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COMPARATIVE STUDY ON THE GROWTH RESPONSE AND REMEDIATION POTENTIAL OF PANICUM MAXIMUM AND AXONOPUS COMPRESSUS IN LEAD CONTAMINATED SOIL

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Abstract

The global problem concerning contamination of the environment as a consequence of heavy metals is on the increase. Soil contamination by heavy metals is a worldwide problem, therefore effective remediation approaches are necessary. Some plants can absorb these toxic metals and help to clean them up from the soil and sediment. This fact may be useful for developing rational forms of environmental safety management and innovative technology which more efficiently clean soils and improve their ecological condition with for agriculture. Phytoremediation is known as an eco-friendly and cost-effective way of reducing pollutants from the soil. Therefore, the present experiment was undertaken to investigate the comparative potential of two grasses, *Panicum maximum* and *Axonopus compressus* to bioremediate lead polluted soils. In addition, the impact of Pb on the antioxidant defense system of the plants was studied. $Pb(NO_3)_2$ salts were mixed with soil at various concentrations 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg in triplicates and control experiment was also setup. After 4 months, the plants were removed and their parts (root, shoot and leaf) separated. They were analysed for morphological, biochemical parameters and Pb concentration. Soil samples were also analyzed for Pb. The root length of both *P. maximum* and *A. compressus* generally decreased as the concentration of Pb in the soil increased. The least shoot length inhibition of *A. compressus* was 7.13 % (5 mg/kg) while the highest shoot length inhibition was 36.29 % (40 mg/kg). The least shoot length inhibition of *P. maximum* was 10.51 % exposed to 5 mg/kg and the highest shoot length inhibition was 42.46 % (40 mg/kg). 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* is a better removal of Pb than *P. maximum*, however, it was not significant. Glutathione (GSH) 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*. Pb caused a decrease in the metallothionein level (10.11 %) in *P. maximum* while *A. compressus* metallothionein level increased by 116.10 % in 5 % treatment.

Keywords: contaminated soil; heavy metals; phytoremediation; environmental safety control.

1. Problem statement and Analysis of the recent researches and publications

Heavy metals are very common contaminants in the environment [1–3]. The widespread contamination of soil with heavy metals represents currently one of the most severe environmental problems that can seriously affect environmental quality and human health [4, 5]. Unlike certain metals such as copper (Cu), zinc (Zinc) and manganese (Mn), which are essential for various physiological processes of plants, lead (Pb) is a highly toxic metal pollutant, which interferes with the plant metabolic processes [6]. Among pollutants, As, Pb, Cd and Hg are included in the top 20 Hazardous Substances of the Agency for Toxic Substances and Disease Registry [7] and the United States Environmental Protection Agency [8]. The adverse environmental impacts from excessive heavy metals include contamination of water and soil, phytotoxicity, soil degradation [9, 10] and pose serious risks to human health [11].

Lead, is well known metal that inhibit some metabolic activities in the plants such as the biosynthesis of nitrogenous compounds, carbohydrate metabolism and water absorption [12]. Pb contamination in soils induced changes in soil microorganisms and their activities resulting in low soil fertility and also directly affects the change of physiological indices, hence, resulting in yield decline [13]. Some plants can absorb these toxic metals and help to clean them up from the soil and sediment. These plants are called hyper-accumulators [14]. The plants have shown resistant to heavy metals toxicity and are capable of accumulating them. This fact may be useful for developing rational forms of environmental safety management and innovative technology which more efficiently clean soils and improve their ecological condition with for agriculture.

The remediation techniques of heavy metals are classified in biological (biodegradation by living organisms), chemical (chelators, chemical immobilization, oxidation, etc.) and physical (electrokinetic remediation, incineration technologies, soil washing, stabilization/solidification, thermal desorption etc.) remediation techniques [15]. However, all of them are expensive, time-consuming and environmentally destructive. Therefore, effective cleanup requires their removal/immobilization to reduce or remove toxicity [16]. Bioremediation is one of the most viable options for remediating soil contaminated by organic and inorganic compounds considered detrimental to environmental health. Bioremediation is a process that makes use organisms to detoxify organic and inorganic xenobiotics from the environment [17]. It is an option that offers green technology solution to the problem of hydrocarbon and heavy metals contamination [18].

Phytoremediation is the use of plants for in situ remediation of contaminated soil, sludge, and groundwater through contaminant removal or containment [19]. It is an emerging technology for cleaning up contaminated sites. It is cost-effective, simple, ecosystem friendly, and offers aesthetic advantages and long-term applicability [20]. The choice of *Panicum maximum* (*P. maximum*) and *Axonopus compressus* (*A. compressus*) in this study stems from the fact that grasses have multiple ramified root systems that give room for rhizospheric degradation [21, 22]. 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 *P. maximum* and *A. compressus* to bioremediate lead polluted soils and the impact Pb on the antioxidant defense system of the plant, measuring some protein and enzyme activities that play a major role in this defense.

