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Growth and phytoremediative capacity of *Axonopus compressus* in a copper-polluted soil

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Abstract

The importance of lawn grasses in both landscaping and recovery of degraded lands is a practice that is becoming somewhat popular in many climes. To better explore the capacity for any known lawn plant, like Axonopus compressus, for landscaping, which ultimately considers its phytoremediative capacities, the presented study investigated growth and phytoremediative capacity of the test plant in a heavy metal-polluted soil. Experimental soils were polluted with copper (as $CuSO_4$) in 4 different concentration; 100, 200, 300 and 400mg/kg. Copper-polluted soils and control soils were sown with tillers of Axonopus compressus. The study was observed for 3 months. Afterwards, results showed that plant growth was adversely impacted as concentration of Cu increased. This reduction intensified as Cu concentration increased. Accumulation of Cu in leaves, stem, sheath, and root of Axonopus compressus in Cu-polluted soil as well as residual soil metal concentration after 3 months of exposure was reported. Leaf accumulation was 18.06–61.08mg/kg, whereas stem accumulations were 13.27–50.87mg/kg. Comparatively, there were differences in the phytoaccumulations of metal across the plant organs. The root had better accumulation levels. However, at higher soil metal levels, this trend was somewhat distorted; the highest accumulation being in the leaf (61.08mg/kg) in the 200mg Cu/kg soil treatment level, when compared with stem (35.15mg/kg), sheath (23.77mg/kg), and root (40.99mg/kg). This suggests perhaps, that the preferred organ of metal deposition depend on the soil concentration of the polluting metal. Antioxidant composition of the leaves of metal-exposed plants showed that contents of CAT, SOD, MDA and to copherol significantly ($p<0.01$) increased. Significant increases in CAT and SOD levels in the roots of the test plant implied that metal effects necessitated the heightened activity of the enzymes in question. Root CAT was 17.01mol/g tissue in the control, compared to 21.05–23.74mol/g tissue in the Cu-exposed plants. SOD contents of root was 49.33mol/g tissue in the 400mg Cu/kg soil treatments compared to 30.12mol/g tissue in the control. Minimal changes ($p>0.05$) in peroxidase and ascorbate concentrations however were. This study has established that Axonopus compressus has the capacity to withstand the deleterious effects of copper contamination and the capability to remove copper from polluted soils, and hence a good candidate for landscaping.

Keywords: *Axonopus*, copper, heavy metal, pollution, phytoremediation, phytotoxicity.

Introduction

The soil is an important aspect of the ecosystem; it is the substrate that both non-natural and natural ecological systems cannot do without. It is also the key sinks for which all heavy metals that emanate from human activities are released into the environment. Heavy metals are non-degradable, unlike the organic pollutants that usually oxidized to carbon (IV) oxide. Most metals may not be bioremediated via microbial or chemical processes[1]. This means that the metals would remain in the soil over lengthier spells than when originally introduced into that environment[2]. This persistent nature of most metals exasperates the hazards they posit to the environment by contact, through the food chain, or via absorption or adsorption[3,4].

Metals remain required for plant growth and development; some necessary for critical biochemical process that guarantee plants survival under stressed conditions. However, these metals are required in minute quantities hence their reference as microelements. At disproportionate quantities, they directly or indirectly become toxic to plants. Among phytotoxic impacts of excessively accumulated heavy metals on plant physiology include inhibition of cytoplasmic enzymes owing to accumulation reactive oxygen species, which ultimately lead cell damage[5]. Essential nutrients have also been reported by Taiz and Zeiger[6] to be replaced at the sites of cation exchange. Additionally, indirect impacts of heavy metal pollution may include adverse effect on the development and activities of beneficial soil microorganisms[7]. Among these microelements which are important for plant and human metabolism but also hazardous when concentrations are excessive, is copper.

