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Seasonal variations in distribution, heavy metal uptake and proline production of native plants growing on Pb-contaminated site in Ibadan, South-Western, Nigeria

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Abstract

Effects of seasonal variations on metal (Lead, Chromium and Cadmium) uptake, proline concentration, density and distribution of metallophytes inhabiting heavy metal polluted site in Ibadan, South-Western Nigeria were investigated. Vegetation surveys and Relative Importance Values (RIV) of different plant species were carried out both at the rainy and dry seasons. The metal uptake and partitioning in plants at different seasons as well as proline content were also determined. The results showed that the Relative Importance Value (RIV), metal uptake and proline production by plants varied depending on the prevailing environmental conditions. During both sampling seasons, same plant species were enumerated on the contaminated site. However, their RIV varied in the two seasons. *Sporobolus. pyramidalis* was prevalent in the rainy season while *Imperata cylindrica* had the highest RIV during the dry season. Heavy metal accumulation in different plant species also differed depending on the sampling period and higher concentrations of metals were accumulated during the rainy season compared to the dry season except in *Gomphrena celosioides* with Pb being the highest at both seasons. The proline concentrations were also species and season dependent. The concentrations varied in different plant species and *G. celosioides* accumulated more during both seasons while *Eleucine indica* and *Rhyncospora corymbosa* had higher concentrations during the dry season compared to rainy season. Plant species abundance, metal uptake and osmolyte production by metallophytes are therefore dependent on the prevailing environmental conditions.

Key words: Metallophytes, Osmolyte, Heavy metals, Season, Proline, Vegetation

Introduction

Heavy metal (HM) contamination of agricultural lands reduces crop production and poses threat to food security. The use of green technology which is otherwise known as phytoremediation is now preferred to other methods for reclaiming metal-polluted sites (Mudgal *et al.,* 2010). It is cost effective and environment-friendly (Baker and Brooks 1989). Plant species growing naturally on metal-polluted soils are currently being studied for their potential uses in phytoremediation technology. These plants are generally tolerant to different abiotic stresses most especially, high HM concentrations and drought. They are able to colonize HM polluted environment and accumulate high concentrations of toxic metals in their tissues (accumulators). These make them important genetic resources for metal and drought tolerance studies (Baker and Brooks, 1989).

Identification of a plant species that is capable of accumulating high amounts of HM in their tissues irrespective of the season is however a required prerequisite for employing them for phytoremediation of contaminated soils (Garc´ıa *et al*., 2003; Tordoff *et al*., 2010). The species must also have the ability to efficiently tolerate high concentrations of metals within the plant tissues and translocate them to the shoot irrespective of the season (Pollard *et al*., 2002). More importantly, HM contamination induces a wide range of morphological, physiological and biochemical responses in plants (Apel and Hirt, 2004). Exposure to HMs has been reported to increase the production of Reactive Oxygen Species (ROS) such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, thereby causing imbalance in cellular metabolism which in turn leads to oxidative stress (Verma and Dubey, 2003). Lead (Pb), most especially has been reported to induce ROS production in plant (Reddy *et al*., 2005). Plants for phytoremediation must therefore have the capacity to scavenge these ROS at an efficient rate irrespective of the prevailing environmental conditions.

The ability to scavenge the toxic ROS through enhanced production of different metabolites/antioxidants has been reported to be the major strategy for the survival of many metallophytes on contaminated soil (Gill and Tuteja, 2010; Hossain *et al*., 2012). Among the antioxidant compounds that have been reported is Proline. The production of which is induced in HM and drought stressed plants (Xiang *et al*., 2001; Smirnoff, 2005; Athar *et al.*, 2008). Proline is considered as a potent antioxidant and osmoprotectant against metal toxicity (Ashraf and Foolad, 2007; Trovato *et al*., 2008). The enhanced synthesis of proline under abiotic stress has been implicated as a mechanism to alleviate cytoplasmic acidosis (Hare and Cress, 1997).