2. Statement of the problem and its solution.

2.1. Materials and methods.

Samples and sources

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 *A. compressus* and *P. maximum* were obtained from University of Lagos, Akoka. Dr Nodza George of the Herbarium Unit of the Department of Botany, University of Lagos, identified them. These tufts were transplanted into loamy soil, watered regularly for 21 days.

Experiment site and treatment applications. The growth study was carried out in the botanical garden of University of Lagos (UNILAG). The $Pb(NO_3)_2$ was purchased from Lazco international scientific and medical supplies Ltd. 14 Shiro street Fadeyi, Lagos Nigeria. This was mixed with soil at various concentrations 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg [23]. Three replicates were made for each treatment combination and for the control setup too.

Three young plants of 3 cm were grown in the different concentration of Pb in soils. They were allowed to grow for four months and data collected were analysed. Those that did not survive (such as those planted in 80 mg/kg) were removed from the experiment.

Analysis of the Experimental Soil. Soil physicochemical parameters such as pH, organic matter, total organic carbon and cation exchange capacity of the vegetated and unvegetated soils were analysed. The pH of the soil sample determined using the procedure of [24]. The total organic carbon was determined according to the procedure of [25] using carbon analyzer. The organic matter content was determined using the loss-on-ignition method as described by [24]. Cation exchange capacity was determined using the procedure of [26].

Measurements were done in 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 Pb concentration. Soil samples were also analyzed for Pb concentration using Atomic absorption spectroscopy [1].

Determination of Plants Growth Performance. 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 [27].

The concentrations of lead (Pb) in the soil and plants parts. The concentration of Pb was determined using atomic absorption spectroscopy (AAS) following the procedure described by [28]. The concentrations of lead (Pb) in the soil, root, Stem, Leaf and shoot of *A. compressus* and *P. 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 unvegetated soil was estimated as percentage loss of heavy metals in the soil.

Biological accumulation coefficient (BAC) and Translocation factor (TF) of A. compressus and P. maximum of lead. 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 [29]. Transolcation 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 [29].

Enzyme and protein analysis. Determination of Reduced Glutathione was analysed as described by [30]. Determination of metallothionein was done using the silver saturation method [31]. Total protein was determined by Biuret Method according the method of [32]. Glutathione S-transferase (GST) activity was calculated as described by [33].

Statistical Analyses. 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.

2.2. Results and discussion.

2.2.1. Effect of the different concentration of Pb on root lengths, shoot lengths and leaf area of P. maximum and A. compressus. Roots are positively geotropic and negatively phototropic. Their function is to fix the plant and absorb the nutrients and water for growth and development. Heavy metals are known to reduce and disturb root system [34]. The root length of both *P. maximum* and *A. compressus* generally decreased as the concentration of lead (Pb) in the soil increased (table 1). These differences in shoot length were significant for both *P. maximum* and *A. compressus* at 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg of lead contamination ($p < 0.05$). This may be due to the accumulation of heavy metals in the plant roots [12]. The highest root inhibition was observed in *P. maximum* while the least was observed in *A. compressus*. The reduction in root length due to accumulation of metals within the root reduces the rate of mitosis in the meristematic zones of roots, especially by blocking the metaphase in meristemetic cells. Therefore, root showed reduction in length as also observed by [35]. In work

[36] reported significantly inhibited root elongation in Mesquite (Prosopis sp.).

Shoot length also generally and significantly decreased as the concentration of lead treatment increased for both *P. maximum* and *A. compressus* ($p < 0.05$). The least shoot length inhibition of *A. compressus* was 7.13 % (5 mg/kg) while the highest shoot length inhibition was 36.29 % (40 mg/kg). The least shoot length inhibition of *P. maximum* was 10.51 % exposed to 5 mg/kg and the highest shoot length inhibition was 42.46 % (40 mg/kg). Retarded shoot length due to the presence of the root environment with excess of Pb was also observed by [37]. The leaf area of *P. maximum* decreased from 58.660 ± 4.14 cm^2 (control) to 30.89 ± 4.11 cm^2 (47.34 % reduction) in 40 mg/kg while that of *A. compressus* decreased from 16.11 ± 1.19 cm^2 to 8.928 ± 0.91 cm^2 (44.57 % reduction) in 40 mg/kg contamination. The decrease in shoot length and leaf areas with increasing concentration of heavy metals may be due to the sensitivity of enzymes of the photosynthetic carbon reduction cycle to Pb, as reported in the study [12].

