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Comparative Study on the Remediation Potential of *Panicum Maximum* and *Axonopus Compressus* in Zinc (Zn) Contaminated Soil

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ABSTRACT: Soil contamination by heavy metals has increased noticeably within the past years. Unlike organic compounds, metals cannot degrade; therefore effective cleanup is required to reduce its toxicity. This experiment was undertaken to investigate the comparative potential of *Panicum maximum* and *Axonopus compressus* to bioremediate zinc polluted soils, the impact of Zn on the antioxidant defense system of the plant, assaying for activities of antioxidants proteins. Zinc salts were mixed with soil at various concentrations 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg in triplicates and control was setup. After 4 months, the plants (root, shoot and leaf) and soil were analyzed for morphological, biochemical parameters and Zn concentration. The root length of *P. maximum* and *A. compressus* decreased as the concentration of zinc increased. The least shoot length inhibition of *A. compressus* was 6.16% (5 mg/kg) while the highest shoot length inhibition was 40.14% (40 mg/kg). The least shoot length inhibition of *Panicum maximum* was 6.16% exposed to 5 mg/kg and the highest shoot length inhibition was 53.13% (40 mg/kg). There was significant reduction of the heavy metals in vegetated soils for *P. maximum* and *A. compressus* at the end of the study compared to the heavy metals in the soils at the beginning of the study ($p<0.05$). *P. maximum,* is a better removal of Zn than *A. compressus*, however, it was not significant. Glutathione levels varied significantly ($p\leq 0.05$) with respect to heavy metals. *A. compressus* has more effects on Glutathione activities than *P. maximum*. Zn caused a decrease in metallothionein level in *P. maximum* while *A. compressus* metallothionein level increased.

Keywords: Remediation, Zinc, contaminated soil, enzymes, organic matter.

INTRODUCTION

Ecosystems are regularly confronted with natural environmental variations and disturbances over time and geographic space due to intense industrial activity and urbanization in recent times, especially in developing countries (Jadia, 2015). One of such disturbances is soil pollution by heavy metal, which refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Nagajyoti *et al.*, 2010). Soils may become contaminated by the accumulation of heavy metals in areas with high anthropogenic pressure (United States Environmental Protection Agency (Fazeli *et al.,* 2018).

The presence of toxic metals in soil can severely inhibit the biodegradation of organic contaminants (Eghbal et al., 2018). Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil, the food chain (soil-plant-

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human or soil-plant-animal-human), drinking of contaminated ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems (Roudposhti *et al.,* 2016). The most commonly heavy metals found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (Ghaemi et al., 2015).

Zinc is a transition metal, the 24th most abundant element in the earth crust and is an essential mineral of exceptional biologic and public health importance. Zinc plays a crucial role in biological processes of all living cells (Swarnalatha and Radhakrishnan 2015)). Because of its unique properties, zinc is used in wide range of consumer, infrastructure, agricultural, and industrial products Wuana and Okeimen, (2011). However, Zinc concentrations are rising unnaturally, because of anthropogenic additions, which have deleterious effect on the environment (Fazeli *et al.,* 2018). Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils. Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms, thus retarding the breakdown of organic matter (Greany, 2005)

Some plants however, can accumulate very high concentrations of metals in their tissues without-showing toxicity (Klassen *et al*., 2000; Bennett *et al*., 2003). These plants are called hyper-accumulators (Aluko *et al*., 2018). Such plants can be used successfully to clean up heavy metal polluted soils if their biomass and metal content are large enough to complete remediation within a reasonable period (Ebbs and Kochian, 1998). This ability of plants to hyper-accumulate heavy metals from contaminated soil is an emerging bioremediation techniques called phytoremediation (Dada *et al*., 2015). Phytoremediation is an integrated multidisciplinary approach to the cleanup of contaminated soils, which combines the disciplines of plant physiology, soil chemistry, and soil microbiology (Cunningham and Ow, 1996). It is cost-effective, simple, ecosystem friendly, and offers aesthetic advantages and long-term applicability (Njoku *et al*., 2012).

For Plants to be used for phytoremediation, such plants must (1) extract large concentrations of heavy metals into their roots, (2) translocate the heavy metal into the surface biomass, and (3) produce a large quantity of plant biomass. In addition, remediative plants must have mechanisms to detoxify and/or tolerate high metal concentrations accumulated in their shoots. The choice of *Panicum maximum* and *Axonopus compressus* in this study stems from the fact that grasses have multiple ramified root systems that give room for rhizospheric degradation (Njoku *et al*, 2016b; Iheme *et al*., 2017). Soil contamination by heavy metals is a worldwide problem; therefore, effective remediation approaches are necessary. Therefore, the present experiment was undertaken to investigate the potential of *Panicum maximum* and *Axonopus compressus* to bio remediate zinc polluted soils and the impact Zinc on the antioxidant defense system of the plant, measuring some protein and enzyme activities that play a major role in this defense.

