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Phytotoxicity Assay of Cu, Pb and Zn on *Launaea cornuta* and *Sporobolus jacquemontii* Grown in Heavy Metal contaminated soil

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ABSTRACT: In this paper, a phytotoxicity assay is used to assess the harmfulness and tolerance of *L. cornuta* and *S. jacquemontii* in phytoremediation of heavy metal-contaminated soils. The effects of Pb, Cu, and Zn concentrations in the soil on the number of leaves generated, root and shoot growth, and tolerance indices of the investigated plant species were analyzed. The experimental plants were grown in soil with 0, 100, 300, 600, 1500, 2000, 2500, and 3000 mg/kg dry soil weight (DW for Pb, Cu, and Zn). Sample preparation and laboratory analysis followed the standard methods. Data were analyzed with one-way analysis of variance (ANOVA) using GraphPad Instat 3.1 software. The growth mean of different treatments was compared using Duncan's Multiple Range Test at $p < 0.05$. Plants grown in soil containing more than 300/kg DW metal contents exhibited a significant effect on the growth of the root and shoot. Concentrations lower than 300 did not show any significant effects. At 1500 mg/kgDW, the numbers and sizes of leaves decreased very significantly, while at 2000 mg/kgDW both plants failed to survive. The toxic effects of the metals on biomass production showed inhibition in the following trend: Zn>Cu>Pb. The results suggest that hyper accumulator plants can also be affected by metals in the soils on which they grow. Therefore, the application of *L. cornuta* and *S. jacquemontii* as phytoremediation plants at higher metal soil concentrations requires the application of soil amendments to minimize the toxicity effect of metal on the plants.

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Heavy metals are naturally occurring elements with their concentration on the earth crust being low in most places (Nagajyoti *et al.,* 2010). Haphazard and rampant disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites (Mwegoha, 2008). These emissions have altered the metal concentrations in various environmental. Soil pollution by heavy metals has become a worldwide problem that is threatening the existence of various ecosystems (Garba and Barminas, 2010). This threat stems from the toxic effects that these metals have on the survival and growth of plants and animals, including humans. Toxicity may involve the oxidation of plant cells or genotoxic mechanisms (Alkorta *et al.,* 2004). Consequently, remediation of heavy metal-polluted soils is one of the significant current discussions in environmental restoration, considering the widespread use of the metal in environments worldwide and the environmental issues it raises (Rivelli *et al.,* 2012). Phytoremediation is a new and promising, cost-effective option as compared to conventional methods, which are energy-intensive and costly to implement (Mwegoha, 2008). This method is environmentally

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friendly, aesthetically acceptable, in harmony with the landscape (Rivelli *et al.,* 2012), and has the possibility of bio-recovery of metals. However, the application of plants for remediation of polluted soils is encountered with the phytotoxicity challenge, whereby plants cannot tolerate extremely high concentrations of metal because of the phytotoxicity of metal (Yadav, 2010). High levels of metal in the soil can interfere with several metabolic processes, causing toxicity to the plants, as exhibited by reduced seed germination, root and shoot growth and phytomess, chlorosis, photosynthetic impairment, stunted growth, and finally plant death. The current investigation uses a range of different metal concentrations (Cu, Zn, and Pb) to assess their phytotoxicity effects on the survival and growth of *L. cornuta* and *S. jacquemontii.*

MATERIALS AND METHODS

Soil samples were collected from the top soil to a depth between 0 and 0.15 m from the soil surface at Ardhi University. Large objects, such as stones, plastic materials, and plants, were removed from the soil. The soil was air-dried in the greenhouse. The dry soil was sieved through a 2 mm polyethylene sieve and stored in a plastic bag at room temperature until use.

Salts containing Pb, Zn and Cu ($(Pb(NO_3)_2)$, $ZnSO_4.7H_2O$ and $(CuCl_2{\cdot}2H_2O)$) were dissolved in 1.5l of distilled deionized water. The solution was poured into the soil and mixed thoroughly to artificially contaminate the soil with the final concentration of 100, 300, 600, 1,000, 1,500, 2,000, 2,500 and 3,000 mg/kg for all the three metals. These pollution levels are within the concentrations levels found in the polluted soils (Mkumbo *et al*., 2012). The polluted soil was left for one month to equilibrate before the experimental plants were transplanted. In order to achieve heavy metals/colloidal fraction equilibrium, samples were wetted and air-dried, every five days, for one-month period, according to a modification of the methodology proposed by Mart´ınez and Motto (2000).