Meanwhile, osmolyte production is a factor of the metal concentration in the growing medium and the subsequent concentration in the plant. However, the bioavailability of metal ions depends on the prevailing environmental conditions. These also determine the plants' distribution and density. To be able to recommend any metallophyte for phytoremediation technology, its distribution, availability, metal uptake and distribution as well as its ability to produce osmoprotectants to scavenge ROS under different environmental factors and at different seasons must be well understood. In this study, the distribution, metal uptake and partitioning at different seasons as well as variations in concentrations of proline which is a popular osmolyte reported for tolerance to abiotic stress were investigated. The study of the behaviour of plants under natural conditions will give broad understanding of how changes in environmental conditions affect plants' distribution, metal uptake and metabolism.

Materials and methods

Experimental site and vegetation survey

The landfill site of lead-acid battery manufacturing company located at Lalupon in Ibadan, Nigeria was used for this study. The site was reported to contain 10-12% Pb apart from other heavy metals (Ogundiran, 2007). Vegetation enumeration was carried out during dry and rainy seasons November-December 2013 and April-June

2014, respectively using quadrat method. For the vegetation survey during the dry season, the site was divided into nine (9) quadrats while for the rainy season, it was divided into eleven (11) quadrats of 2m by 2m and the plant species rooting within each quadrat were identified to species level and each counted. The Relative Importance Value (RIV) of each plant species at each season was determined using the formula:

RIV (%) = [(RD+RF)/2] x 100;
where RD = Relative density; RF = Relative frequency
 - (Awodoyin and Ogunyemi, 2005; Adejumo *et al*., 2012).

Plant samples were also taken in triplicate for heavy metal analysis. Three heavy metals (Pb, Cr and Cd) in the shoot and root were analysed using the method described by Ogundiran and Osibanjo (2008). Translocation factor was also determined for each plant species at different sampling periods.

Laboratory Procedures

Determination of Proline contents

To determine the concentration of proline in different plant species growing on lead contaminated site, plants species were collected in triplicate. These were washed carefully and their leaves were used for osmolyte determination. Proline content was estimated by homogenizing leaf (0.5 g) in 3.0% (w/v) sulphosalycylic acid and the homogenate was filtered. From the filtrate, 2 ml was taken and mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. The mixture was boiled for 60 minutes in water bath, and then the reaction was stopped by placing in an ice bath. The mixture was separated by adding 4 ml of toluene, and the absorbance of the fraction with toluene separated from liquid phase was read at 520 nm using UV 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.

Statistical analysis

The data obtained were analysed by ANOVA using excel and SPSS (`17.0 version) and the means were separated using DMRT at 5% level of probability.

RESULTS

Dry and rainy season's vegetation survey

Eight plant species (*Sporobolus pyramidalis*, *Gomphrena celosioides*, *Rhynchospora corymbosa*, *Chromolaena odorata*, *Imperata cylindrica*, *Eleucine indica*, *Cynodon dactylon* and *Echinochloa obtusiflora*) were enumerated during the dry season. In the season, RIV of *S. pyramidalis* was the highest (23.7%) followed by *G. celosioides* (20.2%), *I. cylindrica* (18.8%), *C. dactylon* (14.3%), *E. indica* (8.5%), *C. odorata* (6.8%) and *R. corymbosa* (4.3%) while *E. obtusiflora* had the lowest RIV (4.3%). Similar plant species were also enumerated during rainy season. Variations were only observed in their RIV. The RIV for *S. pyramidalis* still ranked the highest (28.7%) followed by *G. celosioides*, *I. cylindrical* and *C. dactylon*. *E. obtusiflora* still had the least value (Table 1).

Table 1. The Relative Importance Values of plant species enumerated in the dry and rainy seasons in a heavy metal contaminated site in Ibadan, Nigeria.