MATERIALS AND METHODS

The soil used for this study was sandy loam soil from University of Lagos uncultivated rain forests, identified according to the method specified by the British Standard Institution (BSI) for soil tests for civil engineering purposes, BS1337: part 2 (1990). Tufts of *Axonopus compressus* and *Panicum maximum* were obtained from University of Lagos, Akoka. They were identified by Mr Nodza George of the Herbarium Unit of the Department of Botany, University of Lagos. These tufts were transplanted into loamy soil, watered regularly for 21 days. The heavy metal salt used in this study, Zinc (Zn) was purchased from Lazco international scientific and medical supplies Ltd. 14 Shiro street Fadeyi, Lagos Nigeria. The growth study was carried out in the botanical garden of University of Lagos (UNILAG). The heavy metal salts were mixed with soil at various concentrations 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg (Ali *et al*., 2009). Three replicates were made for each treatment combination and for the control setup too. Three young plants of 3cm were grown in the different concentration of Zn in soils. They were allowed to grow for four months and data collected were analyzed. Those that did not survive (such as those planted in 60 mg/kg and 80 mg/kg) were removed from the experiment.

Soil physicochemical parameters such as pH, organic matter, total organic carbon and cation exchange capacity of the experimental soil in the vegetated and none-vegetated soils were analyzed. The pH of the soil sample determined using the procedure of Soil Survey Staff, (2003). The total organic carbon was determined according to the procedure of Benard *et al*. (2004) using carbon analyzer. The organic matter content was determined using the loss-on-ignition method as described by Mucha *et al*. (2005). Cation exchange capacity was measured by ammonium acetate method at a pH 7 (Soil Survey Staff, 2003). Three replicates of each plant along with control were taken. After completion of the treatment (4 months), the plants were removed from polythene bags and their parts (root, shoot and leaf) separated. These parts were analyzed for morphological, biochemical parameters and heavy metals concentration. Soil samples were also analyzed for Zn concentration using Atomic absorption spectroscopy (Adesuyi *et al*., 2015). Plant height was calculated using meter rule while leaf area of the plants was calculated by measuring the length (L) and width (W) of the plants using the methods of Ogbuehi, *et al.* (2014). The relative concentrations of Zinc (Zn) in the soil, root, Stem, Leaf and shoot of *Axonopus compresus and Panicum maximum* at 120 days after planting were calculated as mean and standard error of the data obtained. The amount of lead (%) lost in the vegetated and none-vegetated soil was estimated as percentage loss of heavy metals in the soil.

Bioconcentration factor (BCF) = concentration of heavy metals in the roots/concentration of the respective soil (Yoon *et al*., 2006). Biological accumulation coefficient (BAC) is the metal $concentration\ in\ shoots/metal\ concentration\ in\ soil$. BAC factors greater than one (>1) indicates that the plant species has the ability to store metals from the soil into the shoots (Khan and Uzair, 2013). Translocation factor (TF) is the metal concentrations in the shoot/metal concentration in root. TF values greater than one (>1) indicate that the species has potential to accumulate heavy metals (Khan and Uzair, 2013). Determination of Reduced Glutathione was analyzed as described by Bulaj *et al*. (1998). Determination of metallothionein was done using the silver saturation method Scheuhammer and Cherian (1991). Total protein was determined by Biuret Method according the method of Al-Moaikal *et al.*, (2012). Glutathione S-transferase (GST) activity was calculated as described by Habig *et al*. (1974). Two-way Analysis of Variance (ANOVA) was employed to test the group means' differences with Turkey's multiple comparison tests was used to determine the significant variations among the means. Statistical significance differences was tested at $p<0.05$. All analyses were carried out, using SPSS 21.0.

RESULTS AND DISCUSSION

Plant roots serve the functions of anchorage, nutrients and water absorption for growth and development. Heavy metals are known to reduce and disturb root system (Singh *et al*., 2013). Zinc though an essential element for plant growth, however at higher concentrations, Zn shows toxicity symptoms that inhibiting root growth (Bradshaw, 1981 and Baker, 1978). The root length of both *P. maximum* and *A. compressus* generally decreased significantly at (P≤0.05) as the concentration of Zn in the soil increased (table 1). For *P. maximum* the root length was 35.00±2.65 cm (control) and it decreased to 20.67±0.68 cm in 40 mg/kg. Root length for *A. compressus* also decreased from 32.00±1.00 cm (control) to 15.000±1.53 cm in 40 mg/kg. Root inhibition was highest in *A. compressus* (53.13%) at 40mg/kg and in *P.maximum* was 40.94% in 40 mg/kg of Zn contamination. 5 mg/kg zinc contaminated soil had the lowest root inhibition of both plants *P. maximum* and *A.compressus* plants, respectively. The reduction in root length is due to the accumulation of heavy metal within the root system, (Barcelo and Poschenrieder, 1990) has reported that Zinc toxicity was marked in root system particularly in root blunt, thickening and caused restraint on both cell division and cell elongation due to accumulation of heavy metal within the root.) Arias *et al*. (2010) reported significantly inhibited root elongation in Mesquite (*Prosopis sp*.).