Pot experiment: Eighty-one plastic pots (20 cm Ø and 17 cm depth) were used as containers for carrying out the phytotoxicity tests. Seven kilograms of soil were filled in each pot for the different treatments, and three replicates were taken for each treatment. An extra set of pots that contained no added HM was also taken as a control. To prevent loss of HM out of the pots through infiltration downwards, the plastic containers were sealed at the bottom. *L. cornuta and S. jacquemontii K.* were used as hyperaccumulator plants in the experiment (Mkumbo *et al*., 2012). These plants were obtained from Geita district, and they were collected in areas that had no influence from mining activities. Five seedlings were sown in each pot, and the seedlings and the pots were irrigated once every three days to supply sufficient moisture for the plant's growth.

Analysis of physical chemical parameters of the soil: The soil particle size distribution was measured by the dry sieving method, and the soil texture was read on the soil texture triangle. For pH and electrical conductivity, the soil samples were dissolved in water at 1:2.5 and 1:5 (w/v) in distilled deionized water suspension for pH and electroconductivity (EC) measurements, respectively. This was done by weighing 10 g of air-dry soil (<2 mm) into a bottle. Then 25 or 50 mL of deionized water were added, and the bottles were shaken manually at 20-minute intervals for 1 hour to allow soluble salts to dissolve and ionic exchange to reach equilibrium prior to measuring pH and electrical conductivity. pH was measured using a Hach H130 Rugged Pocket pH meter, while electrical conductivity was measured using a calibrated Hanna EC meter HI98353 DiST-3. Cation exchange capacity was determined after extraction using standard methods, followed by analysis of magnesium, calcium, potassium, and sodium. The determination of the concentration of these elements was followed by the calculation of their equivalent weight.

Analysis of total heavy metal concentration in the soil: Total heavy metal concentration was determined in soil digested using aqua regia (HNO_3: HCl (1:3)). In this process, 1 g of the soil sample was added to a conical flask with 5 ml of aqua regia, and the mixture was digested on a hot plate at 95 °C for a period of about 1 hour. The resulting mixture was diluted using 30 mL of distilled deionized water and filtered. After filtration (using Whatman No. 1 filter paper), the resulting solution was analyzed using an Atomic Absorption Spectrophotometer (AAS) from PerkinElmer® A Analyst 100.

Heavy Metal accumulation in plant: The plants were harvested by carefully uprooting the whole plant after 84 days. The harvested plants were washed using tap water to remove the residual soil particles from the leaves, stems, and roots, and thereafter rinsed with distilled deionized water. A stainless-steel knife was used to cut the plant samples into different parts (shoot and roots), which were oven-dried separately at 80 °C for 48 hours and then ground using a pestle and mortar. A 1.0 g dry, powdered plant sample was placed in a test tube with 5 mL of the mixture of aqua regia (HNO_3-HCl) and perchloric acid ($HClO_4$). The samples were then digested at 95 °C for approximately 1 hour until complete digestion. The digested samples

were cooled and then transferred to 100-mL volumetric flasks. The test tubes used for digestion were rinsed with distilled deionized water, and the rinsing water was added to the volumetric flasks to bring the volume to 100 mL, filtered using Whatman No. 1 filter paper. Metal analysis was carried out using an Atomic Absorption Spectrometer (AAS).

Measurement of growth and Biomass production: Plant length was measured at an interval of seven days each, while biomass was measured after harvesting all the plants 84 days from the day of transplanting. Plants were washed with flowing tap water, and thereafter they were rinsed with distilled, de-ionized water. Roots and shoots were separated by using a stainless-steel knife, and thereafter, they were dried at 80◦C for 48 hours and then weighed separately.