Plant Species	Dry Season		Rainy Season	
	D/ m^2	RIV (%)	D/ m^2	RIV (%)
Gomphrena celosioides Mart.	230.4	20.2	619.1	22.1
Sporobolus pyramidalis P. Beauv.	225.7	23.7	704.5	28.7
Chromolaena odorata (L.) R.M. K&R	8.8	6.8	590.8	7.3
Imperata cylindrica (Linn.) Raeuschel	222.2	18.5	8.4	19.0
Rhynchospora corymbosa (Linn.) Britt.	18.9	8.6	513.6	2.7
Eleucine indica Gaertn.	4.4	4.1	5.0	4.1
Cynodon dactylon (Linn.) Pers.	194.4	14.4	34.7	13.5
Echinochloa obtusiflora Stapf.	6.7	4.1	10.0	1.3

RIV = Relative Importance Value, D= Density

Heavy metal concentration in different plant parts during dry season

Among the three heavy metals (Pb, Cd and Cr) considered, Pb was mostly accumulated by all the plant species. The highest Pb concentration was found in the shoot of *G. celosioides* (6249 mg/kg) followed by *E. indica* (2318 mg/kg) while the lowest was found in *C. dactylon* (40.4 mg/kg). Similarly, the highest lead concentration was also found in the root of *G. celosioides* (2134.8 mg/kg) while the lowest was found in *C. odorata* (39.2 mg/kg) (Fig 1a).

Compared to Pb, the accumulation of chromium, in the shoot of *C. dactylon, I. cylindrica, S. pyramidalis, E. indica* and *C. odorata* could be considered negligible with values that ranged between 0.00-0.01 mg/kg whereas. *G. celosioides, R. corymbosa* and *Echinochloa obtusiflora* had 0.21, 0.14 and 0.04 mg/kg respectively. The accumulation in the root was more in *Echinochloa obtusiflora* (0.36 mg/kg) compared to other plant species (Fig 1b).

Similarly, cadmium concentrations ranged from 0.00- 0.06 mg/kg in the shoot of most plant species except in *G. celosioides* and *R. corymbosa* that had 0.17 and 0.10 mg/kg respectively. The highest value was also found in the root of *G. celosioides* (0.13 mg/kg) followed by that of *Chromolaena odorata* (0.11 mg/kg) (Fig 1c).

However, for translocation factors, the highest value for Pb was recorded for *E. indica* (9.4) followed by those of *E. obtusiflora*, *G. celosioides*, *C. odorata* and *C. dactylon* having 3.5, 2.9, 1.65 and 1.26 respectively while *S. pyramidalis* had the lowest value of 0.12. With regards to Cr translocation factor, the highest value was found in *G. celosioides* (21) while *R. corymbosa*, *E. indica* and *Echinochloa obtusiflora* had 0.82, 0.125 and 0.11 respectively. The Cd translocation factors for *C. dactylon*, *I. cylindrica* and *S. pyramidalis* were not significant. The highest factor was found in *G. celosioides* and *R. corymbosa* with a value of 1 followed by that of *E. obtusiflora* (0.8), *Chromolaena odorata* (0.55) and *E. indica* (0.125) (Fig 1d).

Heavy metal accumulation in different plant parts during rainy season

The Pb concentration was also the highest during rainy season compared to the concentrations of other heavy metals, with cadmium being the least metal accumulated. Out of all the plants collected, *G. celosioides* also had the highest concentration of Pb in the shoot (4391.2

mg/kg) followed by *E. indica* (3138.8 mg/kg) and the lowest value was obtained in *Chromolaena odorata*. In the root, Pb concentration was more in *G. celosioides* (9404 mg/kg) compared to other plant species. This was followed by that of *E. indica* (4346 mg/kg), *S. pyramidalis* (2991.2 mg/kg), *Echinochloa obtusiflora* (2458.4 mg/kg) and *C. dactylon* (346 mg/kg) (Fig 2a).