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

Concentration of Lead, 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	34.67 ± 2.73^{a}	28.67 ± 1.86^{ab}	84.00 ± 12.22^{a}	15.433 ± 0.54^{a}	58.66 ± 4.15^{a}	16.11 ± 1.19^{a}
5	29.67 ± 2.33^{b} (14.42 %)	28.14 ± 1.76^{b} (1.85 %)	75.17 ± 2.13^{a} (10.51 %)	14.33 ± 0.83^{ab} (7.13 %)	44.09 ± 1.08^{b} (24.84 %)	13.17 ± 1.05^{ab} (18.25 %)
10	28.00 ± 3.52^{b} (23.07 %)	27.00 ± 4.16^{b} (5.82 %)	74.33 ± 6.98^{a} (11.51 %)	13.67 ± 1.92^{b} (11.41 %)	41.33 ± 3.49^{b} (29.54 %)	11.78 ± 1.25^{b} (26.88 %)
20	25.67 ± 0.33^{b} (25.96 %)	26.20 ± 11.50^{b} (8.62 %)	65.50 ± 11.50^{ab} (22.02 %)	12.60 ± 0.46^{b} (18.34 %)	41.04 ± 6.26^{b} (30.04 %)	9.75 ± 0.72^{bc} (39.48 %)
40	20.33 ± 0.33^{c} (41.36 %)	27.67 ± 1.85^{b} (3.49 %)	48.33 ± 12.67^{b} (42.46 %)	9.83 ± 0.44^{c} (36.29 %)	30.89 ± 4.11^{c} (47.34 %)	8.93 ± 0.91^{c} (44.57 %)

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

2.2.2. Effect of P. maximum and A. compressus on soil pH level of the soil. All of the interactions that occur throughout the soil matrix are pH dependent. The soil pH has a significant effect on the mobility of lead and other metals within the soil [38]. The pH values decreased as the lead concentration increases in the contaminated soil (table 2). As observed, there was increment in the pH of the heavy metals contaminated soil without the *P. maximum* and *A. compressus* (non vegetated soil), however, further significant increase in the pH of lead contaminated soils vegetated with the two grasses ($p < 0.05$). Generally, *P. maximum* have more positive impact on soil pH than *A. compressus*. The pH of the soil can greatly influence the equilibrium between speciation of metals, solubility, adsorption, and exchange on solid phase sites [39]. Thus, 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 [39]. This study corroborates the work of [40], that the shoots of Elodea canadensis and Eriophorum angustifolium roots cause an increase in the pH of the surrounding heavy metal contaminated medium.

2.2.3. Effects of P. maximum and A. compressus on the soil total organic matter content of the Pb contaminated soils. The initial organic matter content (table 3) of the soil decreased with increase in the concentration of heavy metals added to the soil from 87.750 ± 0.076 (control) to 84.900 ± 0.001 (40 mg/kg lead contamination).

Table 2 – Effect of *P. maximum* and *A. compressus* on soil pH level of the soil

Concentration of Lead, mg/kg	Initial soil pH	Final pH in soil without plants (% change from initial after120 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	6.597 ± 0.0612^{a}	6.547 ± 0.013^{a} (0.76 %)	6.643 ± 0.012^{a} (0.69 %)	6.647 ± 0.023^{a} (0.75 %)
5	6.507 ± 0.015^{b}	6.523 ± 0.012^{b} (0.25 %)	6.607 ± 0.019^{a} (1.51 %)	6.613 ± 0.019^{a} (1.60 %)
10	6.457 ± 0.018^{b}	6.510 ± 0.058^{b} (0.81 %)	6.587 ± 0.013^{a} (1.97 %)	6.573 ± 0.012^{a} (1.77 %)
20	6.413 ± 0.088^{c}	6.487 ± 0.012^{b} (1.14 %)	6.567 ± 0.015^{a} (2.34 %)	6.543 ± 0.009^{a} (1.99 %)
40	6.403 ± 0.007^{c}	6.480 ± 0.010^{b} (1.19 %)	6.543 ± 0.012^{a} (2.14 %)	6.517 ± 0.018^{ab} (1.75 %)

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 Pb contaminated soils

Concentration of Lead, mg/kg	Initial soil total organic matter content	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} (89.14 %)	17.583 ± 1.458^{b} (79.96 %)
5	85.230 ± 0.020^{a}	7.520 ± 0.538^{b} (91.18 %)	8.800 ± 2.692^{b} (89.67 %)	9.667 ± 1.631^{b} (88.66 %)
10	85.160 ± 0.030^{a}	8.233 ± 1.273^{b} (90.33 %)	11.033 ± 4.113^{b} (87.04 %)	$7.350 \pm 0.701^{b*}$ (91.37 %)
20	$84.900 \pm 0.010^{a*}$	10.323 ± 3.383^{b} (87.84 %)	11.590 ± 3.921^{b} (86.35 %)	10.483 ± 0.361^{b} (87.65 %)
40	$84.900 \pm 0.010^{a*}$	10.323 ± 3.383^{b} (87.84 %)	6.980 ± 2.025^{b} (91.78 %)	14.017 ± 0.848^{b} (83.49 %)

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

There was no significance difference between the soil organic matter of *A. compressus* and *P. maximum* for the different lead contamination in the vegetated soils ($p > 0.05$). Ross (1994) reported that the organic matter in the solid phase, especially the humic compounds of high molecular weight, strongly retain the metals in soils and reduce its availability. Hence, bioavailability of metals is inversely proportional to the organic matter in soils [39]. This finding corroborated that of [41] 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.