Determination phytotoxicity and tolerance index: Phytotoxicity and tolerance index were calculated using the following equations (Chintey *et al.*, 2022)

$$\%\ Phytotoxicity\ of\ shoot = \frac{SLC - SLT}{SLC} \times 100$$

Where SLC = shoot length control; SLT = shoot length treatment

$$\%\ Phytotoxicity\ of\ root = \frac{RLC - RLT}{RLC} \times 100$$

Where RLC = root length control; RLT = root length treatment

$$Tolerance\ Index = \frac{Biomass\ of\ treated\ plant}{Biomass\ of\ control\ plants} \times 100$$

Where

Statistical Analysis: All data were subjected to standard one-way analysis of variance (ANOVA) using Graph pad Instat 3.1 software. The treatment means were separated by Duncan's multiple range test (DMRT) at $p < 0.05$. Dunn's Multiple Comparison tests was used to check the differences whether they are significant or not for different treatments.

RESULTS AND DISCUSSION

The soil pH was 5.68 ± 1.04; this pH favored the growth of plants. The mobility and bioavailability of heavy metals in the soil increase with a decrease in the soil pH (Violante *et al.*, 2010). The organic matter content was 13.4 ± 2.8% (Table 1). Organic matter has a substantial influence on the availability of heavy metals since most metals have a high tendency to adsorb organic matter. A high content of organic matter in the soil can reduce the bioavailable metal species as a result of the complexation of free ions with organic matter (Machiwa, 2010), altering their availability to plants. For example, COO-groups in both solid and dissolved organic matter can form stable complexes with metal (Ren *et al.*, 2015). The organic matter contents in the soil are similar to those reported by Machiwa (2010), in which the organic matter contents in paddy farms in Mwanza and Geita were found to range from 1.8-12.4%.

Table 1. Physicochemical properties of experimental soil

Soil parameters	Units	Values
Clay %	g kg^{-1}	32.35
Silt %	g kg^{-1}	43.76
Sand %	g kg^{-1}	23.45
pH		5.68 ± 1.04
Organic matter %	%	13.4 ± 2.8
Electrical conductivity	mS cm^{-1}	612.06 ± 23.6
CEC*mol/100gm soil	Meq/100g soil	21.14

Effects of lead on L. cornuta stem height: Results show that the stem heights were increasing with time for all soils with metal concentrations ranging from 100 to 1500 mg/kg DW (Figure 1). At a concentration of 2000 mg/kg DW and above, the effects were adverse, leading to the death of the plants. *L. cornuta* planted in soil with Pb metal concentrations ranging from 100 mg/kg to 600 mg/kg DW grew well from the first week of their transplanting to the 9th week. From the 9th week on, the rate of growth was slowing, indicating that the plants were almost reaching the maximum tolerance level for metal accumulation. This is evident when comparing the growth of these plants with the plant grown in the control, which kept on increasing in length (Figure 1).

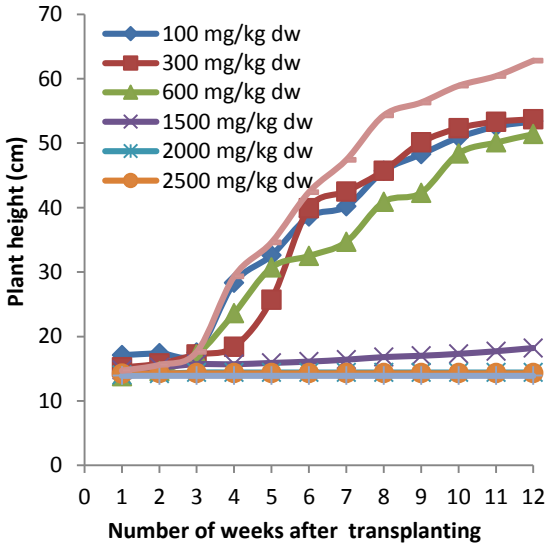

Fig 1. Effects of Pb on L. cornuta stem height

At the concentration of 1500 mg/kg DW, the plant had stunted growth with small yellow leaves; however, the plants survived to the end of the experimental time. Stunted growth and small, yellow leaves are indicators of toxicity effects on the plant. For all concentrations above this, the plants died. Toxicity may be caused by a decrease in mitotic frequency and lead accumulation in cell wall components, especially pectic substances and hemicelluloses. On the other hand, metal stress-induced reactive oxygen species (ROS) influence metabolic processes (Yadav, 2010). Under normal growth conditions, ROS levels in a plant cell are under tight control of scavenging systems that include the antioxidant glutathione (GSH) (Shao *et al.,* 2008). Excess ROS formed within cells can provoke the oxidation and modification of cellular amino acids, proteins, membrane lipids, and DNA. These changes lead to oxidative injuries and result in the reduction of plant growth and development (Branzini and Zubillaga, 2010).