For Cr, the accumulation was high in the rainy season with *E. indica* having the highest values both in the shoot (7.14 mg/kg) and in the root (20.32 mg/kg). This was followed by that of *I. cylindrica*. The Cr values in the shoot of other plant species ranged between 2.22 and 3.49 mg/kg. *Ryncospora corymbosa* and *G. celosioides* had the highest accumulation in the root (0.13 and 6.54 mg/kg respectively). The lowest concentration was found in the root of *Chromolaena odorata* (0.18 mg/kg) (Fig. 2b). Unlike Pb and Cr, the accumulation of Cd was found to be more in the shoot and root of *R. corymbosa* (10.67 and 5.30 mg/kg, respectively) and *G. celosioides* (2.50 and 3.80 mg/kg, respectively). The lowest Cd concentration was found in the shoot of *Chromolaena odorata* (Fig. 2c).

For translocation factor, the highest value for Pb was found in *G. celosioides* (0.47) and the least was obtained in *I. cylindrica* (0.21). The highest Cr translocation value was calculated for *Chromolaena odorata* (17.08) while *E. indica* had the lowest value (0.35). The translocation factor for Cd was the highest in *Rhyncospora. corymbosa* having 2.01 while *Echinochloa obtusiflora* had the lowest.(0.32) (Fig. 2d).

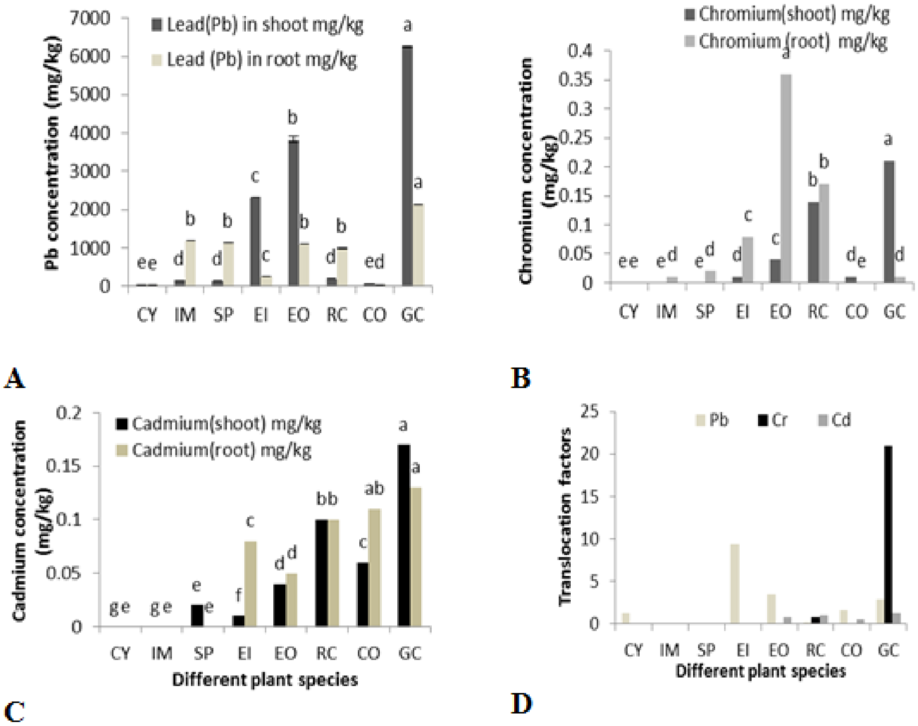

Figure 1. Heavy metal concentrations in the shoot and root of plant species (A – Pb, B- Cr and C - Cd) and their translocation factors (D) during the dry season, in Ibadan, Nigeria.
NB: CY= *C. dactylon*, IM = *I. cylindrica*, SP = *S. pyramidalis* GC= *G. celosioides*, CO = *Chromolaena odorata*, RC = *R. corymbosa*, EI = *E. indica* and EO = *Echinochloa obtusiflora*.
Bars carrying the same alphabet are not significantly different from one another using DMRT at 5% level of probability.