2.2.4. Effects of P. maximum and A. compressus on soil cation exchange capacity of the Pb contaminated soils. Cation exchange capacity (CEC) is a dominant factor in heavy metals retention [42]. The CEC of soils depends on soil types, amounts, and types of different colloids present and on the CEC of the colloids. Fine-textured (clay) soils tend to have higher cation exchange capacity than sandy soils [42]. The effects of *P. maximum* and *A. compressus* on soil CEC at the initial day of treatment with lead and the final day in the vegetated and unvegetated soils are presented in table 4. Soil cation exchange capacity at the beginning of the study was generally and significantly lower than at the end of the study after 120 days ($p < 0.05$). There was also significant difference between the contribution of both *P. maximum* and *A. compressus* to cation exchange capacity of the soils for each level of treatments ($p < 0.05$). In work [43] 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.

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

Concentration of Lead, 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^a	192.877 ± 0.067^a (1105.48 %)	257.157 ± 64.287^a (1507.23 %)	225.013 ± 32.143^a (1306.33 %)
5	34.633 ± 0.149^b	200.870 ± 0.000^a (480.00 %)	265.013 ± 32.143^a (665.20 %)	230.010 ± 0.143^a (564.13 %)
10	34.620 ± 0.310^b	225.013 ± 0.000^a (549.95 %)	272.870 ± 0.000^a (688.19 %)	242.500 ± 0.000^a (600.46 %)
20	35.100 ± 0.049^b	240.133 ± 32.143^a (584.14 %)	285.013 ± 32.143^a (712.00 %)	252.600 ± 0.000^a (619.66 %)
40	35.303 ± 0.074^b	250.870 ± 0.000^a (610.62 %)	287.870 ± 2.025^a (715.43 %)	255.140 ± 0.300^a (622.71 %)

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

2.2.5. Lead level in the soils before and after the growth of the plants. The lead level in the soils before and after the growth of the plants is presented in table 5. The initial lead concentration in the contaminated soils were higher than the final Pb concentration level in all the concentrations in the soil samples. There was more significant reduction of the Pb 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* is a better removal of Pb than *P. maximum*, but with no significant differences. The study corroborates the study of [44] that *A. compressus* has the tenacity to withstand the deleterious effects of pollutants such as waste engine oil contamination.

The relative reduction of lead in the different soils planted with the respect to the soil without plant is shown in table 6. Both *A. compressus* and *P. maximum* reduces the level of lead 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 lead in all the degree of contaminations with the highest (92.90 %) in 5 mg/kg. This study corroborates the report of [45] and [44] who observed that plants of the grass family (Poacea) are particularly suitable for phytoremediation because of their multiple ramified root systems.

Table 5 – Lead level in the soils before and after the growth of the plants

Concentration of Lead, mg/kg	Initial day	Final day without plant	Final day with *A. compressus*	Final day with *P. maximum*
Control	0.0017 ± 0.0003^a	0.0015 ± 0.0003^a	0.0010 ± 0.0015^a	0.0011 ± 0.0018^a
5	$6.4677 \pm 0.2932^{a*}$	$5.8830 \pm 0.4630^{a*}$	0.4593 ± 0.0216^c	$2.7227 \pm 0.1668^{b*}$
10	$12.7823 \pm 0.7477^{a*}$	$11.2870 \pm 0.0450^{a*}$	$5.5567 \pm 0.3663^{b*}$	$6.0987 \pm 0.117^{b*}$
20	$26.3743 \pm 0.6661^{a*}$	$25.0430 \pm 0.0017^{a*}$	$7.3200 \pm 0.3985^{c*}$	$11.2363 \pm 0.2777^{b*}$
40	$52.3533 \pm 1.6917^{a*}$	$48.9700 \pm 0.0012^{a*}$	$11.0000 \pm 0.3495^{b*}$	$11.5540 \pm 0.2870^{b*}$

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

Table 6 – Relative reduction of Lead (Pb) from the soil by *A. compressus* and *P. maximum*

Concentration of Lead, mg/kg	Amt of Metal Lost from soil without plant, mg/kg	Amt of metal lost from soil with *A. compressus*, mg/kg	Amt of Metal Lost from soil with *P. maximum*, mg/kg
Control	0.0002 (11.77 %)	0.0007 (41.18 %)	0.0006 (35.29 %)
5	0.5847 (9.04 %)	6.0084 (92.90 %)	3.7450 (57.90 %)
10	1.4953 (11.69 %)	7.2256 (56.55 %)	6.6836 (52.29 %)
20	1.3313 (5.05 %)	19.0543 (72.25 %)	15.1380 (57.39 %)
40	3.3833 (6.46 %)	41.3533 (78.99 %)	40.7993 (77.93 %)