Shoot length was significantly difference for both plants *A. compressus* and *P.maximum* at the different levels of Zn contamination 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg p≤ 0.05).

The leaf area of *P. maximum* in Zn contaminated soil decreased from 49.55±0.99 cm^2 (control) to 30.89±4.11 cm^2 (37.66% reduction) in 40mg/kg contamination while the Leaf area of *A. compressus* decreased from 16.98±25.25 cm^2 (control) to 13.01±4.97 cm^2 (23.38% reduction) in 40mg/kg contamination. This reduction in leaf area was highest in *P. maximum* compared to *A. compressus*. This suggest that *A. compressus* is more susceptible to Zn toxicity than *P.maximum*.

The decrease in shoot length and leaf areas with increasing concentration of heavy metals may be due the fact that once heavy metals pass through the plasma membrane, they could immediately interact with all metabolic processes in the cytosol. Godbold and Huttermann (1985) reported that increasing zinc levels in culture solution decreased the shoot to root ratios and translocation of Zn, Fe, Mg, K, P and Ca and caused accumulation of these nutrients in the root. Pearson and Rengel (1995) indicated that higher concentration of zinc affected the leaf and the root morphology. They suggested that the zinc supply from the roots into the leaves of different ages might be determined by the relative transpiration rate of the leaves.

Table 1. Effect of the different concentration of Zn on root lengths, shoot lengths and leaf areas of *P. maximum* and *A. compressus*

Conc. Level (mg/kg)	Root Length (cm)		Shoot Length (cm)		Leaf Area (cm^2)	
	P. maximum	*A. compressus*	*P. maximum*	*A. compressus*	*P. maximum*	*A. compressus*
Control	35.00±2.65^a	32.00±1.000^a	75.17±2.13^a	16.00±0.58^a	49.55±0.99^a	16.98±25.25^a
5	33.67±1.20^{ab} (3.80%)	30.03±1.56^a (6.16%)	30.03±1.56^a (6.16%)	54.33±6.33^b (27.72%)	15.03±0.54^a (6.06%)	46.99±4.25^{ab} (5.17%)
10	26.67±1.20^b (23.80%)	27.67±1.53^b (13.53%)	47.43±2.12^b (36.90%)	12.67±1.86^b (20.81%)	41.78±1.86^b (15.68%)	15.46±0.31^a (8.95%)
20	26.00±3.22^b (25.71%)	20.33±2.91^c (36.47%)	46.90±8.39^b (37.61%)	11.50±3.01^b (28.13%)	38.22±4.99^{bc} (22.87%)	11.47±2.18^b (32.45%)
40	20.67±0.67^c (40.94%)	15.00±1.53^d (53.13%)	45.00±6.62^b (40.14%)	10.00±3.28^{bc} (37.50%)	30.89±4.11^c (37.66%)	13.01±4.97^b (23.38%)

Means with the same superscript along the column have no significant difference (p≤ 0.05).

Soil pH is known to affect plant uptake of most trace elements from soil by directly or indirectly influencing the sorption–desorption and complex formation (Kushwaha *et al*., 2015). Table 2 shows that pH value increased as the Zn concentration increases in the contaminated soil. Also there was increment in the pH of the non-vegetated heavy metals contaminated soil, however, further significant increase in the pH of zinc contaminated soils vegetated with the two grasses ($P<0.05$). Generally, *P. maximum* have more positive impact on soil pH than *A. Compressus*. This study corroborates the work of Javed (2011), that the shoots of *Elodea canadensis* and *Eriophorum angustifolium* roots cause an increase in the pH of the surrounding heavy metal contaminated medium.

The soil organic matter at the initial day (day 0) and final day (120 DAP) in the vegetated and non-vegetated soils is presented in Table 3. As the concentration of Zn added to the soil increased, initial soil organic matter decreased from 87.750±0.076 (control) to 84.89±0.052 (40 mg/kg lead contamination). The highest organic matter increase of *P. maximum* was 42.69% in 10 kg/mg zinc contamination and the highest organic matter increase of *A. compressus* was 36.69% in 5 mg/kg zinc contamination. There were significance difference between the organic matter content of *A. compressus* and *P. maximum* at the different contamination in the vegetated soils ($P\leq0.05$). The organic matter in the solid phase, especially the humic compounds of high molecular weight, strongly retains the metals in soils and reduces its availability (Ross, 1994). Hence, bioavailability of metals is inversely proportional to the organic matter in soils (Kushwaha *et al*., 2015). This finding corroborated that of Efe and Elenwo (2014) in their phytoremediating study of crude oil using *A. compressus* and ascribed the enhanced accumulation of organic matter to shielding of leaves from after the 90 days and the decomposition of such leaves increased the organic matter composition of the vegetated soil more than the non-vegetated soil.