Copper is one of the heavy metals of foremost concern with regards to bioavailability studies[8]. It is most likely to be very common in heavy metal-polluted soil[9]. It is very vital for growth improvement and is physiological development of plants. However, it was reported that when copper is introduced into the soil, most of it quickly becomes stable. At soil pH of 5.5, copper becomes rather soluble in soil. Incidentally, this pH level is somewhat close to optimal pH in farmlands as reported by Franklin *et al.*[10]. This invariably implies that at some point, farmlands may have heightened levels of ionic copper, thus imposing phytotoxic effects. Therefore, it becomes very imperious that metal cleanup measures be put forward. Salt *et al.*[11] pointed out that reliance on physicochemical as well as mainstream industrial remediation approaches can be extortionately costly. Alternative less expensive and ecofriendly cleanup approaches like bioremediation therefore suffice. Most bioremediation measure usually relied on plants' inherent capabilities for accumulation of contaminant. However, as pointed out before, metals are hardly degraded, thereby accumulating in plant tissues, even beyond optimal levels, thus imposing direct and indirect undesirable effects on the plant. The present study therefore investigated the remediation capabilities as well as morphological growth responses of *Axonopus compressus* in a copper-polluted soil.

The importance of plants in metal availability in soils cannot be over highlighted. The direct or indirect effects plants have on the soil physic chemistry underscores their ability to regulate the accessibility of heavy metals in soil. Phytoaccumulation of these metals also possibly reduces ion activity and desorbs contaminants from soil surfaces. Other influence of plants on metal availability in soil includes impaction sorption, imposing organic ligand action which affects total concentration of contaminants in solution. Microbial activity stimulated by plants can also alter soil solution chemistry and thus, affect contaminant behavior[3,12]. A number of grass species including *Axonopus compressus* have been previously reported to show these characteristics[13,14]. Bordoloi *et al.*[13], Anoliefo and Ikhajiagbe[14]; Chukwu et al.[15] previously reported the plant to be found in abundant amounts in heavy metal-polluted sites as well as oil-polluted soils[16,17]. Ikhajiagbe et al[18] have also successfully used the plant to remediate a spent lubricating oil-polluted soil; hence the interest.

Axonopus compressus grass is particularly important on a global scale. It has been reported to bear relevance in the Americas, as well as several tropical and subtropical regions of the world[19,20]. Then acceptability for *A. compressus* in management of denuded and degraded lands is based on its capacity for slow growth and tolerance to heavy grazing. The grass can also subsist on a diverse soil types, insofar as there is guaranteed moisture and a favourable pH of not above 7. *A. compressus* used in turf management as well as a pasture[19,21]. Further, as a valuable cover plant, the plant helps to contribute to nitrogen status of the soil[21,22].

Materials and methods

The soils (0–10cm) obtained for the study, which were initially sun-dried to constant weight, were measured (20kg) into round experimental bowls (diameter 66.2cm, 48.4cm height). The bowls were not perforated; this would prevent heavy metal leaching into soil.

Soil Pollution with Cu: Analar grade Copper sulphate was used as the sole source of Cu in the study. Four different concentrations of $CuSO_4$ were adopted for the study; 100, 200, 300 and 400mg/kg of $CuSO_4$. Prior to application of coppers solutions to soil, the water-holding capacity was determined according to the methods of Anoliefo *et al.*[23], to be 0.78L/kg soil. This was necessary for the WHC would form the basis for which the measured heavy metal would be dissolved and used to moisten the soil prior sowing of test plant. Each measured $CuSO_4$ was therefore dissolved in 0.70L of water/kg soil. Therefore, each 20kg soil received 24,6 and 8g of copper sulfate in 1.4L distilled water respectively. The set up was replicated 5 times and left in a well-ventilated screen house overnight prior to sowing.

Sowing of Test Plants: The tillers of *Axonopus compressus* used in the study were earlier raised in nursery (Table-1). Ten tillers were planted per bowl. To ensure that each bowl was adequately watered to guarantee availability of adequate moisture for optimal plant performance, the Soil Moisture Feel Test was utilized[24]. The bowls were arranged following a completely random ized design relying on the conjecture that the experimental plots were homogenized. The study was observed for 3 months. Afterwards, morphological development of test plant measured included plant height, number of leaves per culm, leaf length, leaf width, as well as internode.

Antioxidant activity: Plant tissue samples were assayed for tocopherol activity following the methods of Rosenberg[25]. Catalase[26] and superoxidedismutase[27] activity were also estimated accordingly. Following the Thiobarbituric Acid Assay, malondialdehyde was determined[28]; whereas peroxidase was assayed at 430nm[29].