The percentage loss

2.2.6. The concentrations of lead (Pb) in the soils, roots and shoots of A. compressus and P. maximum at 120 days after planting. The concentrations of lead (Pb) in the soils, roots and shoots of *A. compressus* and *P. maximum* at 120 days after planting are presented in table 7. There was increase in accumulation in the parts of *A. compressus* and *P. maximum* as the level of contamination increased, with significant differences compared to control ($p < 0.05$). Also, there was higher accumulation of heavy metals in the roots than in the other parts of the plants which was significantly higher in the roots. The translocation of lead 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. The finding of the present study of heavy metal accumulation in soil and different plant parts corroborate that of [46].

Comparing the bioaccumulation factors (BF) under treatment for *A. compressus* and *P. maximum*, the trend was observed in the order of 40 > 20 > 10 >5 mg/kg (table 8). In general, plants with increased concentrations of lead presented higher BACs and TFs. The study revealed that a lead-contaminated soil cleanup might be achieved through growing non food crops like grasses. *A. compressus* have a higher biological concentration factor (BCF) and biological accumulation coefficient (BAC) than *P. maximum*.

P. maximum has greater potential to accumulate Pb than *A. compressus* while *A. compressus* has the greater ability to store Pb in their shoots from soils. In work [47] reported that, in 30 *B. pekinensis* cultivars, increased soil lead levels also increased the percent translocation to aerial plant parts as also observed in this study. High concentrations of lead are known to destroy the physical barrier formed by the Casparian strip [48].

Table 7 – The relative concentrations of lead (Pb) in the soils, roots and shoots of *A. compressus* and *P. maximum* at 120 days after planting

Concentration of Lead, 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	0.001 ± 0.002^{a}	0.002 ± 0.003^{a}	0.109 ± 0.000^{a}	0.111 ± 0.004^{a}	0.013 ± 0.003^{a}	0.022 ± 0.000^{a}
5	$2.723 \pm 0.167^{a*}$	0.459 ± 0.002^{b}	$1.557 \pm 0.164^{b*}$	3.807 ± 0.117^{a}	$0.128 \pm 0.004^{c*}$	0.150 ± 0.011^{c}
10	$6.099 \pm 0.118^{a*}$	$5.557 \pm 0.366^{a*}$	2.637 ± 0.135^{b}	3.186 ± 0.553^{b}	$0.191 \pm 0.005^{c*}$	0.199 ± 0.009^{c}
20	$11.236 \pm 0.278^{a*}$	$7.320 \pm 0.399^{a*}$	$4.961 \pm 0.096^{b*}$	$7.351 \pm 0.252^{a*}$	$0.252 \pm 0.009^{c*}$	$0.270 \pm 0.008^{b*}$
40	$11.554 \pm 0.287^{a*}$	$11.000 \pm 0.350^{a*}$	6.339 ± 0.006^{b}	11.700 ± 0.252^{a}	0.294 ± 0.003^{c}	$0.349 \pm 0.030^{b*}$

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

Table 8 – Biological accumulation coefficient (BAC) and Translocation factor (TF) of *A. compressus* and *P. maximum* of Lead

Concentration of Lead, mg/kg	*A. compressus*		*P. maximum*	
	BAC	TF	BAC	TF
5	0.283	1.121	1.379	2.726
10	1.574	2.742	1.432	3.312
20	2.004	1.996	1.442	3.265
40	2.064	1.940	1.549	2.823

2.2.7. Glutathione in the plants parts at varying Pb concentration at 120 days after planting (μmol/ml). The activities of glutathione (GSH) in the different part of the plants are presented in table 9. 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*. GSH, a sulfur containing tripeptide, is considered to be the most important cellular antioxidant involved in cellular defense against toxicants and function directly as a free radical scavenger [49, 50]. GSH is also the precursor for the phytochelatins that act as heavy metal binding peptides in plants [51]. GSH levels in plants are known to change under metal stress [50, 52]. In plant, at cellular level, Pb induces accumulation of reactive oxygen species (ROS), as a result of imbalanced ROS production and ROS scavenging processes by imposing oxidative stress. ROS include superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$), which are necessary for the correct functioning of plants; however, in excess they caused damage to biomolecules, such as membrane lipids, proteins, and nucleic acids among others [53]. This result of this study was similar to that of [54] who observed glutathione content in Sesbania drummondii plant to significantly increase upon exposure to Pb.