Soil cation exchange capacity (Table 4) at the beginning of the study was generally and significantly lower than at the end of the study after 120 days ($p<0.05$). The final day (day 120) cation exchange capacity of the vegetated soils containing *P. maximum* and *A. compressus* respectively were significantly higher at the end of the study than the soils without plants (control) $p\leq0.05$. The highest impact of *P. maximum* and *A. compressus* on the cation exchange capacity of zinc contaminated soil was observed in 40 kg/mg contamination respectively. Also, there was significant difference between the contribution of both *P. maximum* and *A. compressus* to cation exchange capacity of the soils for each level of treatments ($p<0.05$). Hasegawa *et al*., (2016), states that cation exchange capacity (CEC) is a dominant factor in heavy metals retention, which depends on soil types, amounts, and types of different colloids present and on the CEC of the colloids. Fontes *et al*. (2000) reported that the capacity of soils for adsorbing heavy metals is correlated with their CEC, hence the greater the CEC values, the more exchange sites on soil minerals will be available for metal retention.

The quantity of zinc in the soils before and after the growth of the plants is presented in Table 5. The zinc level in the soil at day 0 was significantly higher than the zinc level in the soil without plant at the end of the study ($p<0.05$). Also, there was more significant reduction of the heavy metals in vegetated soils for both *P. maximum* and *A. compressus* at the end of the study compared to the to the heavy metals in the soils at the beginning of the study ($p<0.05$). *A. compressus* has a higher Zn removal potential than *P. maximum*, although, it was not significant. The study corroborates the study of Chijoke-Osuji *et al.*, (2017) that *Axonopus*

Table 2. Soil pH values of the initial day of treatment with Zn and the final day in the vegetated and none-vegetated soils.

Conc. Level mg/kg	Initial soil pH	Final pH in soil without plants (% change from initial after 120 days)	Final pH in soil with *P. maximum* (% change from initial after 120 days)	Final pH in soil with *A. compressus* (% change from initial after 120 days)
Control	6.697±0.064^{b}	6.913±0.029^{a} (3.20%)	6.777±0.022^{b} (2.01%)	6.750±0.020^{b} (2.41%)
5	6.507±0.015^{b}	6.667±0.015^{a} (2.40%)	6.760±0.025^{a} (1.38%)	6.820±0.035^{a} (2.24%)
10	6.457±0.018^{b}	6.697±0.023^{a} (3.58%)	6.737±0.032^{a} (0.59)	6.763±0.063^{a} (0.98%)
20	6.423±0.003^{b}	6.603±0.009^{a} (2.73%)	6.693±0.009^{a} (1.34%)	6.733±.0.064^{a} (1.93%)
40	6.403±0.007^{c}	6.533±0.013^{b} (1.99%)	6.650±0.021^{a} (1.76%)	6.653±.0.026^{a} (1.80%)

Means with the same superscript along the row have no significant difference ($p \leq 0.05$).

Table 3. Effects of *P. maximum* and *A. compressus* on the soil total organic matter content of the Zn contaminated soils

Conc. Level (mg/kg)	Initial soil total organic matter content (%Change)	Final total organic matter content in soil without plants (% change from initial after 120 days)	Final total organic matter content in soil with *P. maximum* (% change from initial after 120 days)	Final total organic matter content in soil with *A. compressus* (% change from initial after 120 days)
Control	87.750±0.076^{a}	7.730±0.096^{c} (91.19%)	9.527±2.182^{c} (18.865%))	17.583±1.458^{b} (56.04%)
5	86.013±0.148^{a}	8.673±1.935^{b} (89.92%)	10.800±0.824^{b} (19.69%)	13.700±1.137^{b} (36.69%)
10	85.693±0.226^{a*}	4.883±1.507^{b*} (93.68%)	8.520±1.417^{b} (42.69%)	8.067±2.085^{b} (2.06%)
20	84.893±0.052^{a*}	12.300±1.400^{b*} (85.51%)	8.863±2.472^{b} (38.78%)	10.100±0.758^{b} (21.78%)
40	84.893±0.052^{a*}	12.300±1.400^{b*} (85.51%)	11.937±1.783^{b} (-3.04%)	6.167±2.009^{b*} (-99.45%)

Means with the same superscript along the row have no significant difference while asterisk have significant difference between the treatments ($p \leq 0.05$)

Table 4. Effects of *P. maximum* and *A. compressus* on soil cation exchange capacity of the Zn contaminated soils