Determination of copper in *Axonopus compressus* tissues: Concentrations of Cu in plant tissues – stem, root and leaves, as well as residual soil concentrations were determined with slight modifications[30]. One gram of dry leaf tissue was ground, sieved and placed inadigestion tube. 10ml of mixed acid (HNO_3:$HClO_4$ mixture: 100ml of nitric acid with 50ml of perchloricacid 1:1 HCl: 50ml of concentration HCl to 50ml of water) was included. The digestion was subjected to heat until dense white fumes were given off and clean solution obtained. Whitman no 42 filter paper was used to filter the solution into a flask. The volume of the resulting solution was made upto 100ml with deionized water. A reagent blank was prepared without the sample. Copper was determined there after by atomic absorption spectrophotometry (AAS Buck scientific210 VGP).

Determination of malic acid in plant tissue: Measured 1g of plant tissue sample (root) was weighed into 250ml beaker. To this sample was added 50ml of $1.5KMnO_4$ and 5ml of 1MKbr solutions respectively. The mixture was homogenized in a vortex mixer. The mixture was later steam distilled into a Dinitrophenyhyrazinein acid solution to form an insoluble condensation product in solution. The condensation product was dissolved in 5% pyridine solution to give an intense blue colour. Working standard solutions of malicacid of range 0-50µg/ml were preparedfrom100µg/ml stock standard malic solution and treated similarly as sample above. The absorbance of working standards as well as sample was read on a UV-Visible Cecil2483 Spectrophotometer at a wavelength of 595nm.

Statistical analysis: Results of the experiment were subjected to statistical analyses using SPSS® software version 20.

Results and discussion

Morphological parameters of tiller used from the nursery have been presented on Table-1. The tillers were 21.44mm in average height and there were no signs of morphological anomaly, chlorosis or necrosis prior to transplanting.

Selected morphological parameters of the test plant after 3 months exposure to Cu-polluted soil have been presented on Table-2. There was significant decrease in plant height ($p<0.05$) in response to copper pollution. Plant height of the control plant (left in the nursery, and not exposed to metal contamination) was 158.23mm, compared to 139.54–105.72mm obtained from Cu-exposed plants. Similar significant differences were obtained for leaf number when control plants were compared to metal-exposed ones. However, values obtained for leaf blade length (9.42–13.22cm), leaf width (1.46–2.01cm) and internode (4.04–4.76cm) were statistically minimal ($p>0.05$).

The importance of Cu in plant growth and metabolism is noted. However, Foy et al.[31] reported that in large amounts, Cu has been identified as having the capacity to adversely affect the growth and yield improvements of plants. In the present study, growth was adversely impacted as concentration of Cu increased. Mengel and Kirby[32] earlier reported that copper at low concentration was important and involved in certain biochemical reactions required for the development of plants; however, it becomes harmful to plants when the concentration is at higher levels. Although Mn is an important plant micronutrient, it inhibited seedling emergence and subsequent growth of *Hordeum vulgare* when concentrations increased[33]. Mrozek[34] indicated that growth was reduced by heavy metal concentration. Significant reduction in plant height was reported in the study (Table-2). This reduction intensified as Cu concentration increased. Bernardo *et al*.[35] observed significant reduction in plant height with increasing Hg stress in cowpea cultivar.

Table-1: Morphological parameters of tiller used from the nursery.

Parameter	Value
Plant height (mm)	21.24±2.11
Flag leaf blade length (cm)	2.01±0.94
Flag leaf blade width (cm)	0.96±0.16
Internode (cm)	2.86±0.43
Number of leaves per culm	6±1
Necrosis?	No
Chlorosis?	No

Table-2: Selected morphological parameters of the test plant after 3 months exposure to Cu-polluted soil.