Effect of Pb concentration on S. jacquemontii stem height: Results of the effects of Pb on S. *jacquemontii* stem height show that the stems were least affected at low metal concentrations, and the magnitude of the effect increased with an increase in metal concentration up to 1500 mg/kg DW (Figure 2).

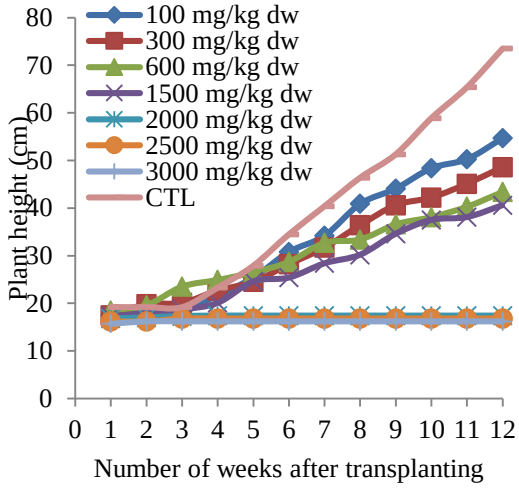

Fig 2 Effects of Pb on the S. jacquemontii stem height

Above 1500 mg/kg DW, the effect was severe, and plants died. Stem height for 100, 300, 600, 1500, 2000, 2500, and 3000 mg/kg DW Pb soil concentrations were reduced by 15.5, 21.2, 23.6, 30.4, 56.7, 58.2, and 59.5%, respectively. Statistical analysis shows that the difference was not significant for plants grown in soils with Pb concentrations between 100 and 1,500 mg/kg DW, but it was significant for plants grown at 2000 mg/kg DW and extremely significant for plants grown at 2,500 and 3,000 mg/kg DW Pb soil concentrations ($P < 0.001$). Pb exerts an effect on the morphology, growth, and photosynthetic processes of plants. These results are in agreement with Munzuroglu and Geckil, (2002) report that Pb toxicity decreased the length and dry mass of roots and shoots; furthermore, the results are similar to Opeolu *et al.,* (2010) findings that the number of branches of each plant (*Lycopersicon esculentum* L.) was reducing with an increase in levels of contamination from 300-1,800 mg/kg DW Pb.

Effects of Zinc on the stem height for L. cornuta: The effects of Zn on L. *cornuta* stem height were high to all the plants in soil with metal concentration above 1500 mg/kg DW (Figure 3). This conforms to what is reported by various literatures that increased Zn concentration contributes to toxic effects on plants. According to Mousan (2011), these effects results into reduction of ATP synthesis and Chloroplast activity leading to decline of photosynthesis process in the plant. Reduction of photosynthesis in the plant hinders the growth of the plant, with limited supply of food to the plant parts and ultimately the plant die. Results show that all the plants grown in soil with 2000 mg/kg DW and above had stunted growth at the starting of the experiment, and dried up on the fifth week. Stem height was decreasing with increase in soil metal concentration. The reduction was 4.8%, 11.5%, 23.6%, 36.3%, 67.5%, 69.3% and 72.4% for 100, 300, 600, 1,500, 2,000, 2,500 and 3,000 mg/kg DW for Zn soil concentration respectively.