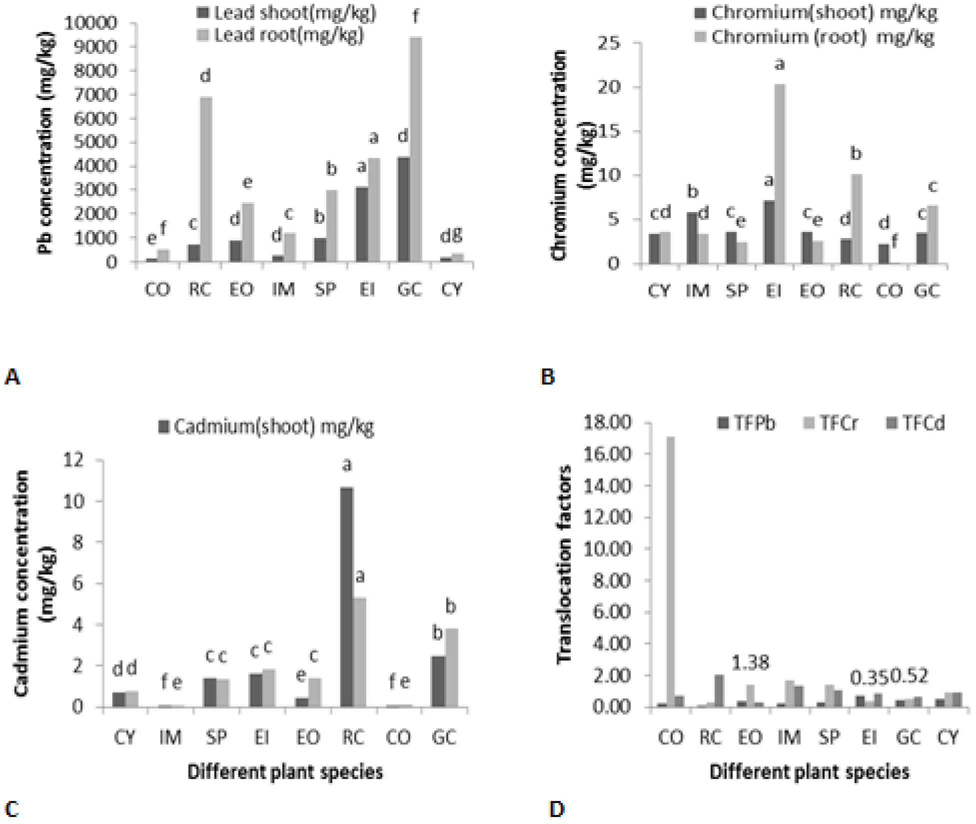

Figure 2. Heavy metal concentrations in the shoot and root of plant species (A – Pb, B- Cr and C - Cd) and their translocation factors (D) during the rainy season, in Ibadan, Nigeria.
NB: CY= *C. dactylon*, IM = *I. cylindrica*, SP = *S. pyramidalis* GC= *G. celosioides*, CO = *Chromolaena odorata*, RC = *R. corymbosa*, EI = *E. indica* and EO = *Echinochloa obtusiflora*.
Bars carrying the same alphabet are not significantly different from one another using DMRT at 5% level of probability.

Comparative analysis of Pb concentrations in the shoots and roots of different plant species at different sampling periods

Comparatively, the accumulation of Pb in the shoot of most plant species was enhanced during rainy season more than dry season except in *G. celosioides* and *Echinochloa obtusiflora* where the accumulation in the shoot during dry season was more than that of rainy season. Similarly, the root Pb concentration was more in all the plant species sampled during the rainy season than that of the dry season except for the *I. cylindrica*. It was observed that plant species that had lowest Pb accumulation during the dry season both in the root and shoot, had increased accumulation in the rainy season. However, the accumulation at both seasons in shoot and root were more in *G. celosioides* than in other plant species. This was followed by those of *E. indica* and *R. corymbosa* (Figure 3).

Proline concentrations in different plant samples collected from contaminated sites

Variations were observed in the proline concentrations depending on the plant species and sampling season. The proline concentration in all the plant species during the dry season were more than that of the rainy season except in *S. pyramidalis* and *I. cylindrica*. Higher concentration was found in *G. celosioides* followed by *E. indica* and *I. cylindrica* during rainy season while *Chromolaena odorata* had the lowest. The concentrations in *R. corymbosa and C. dactylon* were not different from each other but more than that of *S. pyramidalis and Echinochloa obtusiflora*.