Soil metal conc. (mg Cu/kg)	Plant height (mm)	No. of leaves (per cu lm)	Leaf blade width (cm)	Leaf blade length (cm)	Internode (cm)
0	158.43^{ab}	73.54^{a}	2.01^{a}	13.22^{ab}	4.76^{a}
100	139.54^{bc}	60.28^{ab}	1.64^{a}	11.62^{ab}	4.38^{a}
200	113.58^{c}	42.65^{b}	1.58^{a}	10.34^{ab}	4.10^{a}
300	105.72^{c}	40.11^{b}	1.46^{a}	9.42^{b}	4.54^{a}
400	108.32^{c}	43.76^{b}	1.59^{a}	10.72^{ab}	4.04^{a}
P value	0.012	0.008	0.427	0.248	0.736
Sig.	P<0.05	P<0.05	p>0.05	P>0.05	p>0.05

Values with same alphabetic superscripts presented on similar columns do not differ from each other ($p>0.05$).

Accumulation of Cu in leaves, stem, sheath, and root of *Axonopus compressus* in Cu-polluted soil as well as residual soil metal concentration after 3 months of exposure have been presented on Figure-1. The level of metal accumulation in the plant organs differed with metal concentrations in the soil. Increased soil Cu concentration implied increased plant accumulation. For example leaf accumulation was 18.06–61.08 mg/kg (Figure-1a), whereas stem accumulations were 13.27–50.87mg/kg (Figure-1b). The highest accumulation in the plant's sheath (Figure-1c) was 66.92mg/kg in the 400mg Cu/kg soil treatment. Soil residual concentrations were 22.33–131.30 mg/kg, there highest residual concentration being for the 400 mg Cu/kg soil treatment (Figure-1e).

Comparatively, there were differences in the accumulations of metal across the plant organs. Foliar accumulation of Cu in plants sown in the 100mg Cu/kg soil was 18.06mg/kg (Figure-1a), 12.32mg/kg in the stem (Figure-1b), 12.71mg/kg in the sheath (Figure-1c) and 22.76mg/kg in the root (Figure-1d). Obviously, the root had better accumulation levels. However, at higher soil metal levels, this trend was somewhat distorted; the highest accumulation being in the leaf (61.08mg/kg, Figure-1a) in the 200 mg Cu/kg soil treatment level, when compared with stem (35.15mg/kg), sheath (23.77mg/kg) and root (40.99 mg/kg). Perhaps, it is safe to say that the preferred organ of metal deposition depend on the soil concentration of the polluting metal. However, Probst *et al.*[36] previously reported increased metal concentrations in roots, leaves and stems respectively in that order, using *Viciafaba*.

Significant ($p<0.05$) increases in catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and tocopherol contents were reported in plant leaves exposed to Cu-polluted soil (Table-3). Peroxidase and ascorbate contents did not significantly differ from the control ($p>0.05$). Peroxidases have been previously used to indicate metal phytotoxicity[37]. In a study by Wang et al.[38], *Vicia faba* seedlings were exposed to Pb-contaminated soil. Results showed the possibility for peroxidase activity being critical to H_2O_2 degradation. The peroxidase activities in this study increased as the soil metal concentration increased.

CAT content of leaves was 16.34mol/g tissue in the control and 20.37–22.48mol/g tissue in the Cu-exposed plants. The significant increase in malondialdehyde (MDA) indicates significant increases in levels of lipid peroxidation of cells. Similarly, peroxidase is somewhat used to estimate the availability of H_2O_2, as this catalyzes oxidation reactions were H_2O_2 is a substrate. Therefore, any increase in peroxidase may be thought to imply concomitant increase in H_2O_2 levels. However, in the present study, peroxidase contents in the metal-exposed plants (2.02–2.42mM/g) compared statistically ($p>0.05$) with the control (1.89mM/g) (Table-3). Therefore, perhaps, accumulation of Cu by the test plant may not have necessitated any significant increase in H_2O_2 levels as expected.

Significant increases in CAT (Figure-2a) and SOD (Figure-2b) levels in the roots of the test plant implied that metal effects necessitated the heightened activity of the enzymes in question. Root CAT was 17.01mol/g tissue in the control, compared to 21.05–23.74mol/g tissue in the Cu-exposed plants (Table-3). SOD contents of root was 49.33mol/g tissue in the 400 mg Cu/kg soil treatments compared to 30.12mol/g tissue in the control.