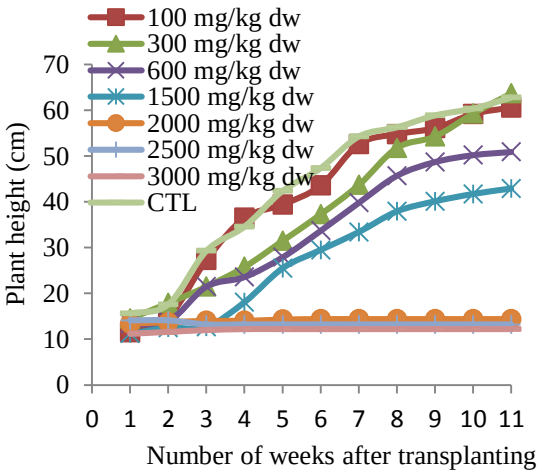

Fig 3 Effects of Zinc on the increase of L. cornuta stems height

Effects of Zinc on the stem height for S. jacquemontii: Figure 4 depicts that the stem height was insignificantly affected in all soils containing low metal concentrations (100 mg/kg–1500 mg/kg), while at higher concentrations, the plants were significantly affected by metal, leading to stunted growth and later on, plant death. This shows that zinc has an effect on the growth of *S. jacquemontii* stems. The stem growth

was reducing with the increase in concentration. A reduction in stem height is due to Zn toxicity. According to Yadav (2010), high levels of Zn in soil inhibit many plant metabolic functions, resulting in retarded growth and the aging of the leaves as the result of the gradual deterioration of the metabolic activities in the plant tissues. Excessive Zn can also give rise to manganese (Mn), phosphorus (P), and copper (Cu) deficiencies in plants, all of which directly affect the metabolic activities leading to stunted plant growth (Yadav, 2010). Several authors have reported the phytotoxicity of Zn in plants, indicated by a decrease in growth and development, metabolism, and the induction of oxidative damage in various plant species such as Tobacco (Branzini and Zubillaga, 2010).

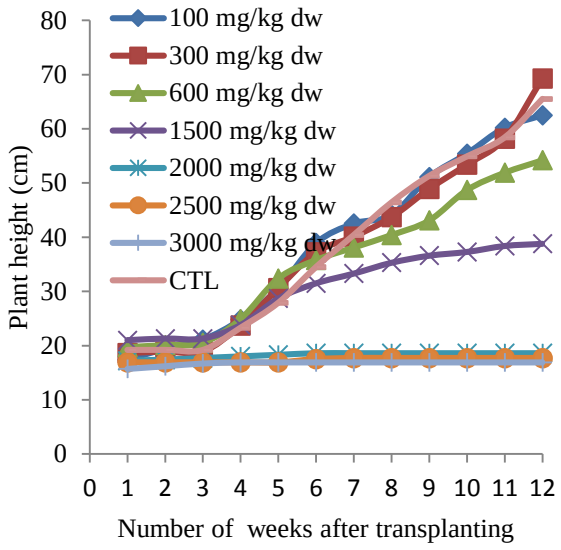

Fig 4 Effects of Zinc on the stems height

Effects of Copper on the stem height growth of L. cornuta: Copper at a higher concentration had retardation effects on the stems of *L. cornuta* (Figure 5). The level of toxicity was increasing with an increase in the soil metal concentration. The reason is that copper in soil plays a cytotoxic role, induces stress, and causes injury to plants (Gill, 2014).

These effects depend on the total concentration and fraction of metal that is bioavailable. Both plants grew well for metal concentrations between 100 and 300 mg/kg. From 600 to 1,500 mg/kg Cu content, the effect on the plant's stem growth increased with an increase in Cu concentration in the soil. According to Gill (2014), excess Cu in soil plays a cytotoxic role, induces stress, and causes injury to plants. Above 1,500 mg/kg Cu plants died.

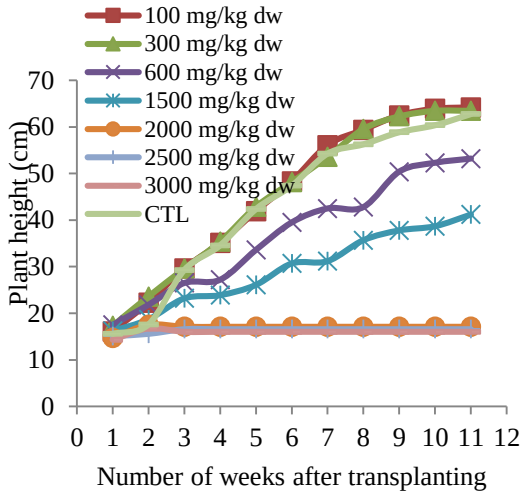

Fig 5 Effects of Cu on L. cornuta stem height growth

Effects of Copper on the stem height growth for S. Jacquemontii: Figure 6 shows the variation in plant growth with time subjected to different metal concentrations. Results show that *S. jacquemontii* has stunted growth for all soil metal concentrations above 1500 mg/kg DW and dies after the fourth week.