Conc. Level (mg/kg)	Initial soil cation exchange capacity	Final cation exchange capacity in soil without plants (% change from initial after 120 days)	Final cation exchange capacity in soil with *P. maximum* (% change from initial after 120 days)	Final cation exchange capacity in soil with *A. compressus* (% change from initial after 120 days)
Control	16.000±0.116^{b}	192.877±0.007^{a} (1105.48%)	257.157±64.287^{a} (1507.23%)	225.013±2.133^{a} (1306.33%)
5	38.747±1.006^{b*}	225.013±0.000^{a} (480.72%)	272.800±0.000^{a} (604.06%)	235.013±2.143^{a} (506.53%)
10	43.747±0.081^{b*}	230.870±3.143^{a} (427.74%)	282.800±0.000^{a} (546.44%)	245.133±3.430^{a} (460.34%)
20	43.413±0.058^{b*}	257.157±0.000^{a} (492.35%)	292.700±0.000^{a} (574.22%)	262.700±0.000^{a} (505.12%)
40	43.007±0.094^{b*}	277.567±2.143^{a} (545.40%)	292.870±0.000^{a} (580.98%)	265.133±2.433^{a} (516.49%)

Means with the same superscript along the row have no significant while asterisk have significant difference within the column ($p \leq 0.05$)

compressus has the tenacity to withstand the deleterious effects of pollutants such as waste engine oil contamination.

The relative reduction of Zn in the different soils planted with the respect to the soil without plant is shown in Figure 1. Both *A. compressus and P. maximum* reduces the level of Zinc in the soil and they were significant for all the contamination level ($p<0.05$). The growth of *A.compressus* led to highest reduction of Zn in all the degree of contaminations with the highest (74.10%) in 40 mg/kg. This study corroborates the report of USEPA (2000) and Chijoke-Osuji *et al*. (2017) who observed that plants of the grass family (Poacea) are particularly suitable for phytoremediation because of their multiple ramified root systems.

The results of Table 6 shows that at 120 days after planting, the accumulation of Zn in the roots and shoots of *A. compresus and P. maximum* increased at higher concentrations of Zn in the soils. At higher concentrations of Zn in the soil, the accumulation of Zn in *A.compresus and P. maximum* increased significantly from that of the control ($p<0.05$). Also, accumulation of Zn in the roots was significantly higher than in the other parts of the plants. *A.compresus* had the highest accumulation of Zn in the root (4.615±0.041) at 40mg/kg zinc contamination. *P.maximum* shoot had the highest accumulation in 40mg/kg zinc contamination and same with *A.compresus* shoot having the highest accumulation of zinc in 40mg/kg zinc contamination. The finding of the present study of heavy metal accumulation in soil and different plant parts corroborate the findings of Baker, (1978) and Mani *et al*. (2015) that zinc is accumulated to a high degree in the roots relative to the shoots. The translocation of zinc ions to the aerial tissues occurs because with plant development, root endoderm may become weak barrier. For this reason, metals easily penetrate xylem and then the above-ground parts of plants. Consequently, plants accumulate higher levels of metal in the roots with slow translocation to the shoots.

Table 5. Zinc level in the soils before and after the growth of the plants

Conc. Level (mg/kg)	Initial day	Final day without plant	Final day with *A. compressus*	Final day with *P. maximum*
Control	1.070±0.006[a]	0.211±0.001[b]	0.113±0.001[b]	0.111±0.0003[b]
5	6.930±0.017[a*]	6.311±0.006[a*]	0.240±0.001[b]	0.401±0.001[b*]
10	12.420±0.012[a*]	11.960±0.029[a*]	0.318±0.003[c]	1.094±0.264[b*]
20	24.680±0.012[a*]	23.640±0.029[a*]	0.873±0.059[c*]	2.328±0.046[b*]
40	47.240±0.023[a*]	46.050±0.029[a*]	1.627±0.082[c*]	3.425±0.318[b*]

Means with the same superscript have no significant difference along the row ($p\leq 0.05$) and means with asterisk have significance difference with the control along the column

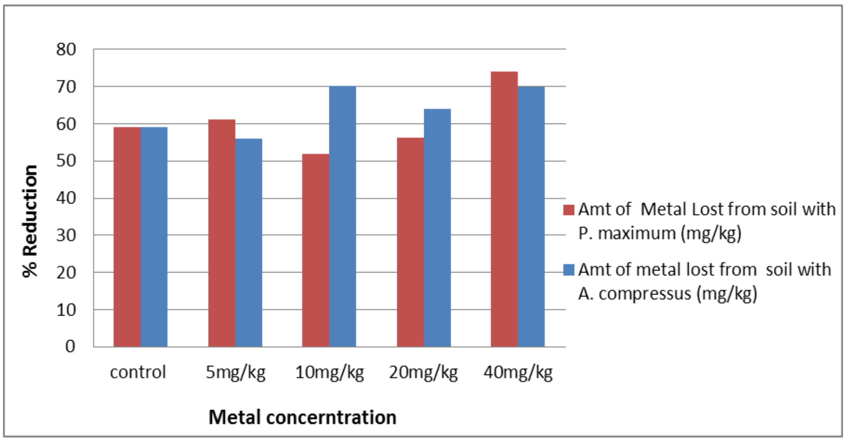

Fig. 1. Relative reduction of Zinc and Lead from the soil by the plants

Table 6. The relative concentrations of Zinc (Zn) in the soils, roots and shoots of *Axonopus compresus and Panicum maximum* at 120 days after planting