Table 9 – Glutathione in the plants parts at varying Pb concentration at 120 days after planting (μmol/ml)

Concentration of Lead, 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	$0.013 \pm 0.0004^{b*}$ (56.66 %)	0.025 ± 0.000^{b} (4.17 %)	$0.038 \pm 0.000^{a*}$ (51.85 %)	0.0140 ± 0.000^{c} (78.13 %)	$0.013 \pm 0.000^{b*}$ (48.00 %)	$0.040 \pm 0.000^{a*}$ (132.50 %)
10	$0.053 \pm 0.000^{a*}$ (76.67 %)	0.029 ± 0.019^{b} (20.83 %)	0.035 ± 0.000^{b} (29.63 %)	$0.019 \pm 0.000^{b*}$ (70.31 %)	$0.001 \pm 0.000^{b*}$ (96.00 %)	$0.037 \pm 0.000^{a*}$ (60.22 %)
20	0.041 ± 0.000^{b} (36.67 %)	0.037 ± 0.000^{b} (35.13 %)	0.028 ± 0.000^{b} (3.70 %)	0.084 ± 0.002^{a} (31.25 %)	0.054 ± 0.000^{b} (53.70 %)	0.036 ± 0.000^{b} (158.33 %)
40	$0.0162 \pm 0.0040^{b*}$ (30.23 %)	$0.046 \pm 0.0002^{a*}$ (91.67 %)	$0.0486 \pm 0.0003^{a*}$ (70.37 %)	$0.046 \pm 0.000^{a*}$ (28.13 %)	0.037 ± 0.000^{a} (32.43 %)	$0.035 \pm 0.000^{a*}$ (165.71 %)

2.2.8. Metallothionein in the plants parts at varying Pb concentrations. Plant metallothioneins (MTs) are cysteine-rich, low-molecular-weight and metal-binding proteins, synthesized due to mRNA translation [55]. They play roles in cellular sequestration, homeostasis of intracellular metal ions as well as adjustment of metal transport, also they play a role in maintenance of the redox level, repair of plasma membrane, cell proliferation and its growth, repair of damaged DNA, and scavenge ROS [56–58]. Metallothionein levels differed significantly among the plant parts and at the treatment levels ($p < 0.05$). Pb caused a decrease in the metallothionein level (10.11 %) in

P. maximum while *A. compressus* metallothionein level increased by 116.10 % in 5 % treatment. 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 [59, 60]. Overexpression of MTs in plants improves heavy metal tolerance [61].

Table 10 – Metallothionein in the plants parts at varying Pb concentrations

Concentration of Lead, 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	29.233 ± 0.2367^{b} (10.11 %)	50.660 ± 0.0808^{a*} (116.10 %)	25.597 ± 0.043^{b*} (487.60 %)	14.577 ± 0.052^{b*} (76.61 %)	23.930 ± 0.040^{b} (18.98 %)	24.757 ± 0.252^{b*} (74.22 %)
10	4.093 ± 0.030^{c*} (87.28 %)	36.2567 ± 0.0448^{a*} (54.66 %)	40.400 ± 0.266^{a*} (827.24 %)	20.957 ± 0.062^{b*} (66.38 %)	1.173 ± 0.055^{c*} (96.03 %)	43.593 ± 0.061^{a*} (5.61 %)
20	3.847 ± 0.073^{c*} (88.05 %)	26.1900 ± 0.0723^{b} (1.079 %)	17.7967 ± 0.1017^{b*} (300.47 %)	43.903 ± 0.092^{a} (29.57 %)	37.523 ± 0.084^{a} (27.04 %)	31.123 ± 0.828^{a*} (67.58 %)
40	26.1167 ± 0.156^{a} (23.24 %)	2.9800 ± 0.0404^{b*} (87.28 %)	25.2633 ± 0.0727^{a*} (479.82 %)	1.443 ± 0.033^{b*} (97.69 %)	23.597 ± 0.172^{a} (19.50 %)	7.577 ± 0.514^{b*} (92.1 %)

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

2.2.9. Total protein level in the plants parts at varying Pb concentrations. Proteins are important constituents of the cell; however, they can be easily damaged under a stressed environmental condition. Hence, any change in these compounds can be considered an important indicator of oxidative stress in plants. The results of this study showed varied changes in protein content in the different parts of *A. compressus* and *P. maximum* ($p < 0.05$) in the different treatments. Low concentrations of lead increase total protein content the most [48] as observed in the 5 and 10 mg/kg contamination. This protein accumulation may defend the plant against lead stress [62], 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 GSH [47]. It has been reported that Pb is able to decrease protein content by inhibiting the uptake of Mg and K ions and promote postranslational modification [12].