The effect of Cu on the stem height growth was increasing with an increase in the soil Cu concentration. Statistical analysis revealed that the stem growth was insignificantly affected by the soil Cu concentration between 100 and 1500 mg/kg, while for 2000 mg/kg the effects were very significant, and at 2,500 and 3,000 mg/kg the effect was significantly different from the control.

These effects are contributed by the copper toxicity in the plants. Figure 6 shows the variation in plant growth with time subjected to different metal concentrations. Results show that *S. jacquemontii* has stunted growth for all soil metal concentrations above 1500 mg/kg DW and dies after the fourth week.

The effect of Cu on the stem height growth was increasing with an increase in the soil Cu concentration. Statistical analysis revealed that the stem growth was insignificantly affected by the soil Cu concentration between 100 and 1500 mg/kg, while for 2000 mg/kg the effects were very significant, and at 2,500 and 3,000 mg/kg the effect was significantly different from the control. These effects are contributed by the copper toxicity in the plants. According to Jaishankar *et al.*, (2014), metal concentrations in excess of the natural requirements can lead to toxicity.

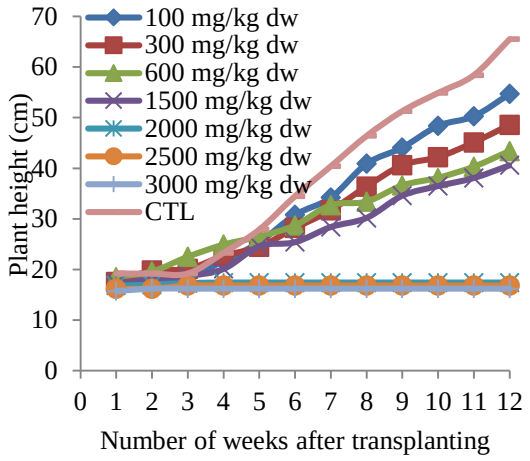

Fig 6 Effects of Cu on the S. Jacquemontii stem height growth

Phytotoxicity of shoots and roots of L. cornuta: Figure 7 shows the phytotoxicity of shoots subjected to different metal concentrations. The phytotoxicity of the shoot ranged between 14.6-78.6%, 3.0-70.9%, and 1.6-73.35% for Pb, Zn, and Cu, respectively. At higher metal concentrations, metals exert toxic effects on plants that interfere with the normal metabolic activities of the plant, leading to stunted growth. These reductions in shoot growth may be due to a decrease in photosynthesis, upsets in mineral nutrition and water balance, changes in hormonal status, effects on membrane structure and permeability (Sharma and Dubey, 2005), and secondary stress such as oxidative stress. Similar findings have been reported by Eun *et al*., (2000) that excess Pb causes a variety of toxicity symptoms in plants, such as reduced growth, chlorosis, and darkening of the root system. The order of toxicity was Pb > Cu > Zn. It is evident from Figure 7 that shoots exhibited significant effects on stem height growth at 1500 mg/kg DW metal concentrations and above for all three metals.