The increase in root concentration of malate in *Axonopus compressus* with increase Cu concentration in the soil was observed (Figure-3). Significant ($p<0.01$) increase in malic acid content of roots increased from 0.399% in the 100mg Cu/kg soil treatment, to 0.562% in the 400mg Cu/kg soil metal concentration. Similar outcomes were obtained by Qin et al.[39] who confirmed exudation of oxalate, malate and formate from the roots of poplar plants that were exposed to copper. Bio availability and mobility of metals are known to be enhanced by root exudates[40]. These organic acid chelators could be implicated in heavy metal transport through thexylem[41].

Table-3: Antioxidant concentrations in leaves of *Axonopus compressus* in response to Cu accumulation in Cu-polluted soil after 3 months of exposure.

Soil metal conc. (mg Cu/kg)	CAT (mol/g tissue)	SOD (mol/g tissue)	MDA (x 10^8 mol/g tissue)	Peroxidase (mM/g)	Ascorbate (µg/g)	Tocopherol (µg/g)
0	16.34^{b}	38.33^{b}	1.44^{b}	1.89^{a}	0.78^{a}	0.11^{b}
100	22.48^{a}	62.17^{a}	3.44^{a}	2.21^{a}	0.68^{a}	0.16^{b}
200	20.37^{ab}	65.32^{a}	3.89^{a}	2.18^{a}	0.73^{a}	0.17^{b}
300	20.79^{ab}	68.31^{a}	3.79^{a}	2.02^{a}	0.72^{a}	0.37^{b}
400	22.11^{ab}	85.67^{a}	3.74^{a}	2.42^{a}	0.70^{a}	1.02^{a}
F-value	7.68	10.46	4.48	0.70	0.29	93.50
p-value	0.004	0.001	0.025	0.607	0.874	<0.001

Values with same alphabetic superscripts presented on similar columns do not differ from each other ($p>0.05$).