Table 11 – Total protein level in the plants parts at varying Pb concentration

Concentration of Lead, 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	63.215 ± 0.183^{a*} (172.51 %)	54.413 ± 0.264^{a*} (254.09 %)	30.193 ± 0.047^{b*} (166.88 %)	10.140 ± 0.1200^{b} (3.71 %)	40.747 ± 0.162^{a*} (149.42 %)	54.670 ± 0.093^{a*} (311.15 %)
10	31.153 ± 0.107^{a} (34.30 %)	40.140 ± 0.371^{a*} (161.21 %)	21.800 ± 0.060^{b*} (98.36 %)	9.597 ± 0.162^{c} (1.84 %)	47.183 ± 0.107^{a*} (188.81 %)	34.490 ± 0.060^{a*} (159.38 %)
20	30.423 ± 0.315^{a} (31.15 %)	51.187 ± 0.107^{a*} (233.10 %)	19.010 ± 0.060^{b*} (72.98 %)	7.497 ± 0.107^{b} (23.32 %)	58.237 ± 0.265^{a*} (256.47 %)	45.730 ± 0.104^{a*} (243.91 %)
40	29.497 ± 0.352^{b} (27.16 %)	27.203 ± 0.162^{a*} (77.02 %)	12.633 ± 0.265^{b*} (14.95 %)	5.850 ± 0.208^{b} (44.25 %)	53.987 ± 0.423^{a*} (230.46 %)	14.253 ± 0.211^{b} (7.19 %)

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

2.2.10. GST level in the plants parts at varying Pb concentration. 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 [63]. 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 [64]. Analysis of Glutathione S-transferase activity in the different parts of *A. compressus* and *P. maximum* in the Pb 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 [65].

Table 12 – GST in the plants parts at varying Zn and Pb concentration at 120 days

Concentration of Lead, 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	2.127 ± 0.007^{b} (26.83 %)	6.677 ± 0.047^{a} (35.90 %)	4.740 ± 0.010^{b*} (36.69 %)	3.560 ± 0.040^{b} (19.46 %)	4.757 ± 0.018^{b} (32.14 %)	9.497 ± 0.144^{a*} (217.00 %)
10	4.327 ± 0.013^{b*} (48.85 %)	3.817 ± 0.032^{b*} (22.31 %)	3.090 ± 0.012^{b*} (58.73 %)	10.377 ± 0.161^{a*} (248.22 %)	3.813 ± 0.009^{b*} (45.61 %)	4.663 ± 0.012^{b*} (55.80 %)
20	8.483 ± 0.092^{a*} (191.00 %)	4.257 ± 0.007^{b*} (13.35 %)	5.633 ± 0.012^{b*} (24.76 %)	12.490 ± 0.206^{a*} (319.13 %)	4.923 ± 0.020^{b*} (29.77 %)	4.310 ± 0.012^{a*} (44.00 %)
40	4.883 ± 0.055^{a*} (67.97 %)	3.720 ± 0.015^{a*} (24.28 %)	1.933 ± 0.041^{b*} (74.18 %)	6.683 ± 0.055^{a} (124.26 %)	2.920 ± 0.021^{b*} (58.35 %)	2.700 ± 0.000^{b} (9.8 %)

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

Conclusion

A. compressus and *P. maximum* growing on Pb polluted soils show a slight reduction in growth due to changes in their physiological and biochemical activities. However 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 ($p < 0.05$). This study suggests *A. compressus* is a better removal of Pb than *P. maximum*, however, it was not significant. Glutathione (GSH) 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*. Pb caused a decrease in the metallothionein level (10.11 %) in *P. maximum* while *A. compressus* metallothionein level increased by 116.10 % in 5 % treatment. Then both *A. compressus* and *P. maximum* have the tenacity and phytoremediating capacity to remediate Pb in soil effectively.

Given the results of this study, we can distinguish the following prospects for further research in this direction:

– development rational forms of environmental safety management and innovative technology which based on the method of bioremediation using *A. compressus* and *P. maximum*;

– study of the genes responsible for remediating potential of these grasses, to avoid the extinction of these species due to the systematic use of these herbs for soil recovery from heavy metals.

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Conflicts of Interest.

None of the authors have any potential conflicts of interest associated with this present study.

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ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ВІДГУКУ ЗРОСТАННЯ І ВІДНОВНОГО ПОТЕНЦІАЛУ PANICUM MAXIMUM Й AXONOPUS COMPRESSUS В ҐРУНТІ, ЗАБРУДНЕНОМУ СВИНЦЕМ