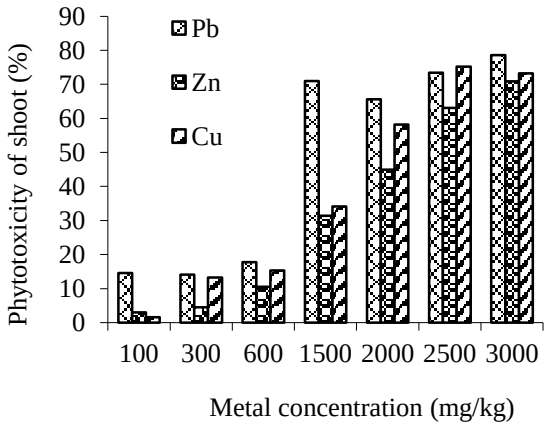

Fig 7. Phytotoxicity effects of metal on shoot *(%) of L. cornuta*

Figure 8 presents the phytotoxicity effects of Pb, Zn, and Cu on the elongation of the root of *L. cornuta* growth. The root phytotoxicity of *L. cornuta* increased from 40.4–84.6, 21.8–76.0, and 4.4–79.1% for Pb, Zn, and Cu, respectively. The order of toxicity was Pb > Cu > Zn for roots. The effect on both shoots and roots increased with an increase in soil metal concentration. Generally, the phytotoxicity effects of the root were greater than the toxicity effects of the shoot. This may have been contributed by the low transfer rate of the metal from the root to the shoot. In addition to the low translocation of metal, the roots are always in contact with the pollutants in the soil matrix, all of which might have contributed to the toxic effects on the cells of the root.

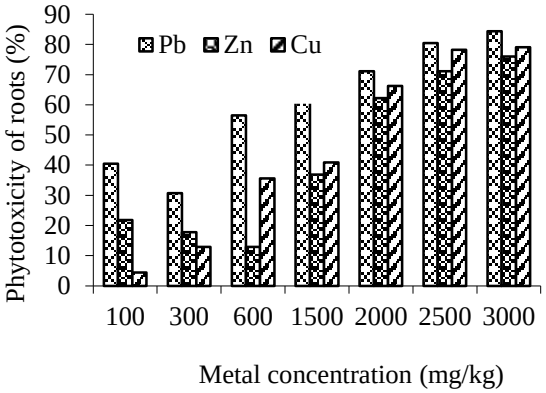

Fig 8 Phytotoxicity effect of metal on of root (%) in *L. cornuta*

Phytotoxicity of shoots and roots of S. jacquemontii: Figures 8 and 9 show the phytotoxicity of *S. jacquemontii* shoot and root. Results show generally that there were reductions in shoot and root growth of *S. jacquemontii* with an increase in the metal contents for all three metals used in the study. The length of shoots and roots for all three metals decreased insignificantly at concentrations above 1500 mg/kg. The toxicity of both the root and shoot of *S. jacquemontii* followed the same pattern: Pb > Cu > Zn. The reason for this trend is that Pb has no function in the plant; hence, it is toxic even at low concentrations. On the other hand, Cu and Zn are essential micronutrients. Zn is required in the enzyme composition catalyzing important life processes (Isak *et al*., 2013), Cu, as a redox-active transition metal, has many functions, such as being a cofactor for many enzymes, photosynthesis, and lignin formation in cell walls; however, the amount required for the normal functioning of the plant differs for this metal. A large amount of Zn (maximum 150 µg/g) is required, compared to a maximum of 50 µg/g of Cu (Pendias, 2010). This may have contributed to the low toxicity effect of the metal on *S. jacquemontii*.