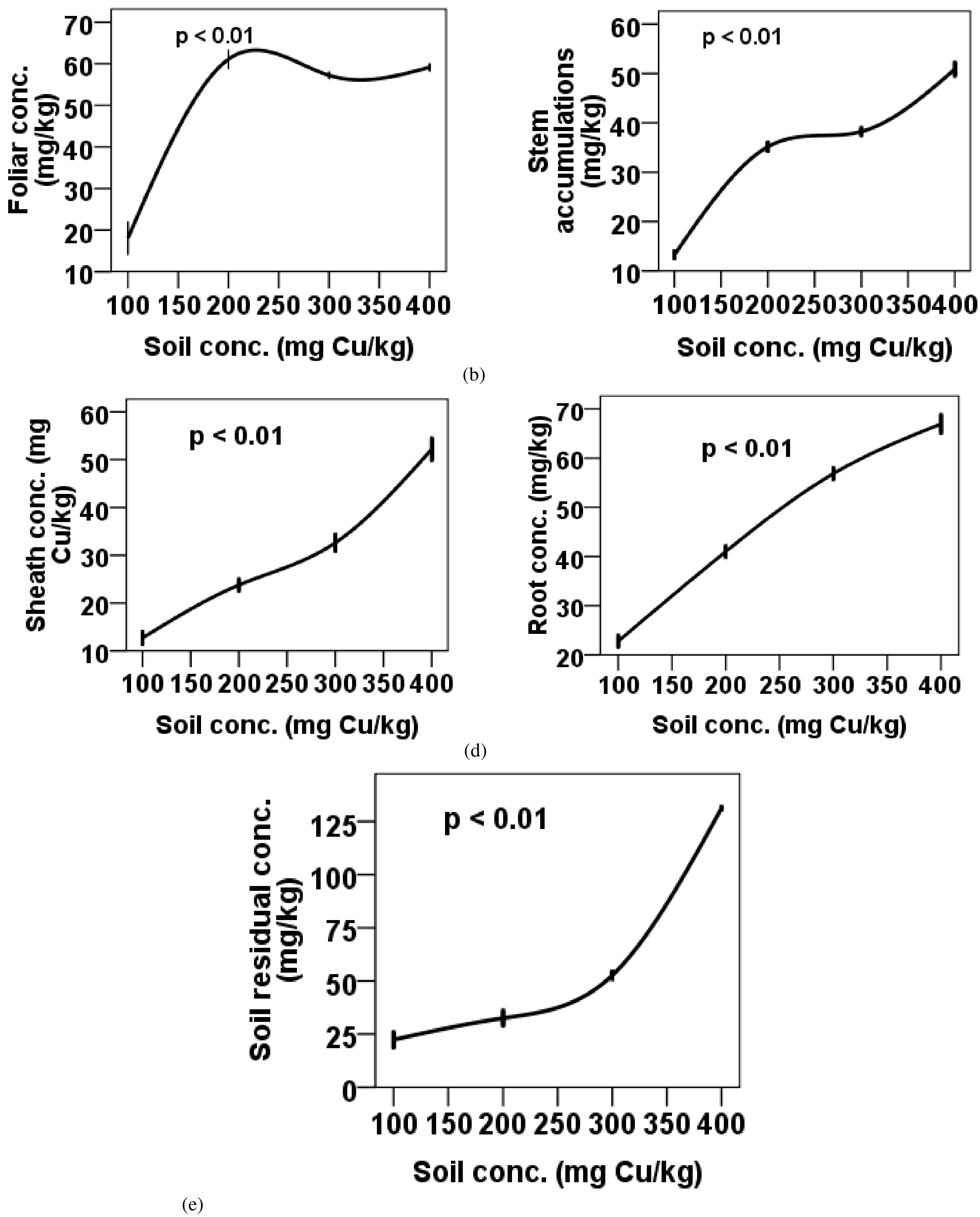
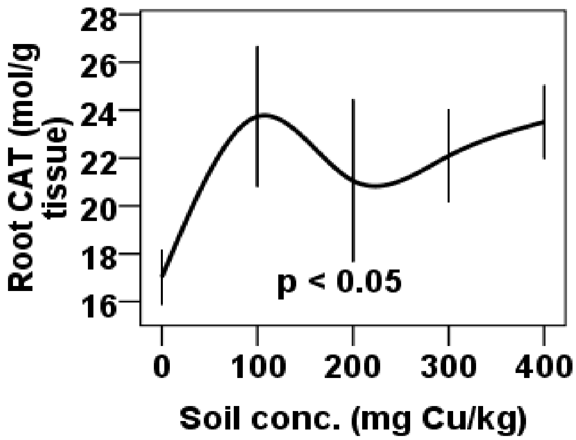
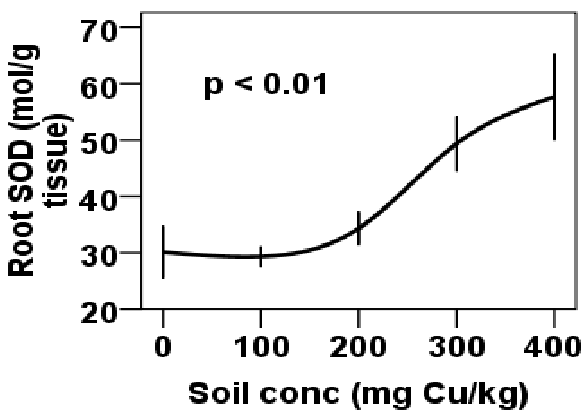


Figure-1: Accumulation of Cu in (a) leaves, (b) stem, (c) sheath, and (d) root of *Axonopus compressus* in Cu-polluted soil as well as (e) residual soil metal concentration after 3 months of exposure.



a)



(b)

Figure-2: Accumulation of (a) SOD and (b) CAT in the roots of *Axonopus compressus* in response to Cu accumulation in Cu-polluted soil after 3 months of exposure

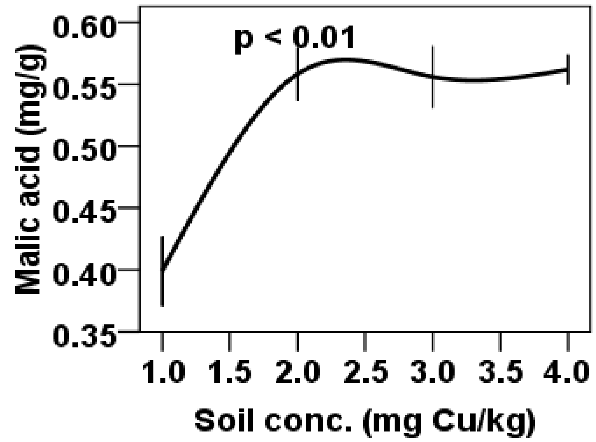


Figure-3: Accumulation of malate in the roots of *Axonopus compressus* in response to Cu accumulation in Cu-polluted soil after 3 months of exposure.