Conc. Level (mg/kg)	Root Length (mg/kg)		Shoot Length (mg/kg)		Leaf Area (mg/kg)	
	P. maximum	*A. compressus*	*P. maximum*	*A. compressus*	*P. maximum*	*A. compressus*
Control	0.111±0.000[a]	0.113±0.001[a]	0.022±0.007[a]	0.023±0.004[a]	0.020±0.007[a]	0.033±0.015[a]
5	0.401±0.001[c*]	0.240±0.001[d]	0.727±0.007[bc*]	0.400±0.008[c]	1.127±0.041[b*]	1.828±0.058[b*]
10	1.094±0.264[c*]	0.318±0.003[c]	1.239±0.020[c*]	0.489±0.008[c]	1.764±0.007[b*]	2.465±0.042[b*]
20	2.328±0.046[d*]	0.873±0.059[c*]	2.805±0.054[c*]	1.111±0.076[c*]	4.514±0.070[b*]	5.286±0.007[b*]
40	3.425±0.318[d*]	1.627±0.0819[d*]	4.615±0.041[c*]	3.311±0.167[c]	5.509±0.009[b*]	7.179±0.130[b*]

Means with the same superscript have no significant difference along the row ($p \leq 0.05$) and means with asterisk shows significance difference with the control along the column

The biological concentration factor (BCF), biological accumulation coefficient (BAC) and translocation factor (TF) of *Axonopus compressus* and *Panicum maximum* of zinc contaminated soil is presented in Table 7. *Axonopus compresus* has the highest biological accumulation coefficient (BAC) value in 5mg/kg Zinc contamination (2.663) and the least biological accumulation coefficient (BAC) was in *Panicum maximum* (1.551) in 5mg/kg Zinc contaminated soils. *Axonopus compresus* also had the highest biological concentration factor (BCF) value (2.035) in 40mg/kg Zinc contamination. The two plants showed variation in their translocation factor (TF) level greater than one. This implied that the two plants are efficient in translocating Zn and Pb though *Panicum maximum* does it better. There was also variation in the biological concentration factor (BCF) level across the different contamination treatments. This can be concluded that both plants have good potential to remediate heavy metal, though *A.compressus* does it better compared to *P.maximum*.

The activities of Glutathione (GSH) in the different part of the plants are presented in Tables 8. Glutathione levels varied significantly ($p \leq 0.05$) with respect to concentration of heavy metals as well as different part of the plants. *A. compressus* has more effects on the Glutathione activities than *P. maximum*. Glutathione is a low molecular weight, water soluble thiol compound distributed widely in plants tissues, responsible for the formation of phytochelatins (PCs) that binds heavy metals for safe transport and sequestration in the vacuole. (Sharma and Dietz, 2006). It also plays a vital role in detoxifying heavy (Srivalli and Khanna-Chopra 2008) GSH levels in plants are known to change under metal stress (Koricheva *et al*., 1997; Sarma, 2011). One major consequence of Zn toxicity is the excess generation of ROS and subsequent oxidative stress (Hasanuzzaman and Fujita 2013). Glutathione presence can protect the plant cells from metal toxicity by direct quenching of ROS, conjugation of toxic metals and other xenobiotics to GST; and or acting as a precursor for the synthesis of phytochelatins (PCs) (Sarma, 2011). This result of this study was similar to that of Ruley *et al*. (2004) who observed Glutathione content in *Sesbania drummondii* plant to significantly increase upon exposure to Pb.

Table 7. Biological concentration factor (BCF), Biological accumulation coefficient (BAC) and Translocation factor (TF) of Axonopus compressus and Panicum maximum of Lead

Conc. Level (mg/kg)	*Axonopus compressus*			*Panicum maximum*		
	BAC	BCF	TF	BAC	BCF	TF
0	2.000	1.029	1.943	1.914	1.094	1.914
5	2.663	1.663	1.601	1.551	1.812	1.551
10	2.537	1.537	1.651	1.882	1.133	1.882
20	2.273	1.273	1.786	1.830	1.205	1.830
40	3.035	2.035	1.491	1.742	1.348	1.742

Table 8. Glutathione level in the plants parts at varying Pb concentration at 120 days after planting (µmol/ml)

Conc. Level mg/kg	Root		Stem		Leaf	
	P. maximum	*A. compressus*	*P. maximum*	*A. compressus*	*P. maximum*	*A. compressus*
Control	0.030±0.000[b]	0.024±0.0010[b]	0.027±0.0003[b]	0.064±0.001[a]	0.025±0.000[b]	0.0930±0.000[a]
5	0.071±0.000[a*] (133.33%)	0.011±0.000[b*] (54.17%)	0.035±0.000[b] (29.63%)	0.026±0.000[b*] (59.38%)	0.028±0.000[b] (12.00%)	0.020±0.000[b*] (78.49%)
10	0.016±0.000[b*] (46.67%)	0.063±0.000[a*] (162.50%)	0.023±0.000[b] (14.81%)	0.018±0.000[b*] (71.88%)	0.056±0.000[a*] (124.00%)	0.051±0.000[a] (45.16%)
20	0.047±0.000[a*] (56.67%)	0.055±0.000[a*] (129.17%)	0.015±0.000[b*] (44.44%)	0.056±0.000[a] (12.50%)	0.027±0.000[b] (8.00%)	0.048±0.000[a] (48.39)
40	0.043±0.000[a*] (30.23%)	0.020±0.000[b*] (16.67%)	0.026±0.000[b] (3.70%)	0.025±0.000[b] (60.94%)	0.055±0.000[a*] (54.55%)	0.045±0.000[a*] (106.67%)