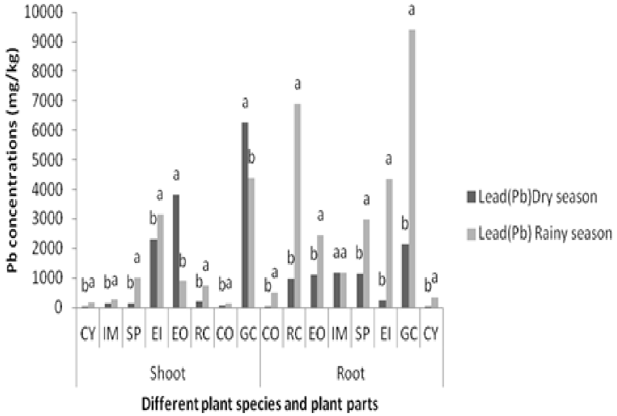

Fig. 3. Comparative analysis of Pb concentrations in the shoots and roots of plant species growing in heavy metal contaminated soil during the dry and rainy seasons in Ibadan, Nigeria.
NB: CY= *C. dactylon*, IM = *I. cylindrica*, SP = *S. pyramidalis* GC= *G. celosioides*,
CO = *Chromolaena odorata*, RC = *R. corymbosa*, EI = *E. indica* and
EO = *Echinochloa obtusiflora*. Bars representing either root or shoot for each plant species and are carrying the same alphabet are not significantly different from one another using DMRT

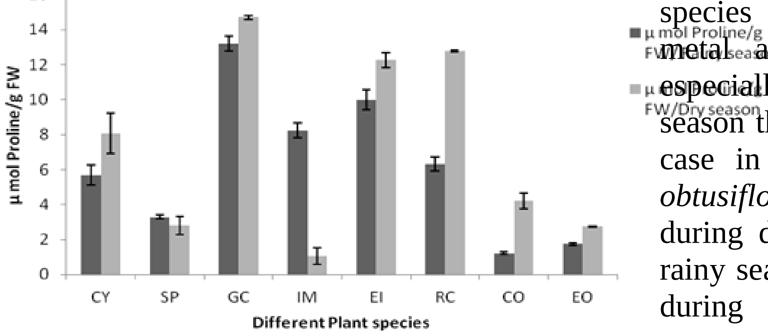

Figure 4. Proline concentrations in plant species growing on heavy metal contaminated sites during the rainy and dry seasons in Ibadan, Nigeria.

Discussion

The findings from this study showed that plant distribution and accumulation of ions are directly related to the prevailing environmental conditions. Though similar types of plant species were enumerated in the two seasons, their RIV varied. This shows that some of these plants are extremely tolerant to both drought and metal stresses while some are less tolerant. The highest concentration was still found in *G. celosioides* during the dry season compared to others. This was followed by the concentrations in *R. corymbosa* and *E. indica*. *Cynodon dactylon* had the lowest concentration in the rainy season (Figure 4).

The higher RIV during rainy season compared to the dry season was due to availability of water as plants generally thrive better and reproduce faster during rainy season. The *S. pyramidalis* with highest RIV during the two seasons coupled with its low metal accumulation at both seasons compared to other plant species may mean that it is drought and metal tolerant, but non-accumulator. *Gomphrena celosioides,* with its high RIV during the two seasons coupled with high metal accumulation, most especially Pb, could be described as a metal and drought tolerant plant as well as a good metal accumulator.
The accumulation of metals by different plant species varied with season, plant species and plant parts. In most species, metal accumulation in the shoot, most especially Pb, was more during the rainy season than dry season but reverse was the case in *G. celosioides* and *Echinochloa obtusiflora* where accumulation in shoot during dry season was more than that of rainy season. The increase in accumulation during rainy season in some of these species, was probably due to increase in solubility and availability of metal ions (Manab and Subodh, 2007). The effect of solubility factor on metal availability was more pronounced in the case of chromium. The results agreed with the previous reports where the levels of heavy metals in crops were found to be higher in the dry season than in the rainy season (Lee *et al*., 2001; Oluyemi *et al*., 2008). This, according to Oluyemi *et al*. (2008), was partly attributed to increase in transpiration rate during dry season which in turn enhances