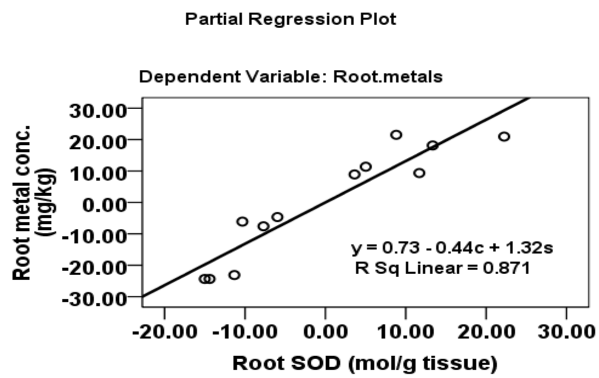
Table-4 shows the bivariate correlation between selected parameters, using Pearson's coefficient of correlation, during plant's 3-month exposure to Cu-polluted soil. Highly significant ($p<0.01$) correlation existed between SOD and stem heavy metal accumulation ($r=0.657$) and between tocopherol concentrations and sheath metal concentration ($r=0.834$). Generally, SOD activity correlated positively metal accumulation in all plant tissues assessed. The implications of positive correlation are that any increase in one parameter is likely to favour the positively correlated parameter, much as a decrease in one resulting in the decrease of the correlated parameter.

Table-4: Bivariate correlation between selected parameters, using Pearson's coefficient of correlation, during plant's 3-month exposure to Cu-polluted soil

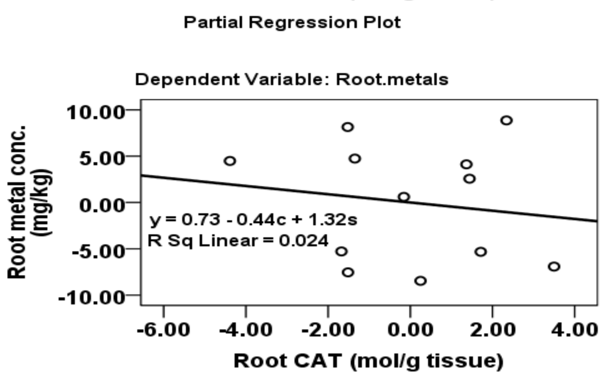
Parameters	Heavy metal accumulated within plant tissues			
	Leaves	Stem	Sheath	Root
Foliar CAT	-0.323	-0.14	0.016	-0.062
Foliar SOD	0.374	***0.657****	***0.779*****	***0.682****
Foliar MDA	0.517	0.369	***0.201*****	0.338
Foliar Peroxidase	0.025	0.162	0.245	0.155
Foliar AsA	0.235	0.166	0.064	0.141
Foliar Tocopherol	0.410	***0.712*****	***0.834*****	***0.693****
Root CAT	-0.352	-0.096	0.094	-0.027
Root SOD	***0.606****	***0.846*****	***0.928*****	***0.932*****

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Figure-4a and b present the partial regression plot of root SOD (a) and root CAT (b) against root metal accumulation (dependent var.) for the test plant. The significant relationship between root SOD and root CAT (r=0.871) is represented by the regression model y = 0.73 – 0.44x + 1.32z (where y=dependent var. or root metal accumulation; x=CAT conc., and z=SOD conc.).Increase in root SOD levels implies a concomitant increase in root metal content, and this can be predicted from then model with known values of CAT and SOD. However, this model is not reliable for predicting level of metal accumulation in the root using root CAT; the reason being that the correlation coefficient is very low (2.40%) (Figure-4b).