Means with the same superscript along the row have no significant difference while asterisk shows significant difference between control and treatments down the column (p≤ 0.05).

Table 9. Metallothionein level in the plants parts at varying Zinc (Zn) concentrations

Conc. Level mg/kg	Root		Stem		Leaf	
	P. maximum	*A. compressus*	*P. maximum*	*A. compressus*	*P. maximum*	*A. compressus*
Control	32.187±0.853[b]	23.443±1.106[b]	4.357±0.954[c]	62.337±0.331[a]	29.537±0.3491[b]	96.050±0.466[a]
5	47.960±0.056[a*] (49.00%)	52.860±0.063[a*] (54.86%)	53.653±0.052[a*] (1130.96%)	58.290±0.064[a] (6.49%)	12.567±0.2405[b*] (57.40%)	43.250±0.113[a*] (54.97%)
10	1.857±0.023[b*] (94.23%)	26.923±0.093[b] (14.84%)	6.270±0.032[ab] (43.91%)	44.8500±0.3118[a] (28.05%)	21.350±0.032[b] (27.72%)	22.367±0.205[b*] (76.71%)
20	10.960±0.139[b*] (65.95%)	15.1500±0.3118[b] (35.38%)	31.843±0.127[a*] (630.85%)	33.017±0.094[a*] (47.03%)	27.597±0.124[a] (6.57%)	31.657±0.093[a*] (67.04%)
40	10.403±0.194[b*] (67.67%)	1.1500±0.0656[b*] (95.09%)	6.997±0.114[b*] (308.47%)	26.813+0.120[a*] (56.99%)	6.960±0.129[b*] (76.44%)	24.827±0.209[a*] (74.15%)

Means with the same superscript along the row have no significant difference while asterisk shows significant difference between control and treatments down the column (p≤ 0.05).

Plant metallothioneins (MTs) are a group of small proteins containing 61-68 amino acids that play a major role in heavy metal detoxification, synthesized due to mRNA translation (Ojuederie and Babalola, 2017). Metallothionein levels differed significantly among the plant parts and at the treatment levels (p<0.05). Zinc caused an increase in the metallothionein level (49.00%) in *P. maximum* and in *A. compressus (*54.86%) at 5% treatment in the root. This increase in metallothionein level in the test plants helps to improve their tolerance to heavy metal (Du *et al*., 2012). The involvement of MTs in response to plant water stress and recovery was assessed by analyzing gene expression in leaves and the cambial zone of white poplar. Expression of *Populus alba* MT2a and MT3a in leaves and roots was higher as water stress increased (Street *et al*., 2006; Bogeat *et al*., 2007)

Proteins are important constituents of the cell; however, under a stressed environmental condition they can be easily denatured. Hence, any change in these compounds can be considered an important indicator of oxidative stress in plants. Total protein levels in the plants parts at varying Zn concentration at 120days after planting are shown in Table 11. There was significant difference between the total protein level in the different parts of the plants and treatment levels (p≤ 0.05). Treatment at 5mg led to an increase in total protein levels in the different plants. In root of *P .maximum*, Zn contamination caused an increase in total protein levels from 23.197±0.2120 µmol/ml in control to 66.680±104 µmol/ml in 5mg treatment and in *A. compressus* protein levels increased from 15.367±0.220 µmol/ml in control to 43.663±0.161 µmol/ml in 5mg/kg treatment. However there was a significant reduction in protein level at 40mg/kg treatment (7.410±0.216 µmol/ml). Low concentrations of zinc increase total protein content the most (Pourrut *et al*., 2011) as observed in the 5 and 10 mg/kg contamination. This protein accumulation may defend the plant against lead stress (Gupta *et al*., 2010), particularly for proteins involved in cell redox maintenance. Thus, such proteins act in a way similar to how ascorbate functions or similar to how metals are sequestered by glutathione (GSH) (Liu *et al*., 2010). It has been reported that Zn is able to decrease protein content by inhibiting the uptake of Mg and K ions and promote post translational modification (Pant and Tripathi, 2014).