Глобальна проблема, пов'язана із забрудненням довкілля важкими металами, зростає. Тому необхідні ефективні підходи до його відновлення. Деякі рослини можуть поглинати токсичні метали й очищують від них ґрунт. Цей факт може бути корисний для розробки раціональних форм управління екологічною безпекою та інноваційних технологій, які більш ефективно очищують ґрунти й поліпшують їхній екологічний стан для ведення сільського господарства. Фіторемедіація відома як екологічно й економічно ефективний спосіб зниження забруднення грунту. Тому, цей експеримент був проведений для вивчення порівняльного потенціалу двох трав, *P. maximum* й *A. compressus*, для біоремедіації ґрунтів, забруднених свинцем (Pb). Крім того, було вивчено вплив Pb на систему антиоксидантного захисту рослин. Солі $Pb(NO_3)_2$ були змішані з ґрунтом в різних концентраціях 5 мг/кг, 10 мг/кг, 20 мг/кг, 40 мг/кг, 80 мг/кг, взяті по три зразка різної забрудненості й контрольний зразок. Через 4 місяці рослини видаляли з ґрунту і відокремлювали корінь, пагін й листок. Частини рослин були проаналізовані на морфологічні, біохімічні параметри і концентрацію Pb. Зразки ґрунту також були проаналізовані на вміст Pb. Довжина коренів *P. maximum* й *A. compressus*, як правило, зменшувалася зі збільшенням концентрації Pb в ґрунті. Найменше інгібування довжини пагона *A. compressus* склало 7,13 % (5 мг/кг), тоді як найбільше – 36,29 % (40 мг/кг). Найменше інгібування за довжиною пагона *P. maximum* склало 10,51 % (5 мг/кг), а найбільше – 42,46 % (40 мг/кг). В кінці дослідження спостерігалося більш значне зниження вмісту важких металів у рослинних ґрунтах як для *P. maximum*, так і для *A. compressus*, у порівнянні із ґрунтами на початку дослідження ($p < 0{,}05$). *A. compressus* краще видаляє Pb, ніж *P. maximum*, однак ця різниця не значна. Рівні глутатіону (GSH) значно варіювалися ($p \leq 0{,}05$) по відношенню до концентрації важких металів, а також різних частин рослин. *A. compressus* більше впливає на активність глутатіону, ніж *P. maximum*. Свинець викликав зниження рівня металлотіонеінов (10,11 %) у *P. maximum*, в той час як рівень металлотіонеінов у *A. compressus* підвищився на 116,10 % (5 мг/кг).

Ключові слова: забруднений ґрунт; важкі метали; фіторемедіація; контроль екологічної безпеки.

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СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ОТКЛИКА РОСТА И ВОССТАНОВИТЕЛЬНОГО ПОТЕНЦИАЛА PANICUM MAXIMUM И AXONOPUS COMPRESSUS В ПОЧВЕ, ЗАГРЯЗНЕННОЙ СВИНЦОМ

Глобальная проблема, связанная с загрязнением окружающей среды тяжелыми металлами, возрастает. Поэтому необходимы эффективные подходы по ее восстановлению. Некоторые растения могут поглощать токсичные металлы и очищают от них почву. Этот факт может быть полезен для разработки рациональных форм управления экологической безопасностью и инновационных технологий, которые более эффективно очищают почвы и улучшают их экологическое состояние для ведения сельского хозяйства. Фиторемедиация известна как экологически и экономически эффективный способ снижения загрязнения почвы. Поэтому, этот эксперимент был проведен для изучения сравнительного потенциала двух трав, *P. maximum* и *A. compressus*, для биоремедиации почв, загрязненных свинцом (Pb). Кроме того, было изучено влияние Pb на систему антиоксидантной защиты растений. Соли $Pb(NO_3)_2$ были смешаны с почвой в различных концентрациях 5 мг/кг, 10 мг/кг, 20 мг/кг, 40 мг/кг, 80 мг/кг, взяты по три образца различной загрязненности и контрольный образец. Через 4 месяца растения удаляли из почвы и отделяли корень, побег и лист. Части растений были проанализированы на морфологические, биохимические параметры и концентрацию Pb. Образцы почвы также были проанализированы на содержание Pb. Длина корней *P. maximum* и *A. compressus*, как правило, уменьшалась с увеличением концентрации Pb в почве. Наименьшее ингибирование длины побега *A. compressus* составило 7,13 % (5 мг/кг), тогда как наибольшее – 36,29 % (40 мг/кг). Наименьшее ингибирование по длине побега *P. maximum* составило 10,51 % (5 мг/кг), а наибольшее – 42,46 % (40 мг/кг). В конце исследования наблюдалось более значительное снижение содержания тяжелых металлов в растительных почвах как для *P. maximum*, так и для *A. compressus*, по сравнению с почвами в начале исследования ($p < 0,05$). *A. compressus* лучше удаляет Pb, чем *P. maximum*, однако эта разница не значительная. Уровни глутатиона (GSH) значительно варьировались ($p \leq 0,05$) по отношению к концентрации тяжелых металлов, а также различных частей растений. *A. compressus* оказывает большее влияние на активность глутатиона, чем *P. maximum*. Свинец вызывал снижение уровня металлотионеина (10,11 %) в *P. maximum*, в то время как уровень металлотионеина в *A. compressus* повысился на 116,10 % (5 мг/кг).

Ключевые слова: загрязненная почва; тяжелые металлы; фиторемедиация; контроль экологической безопасности.