(a)



(b)

Figure-4: Partial regression plot of (a) root SOD and (b) root CAT against root metal accumulation (dependent var.) for the test plant.

An attempt was made to attempt a linear regression of selected leaf antioxidant parameters, using leaf metal concentration as the dependent variable (Table-5). Results showed that the selected predictors (Tocopherol, MDA, AsA, CAT, Peroxidase, SOD) did not provide a significant reliable model against the dependent variable (leaf metal) ($p>0.05$).

In order to conveniently determine which parameter was most reliable for determining the outcome of the aim of the study, as well as to suggest possible conditions that predispose the plants to achieving set objects as laid down in the study, a PCA was conducted using only selected significant results (Table-6 a,b).

From component-1, the parameters with the highest component matrices (>0.92) were those related to metal accumulations (sheath, stem and root accumulations). This means that plant performance in the study is a factor of metal accumulations. Secondly, the antioxidants SOD and tocopherol loaded highly positively on component-1 (>0.7) (Table-6a); an indication that plant performance in the context of the present study could also be monitored using these two parameters. Component-1 was selected for the study for determining characteristic measure for determining plant performance in Cu-polluted soil (Table-6b, Figure-9).

It was noted that Cu-treated plants had significantly increased CAT and SOD activities. This supports the earlier reports of Kafel *et al.*[42] on CAT activity in the above ground parts of *Philadelphus coronarius*. Lin *et al.*[43] also demonstrated increased CAT activities in *Viciafaba* in Cd-polluted medium. Increased Cu concentration enhances the development of ROS and phytotoxichydroxyl radicals[44], resulting to increased MDA levels in plant tissues. In the study, both increase and decrease in MDA content were dependent on concentration levels of Cu in soil (Table-3).

SOD, POD, and CAT are key to protecting plants during oxidative damage due to increased heavy metal concentration. These enzymes perhaps may have contributed to possible removal of ROS[45,46], although the mechanisms were not studied herein. The dismutation of superoxide molecules in hydrogen peroxide and water is catalyzed by SOD. CAT and POD would eventually break down any hydrogen peroxide formed to useful water and oxygen molecules. These ROS-remediating enzymes are therefore important go-between of oxidative impairment in plants and might be sufficient to protect biomolecules necessary for plant survival[47].

Table-5: Summary ANOVA for Regression analysis of leaf contents of antioxidants against leaf metal accumulations.

Model	ANOVA^b					
		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2606.232	6	434.372	1.741554	0.279601
	Residual	1247.082	5	249.4163		
	Total	3853.314	11			

a. Predictors: (Constant), Tocopherol, MDA, AsA, CAT, Peroxidase, SOD, b. Dependent Variable: Leaf. Metals.

Table-6a: Rescaled rotated component matrix for principal component analyses of leaf contents of antioxidants against plant metal accumulations.

	Component 1	Component 2
Sheath. Metals	0.968348	0.098694
Stem. Metals	0.944457	0.28608
Root. Metals	0.937948	0.307498
Root. SOD	0.915661	0.129401
SOD	0.780392	-0.0786
Tocopherol	0.756625	-0.02333
Leaf. Metals	0.737584	0.481823
Peroxidase	0.224135	-0.03115
CAT	-0.1055	0.066993
Plant. Height	-0.04309	-0.98598
MDA	0.160057	0.747182
Root. CAT	0.065507	-0.45199
AsA	0.047169	0.355132

Extraction Method: Principal Component Analysis., Rotation Method: Varimax with Kaiser Normalization. a. Rotation converged in 3 iterations.

Table-6b: Component transformation matrix for principal component analyses of leaf contents of antioxidants against plant metal accumulations.

Component	1	2
1	0.892386	0.451272
2	0.451272	-0.89239

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.

Conclusion

This study has established that *Axonopus compressus* has the capacity to withstand the deleterious effects of copper contamination and the capability to remove copper from polluted soils. Generally, there was over 50% loss of copper from the soil at the end of the 3-month observation. On the premise of these findings, the study therefore suggested that *Axonopus compressus* should be adopted for the remediation of copper polluted soils.

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