metal/nutrient uptake. It was also proposed that high metal concentration in the plant tissues during dry season could be a kind of survival strategy being employed by these plants to maintain their osmotic potentials thereby preventing water loss during the dry season. The reduction in water content is always accompanied by increase in salt concentration in the growing medium and increase in the osmotic pressure of the cell sap which might have contributed to the accumulation of metals in plant tissues.

However, the contrary behaviour of *G. celoisoides*, having higher Pb concentration in shoot during dry season than rainy season, is typical of a good and effective hyperaccumulator and tolerant plant in which metal accumulation is not expected to be water-dependent. Carvalho *et al*. (2013) also reported t*hat Gomphrena spp* is an hyper-accumulator and tolerant plant. These plant species called accumulators have been reported to be secreting different chelating substances into the soil environment which aids in the dissolution of metal ions for easy uptake (Fahr *et al*., 2013). The ability of many metal accumulators in releasing chelating compounds into the rhizosphere for solubilization and uptake of metal has also been reported by Ma *et al (*2001). Many hyperaccumulator plants excrete organic acids which can facilitate metal uptake (Huang *et al.,* 1998) while non-accumulators can inhibit metal uptake by forming a complex with metal outside the root, thereby preventing its uptake (Pineros and Kochian, 2001; Murphy *et al*., 1999).

The increase in the shoot Pb concentration during the dry season in *G. celosioides* might also be due to reduction in metal precipitation (Lee *et al*., 2001). This is because heavy metals undergo reduction reactions during raining season with the availability of more hydrogen ions, hydride, sulfide and carbonate which are not soluble. Rainy season is therefore described as reducing condition and dry season as oxidizing condition. The precipitation resulting from formation of hydride, carbonate, sulfide and iron compounds could have decreased the availability of metals and hence lower metal accumulation by plants during rainy season (Kitagishi and Yamane, 1981; Dutta *et al*., 1989). Jung and Thornton (1997) reported that metal concentrations in rice stalks and leaves grown under oxidizing conditions are higher than those grown under reducing conditions. Kumar and Srikantaswamy, (2012) in their report concluded that the dilution effect on metal during rainy season could be responsible for reduction in the metal concentration in plant tissue.

Meanwhile, the general reduction in metal uptake by most plant species during dry season might be due to decrease in leaf growth, or increasing senescence of leaves under drought conditions which in turn might reduce photosynthesis and internal concentration of photosynthates (Dogan and Akıncı, 2011). Reduction in photosynthate means lower osmotic potential in the stressed plant and lower metal uptake (Brown *et al*., 2006). During dry season, some plant species have also evolved the mechanism of closing their stomatal thereby reducing the internal carbondioxide concentration and hence, decrease in photosynthesis.

The proline concentration which was more in all the plant species during the dry season than the rainy season corroborates previous reports that proline is an important osmoprotectant used by plants under extremely stressful conditions (Chaves and Oliveira, 2004; Gajewska and Sk1odowska, 2005; Ashraf and Foolad, 2007; Hoque *et al*., 2007; Kumar *et al*., 2010). Accumulation of solutes such as proline during the period of stress is otherwise known as osmotic adjustment and is a mechanism being displayed for survival under unfavourable weather conditions (Kumar *et al*., 2003; Mullineaux and Rausch, 2005; Tateishi *et al*.. 2005; Trovato *et al*., 2008). Osmotic adjustment has been found in many species and has been implicated in the maintenance of stomatal conductance, photosynthesis, leaf water volume and growth. It leads to rapid responses to decrease the effect of water stress. The greater capacity for osmoregulation ensures that growth continues under stress and at low water potential.

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