Glutathione *S*-transferases (GSTs) are multifunctional proteins encoded by a large gene family that is found in most organisms. As classical phase II detoxification enzymes, GSTs mainly catalyze the conjugation of reduced glutathione (GSH) with a wide variety of reactive electrophiles (Hayes *et al*., 2005). GST proteins are involved in several crucial physiological and developmental processes, including xenobiotic (e.g., herbicides) detoxification, signal transduction, isomerization, and protection against oxidative damages, UV radiation, and heavy metal toxins (He *et al*., 2016). Analysis of Glutathione S-transferase activity in the different parts of *Axonopus compresus and Panicum maximum* in the Zn contaminated soil showed significant stimulation (P<0.05) with this activity decreasing with concentrations of the metal. These results were consistent with previous research in which Pb were found to induce GST expression in *Salicornia iranica* (Kaviani *et al*., 2017).

Table 10. Total protein level in the plants parts at varying Zn concentration

Conc. Level mg/kg	Root		Stem		Leaf	
	P. maximum	***A. compressus***	***P. maximum***	***A. compressus***	***P. maximum***	***A. compressus***
Control	23.197±0.2120^{a}	15.367±0.220^{ab}	10.990±0.120^{b}	9.777±0.219^{b}	16.337±0.162^{b}	13.297±0.278^{b}
5	66.680±104^{a*} (187.45%)	43.663±0.161^{a*} (184.13%)	29.233±0.237^{b*} (166.00%)	12.570±0.1034^{b} (28.57%)	60.910±0.159^{a*} (272.83%)	14.757±0.1067^{b} (10.980%)
10	73.233±278^{a*} (215.70%)	26.233±0.107^{b*} (70.71%)	4.093±0.030^{c*} (62.76%)	14.570±0.000^{b} (49.02%)	14.150±0.159^{b} (13.387%)	51.190±0.104^{a*} (284.97%)
20	89.570±216^{a*} (286.13%)	40.503±0.265^{b*} (161.30%)	3.847±0.073^{c*} (65.00%)	4.550±0.000^{b*} (53.46%)	93.580±0.340^{a*} (472.81%)	19.373±0.597^{c} (45.69%)
40	89.697±336^{a*} (286.68%)	7.410±0.216^{c*} (51.78%)	2.117±0.156^{c*} (137.64%)	16.883±0.264^{c*} (72.17%)	48.157±0.220^{b*} (238.58%)	13.540±0.120^{c} (1.83%)

Means with the same superscript along the row have no significant difference while asterisk shows significant difference between control and treatments down the column (p≤ 0.05).

Table 11. GST level in the plants parts at varying Zn concentration at 120days

Conc. Level mg/kg	Root		Stem		Leaf	
	P. maximum	***A. compressus***	***P. maximum***	***A. compressus***	***P. maximum***	***A. compressus***
Control	2.907±0.032^{b}	4.913±0.073^{b}	7.487±0.072^{a}	2.980±0.021^{b}	7.010±0.059^{a}	2.993±0.054^{b}
5	4.000±0.006^{b*} (37.60%)	9.567±0.242^{a*} (94.73%)	2.140±0.006^{b*} (71.42%)	3.370±0.035^{b*} (13.09%)	3.543±0.007^{b} (49.46%)	3.473±0.023^{b} (16.04%)
10	3.660±0.015^{c*} (25.90%)	3.783±0.023^{c} (23.00%)	3.987±0.020^{c*} (46.75%)	19.573±0.038^{a*} (556.81%)	12.700±0.134^{a*} (81.17%)	8.243±0.250^{b*} (175.01%)
20	2.890±0.006^{a} (5.88%)	2.503±0.003^{a*} (49.05%)	3.757±0.015^{a} (48.82%)	2.183±0.022^{a*} (26.74%)	1.527±0.007^{b*} (78.22%)	3.427±0.009^{a} (14.50%)
40	2.723±0.127^{b} (6.33%)	2.457±0.007^{b} (49.99%)	9.740±0.064^{a*} (30.10%)	1.087±0.017^{b} (63.52%)	0.707±0.015^{c*} (89.91%)	1.657±0.023^{b*} (44.64%)

Means with the same superscript along the row have no significant difference while asterisk shows significant difference between control and treatments down the column (p≤ 0.05).

CONCLUSION

Metal toxicity issues in plants and soils are of significant environmental due to growing anthropogenic pressure on the environment. Zinc acts as a plant nutrient but at higher concentrations it is toxic. Some plants can accumulate very high concentrations of metals in their tissues without-showing toxicity, such plants serves as bio agents for the bioremediation of heavy metals form contaminated soil. *Axonopus compresus and Panicum maximum* growing on Zinc polluted soils show a slight reduction in growth due to changes in their physiological and biochemical activities. However, *Axonopus compresus and Panicum maximum* both significantly reduced greater percentage of Zn in the polluted soil. This study suggests *Axonopus compressus* to have greater impacts on Zn polluted soil than *Panicum maximum*. *A. compressus* is a better removal of Pb than *P. Maximum.* However, both *Axonopus compresus and Panicum maximum* have the tenacity and phytoremediation capacity to remediate Zn in soil effectively.

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