunae within them compared to control plants, this was more severe in plants collected from Kumapayi site when compared to the Lalupon sites. The abundance of lacunae formed might be a mechanism for minimizing excessive water loss during transpiration and translocation. It has been reported that air spaces are essential for the aeration of the internal tissues (Evert 2006).

Conclusion

This study shows that lead stress induced changes in the biochemical and anatomical characteristics of the different plant species. The accumulation of osmolytes and anatomical development like thickened epidermal cell, abundance of trichomes and lacunae are some of the mechanisms being employed by these metallophytes for tolerance and survival on heavy-metal-contaminated sites. The accumulation of these metabolites is dependent on the plant species and plant parts. More of these osmolytes are produced in the shoot than in the root, whereas Pb was more accumulated in the root than in the shoot. *Sporobolus pyramidalis* and *Gomphrena celosioides* accumulated more of these osmolytes compared to other metallophytes. It could also be concluded from this study that any land/site found to be colonized by *Gomphrena celosioides*, *Sporobolus pyramidalis*, *Imperata cylindrical* and *Cynodon dactylon* in association is a strong indication of contamination as these plants species were commonly found together in all our sampled heavy-metal-contaminated sites.

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