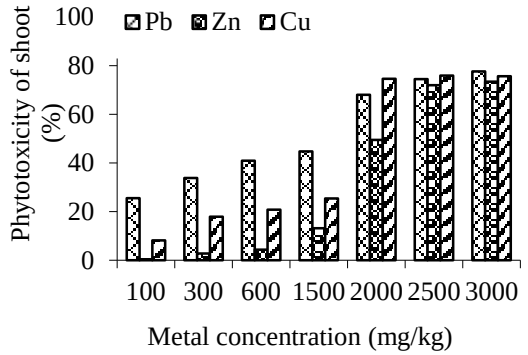

Fig 9 Phytotoxicity effect of metals on shoot (%) of *S. jacquemontii*

Phytotoxicity of shoots and roots was lower at lower heavy metal concentrations, and it was increasing with an increase in metal concentrations. The shoot phytotoxicity on *S. jacquemontii* increased from 26–78, 0–73.5, and 8.2–75.7% for Pb, Zn, and Cu, respectively. The root phytotoxicity increased from 10.3–82, 0–77.3, and 5.2-73.2% as a result of increasing metal concentrations for Pb, Zn, and Cu, respectively. Pb showed a greater impact on both the roots and shoots of *L. cornuta* and *S. jacquemontii*. According to Islam *et al.*, (2007), Pb at low concentrations inhibits the growth of both the roots and aerial parts of the plants. But this is more pronounced in roots. The reason for this is that Pb contributes to plant growth retardation by introducing nutrients, metabolic disturbances, and disturbances in photosynthesis (Islam *et al*., 2007). However, the toxic effects of Pb on root and shoot growth were observed to depend on the dose of metal in the soil. The increasing metal concentration increased the phytotoxicity of roots and shoots. These findings are similar to those of Isak *et al*., (2013), who reported that the phytotoxicity of metal on wheat was increasing with an increase in metal concentration. Elloumi *et al.*, (2007) also observed that the phytotoxicity of heavy metals on the root was greater than in the shoot, leading to a greater reduction in its length and biomass production. It could have been expected that the plants could have suffered a great deal of stress at the metal concentrations of 300 and higher, considering that the standard recommends that the soil concentration should not exceed 200 mg/kg for Pb and Cu and 150 mg/kg for Zn, but the results show that the effects were not significant at 300 mg/kg; the reason for this is that the soil that was used had significant contents of clay (32.35%) and a significant quantity of organic matter (13.4%). High organic matter in the soil has a high capacity to immobilize metals, leading to their low availability in the soil, thus lowering their uptake and hence their toxicity effects. On the other hand, the increase in clay contents increases the surface for sorption of the metal in the soil; this led to lower bioavailability of the metal for plant uptake, leading to low toxicity levels of metal (Usman *et al*., 2005).

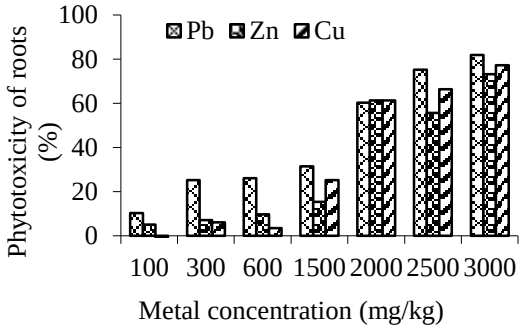

Fig 10 Phytotoxicity effects of metal on root (%) of *S. jacquemontii*

Tolerance Index of L. cornuta: The tolerance index of *L. cornuta* shows that Pb had a significant negative effect on biomass production at all metal concentrations, while Zn and Cu significantly affected the plant at concentrations above 600 mg/kg (Figure 11). This implies that despite the positive growth shown by *L. cornuta* on the number of leaves and stem height for all the concentrations from 100 up to 1500 mg/kg DW, these metal concentrations had some effects on biomass generation. This was due to the interference of metal with the normal metabolic activities of the plants. Excessive metal concentration in plants introduces an imbalance between the production of reactive oxygen species (ROS) and their consumption and interferes with photosynthesis processes (Yadav, 2010).

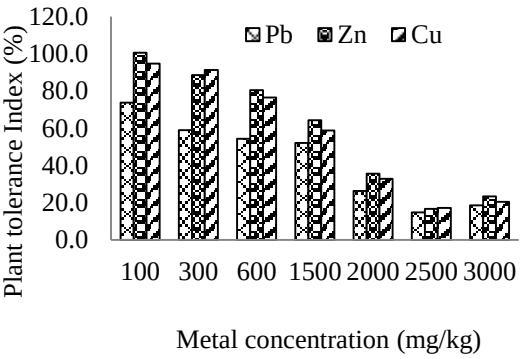

Fig 11 Metal tolerance index for L. cornuta

ROS are a product of normal cellular metabolism, but under stress conditions, the balance between the production and elimination of ROS is disturbed in cellular components of plants, leading to rapidly inactivating enzymes, damaging vital cellular organelles in plants, and destroying membranes by inducing the degradation of pigments, proteins, lipids, and nucleic acids and cell death (Hussain *et al.*, 2013).

The ROS include the superoxide radical ($O2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl radical ($HO2^{\cdot}$), hydrogen peroxide (H2O2), alkoxy radical ($RO^{\cdot}$), peroxy radical ($ROO^{\cdot}$), singlet oxygen (1O2) and excited carbonyl (RO*), all of which are cytotoxic to plants (Gill, 2014). The tolerance index (%) of metal in roots and shoot biomass production were decreasing with increase in metal concentration and they were in the following order: Zn > Cu > Pb. This signifies that the effects of metal on biomass production depend on the type and level of metal concentration in the soil.

Tolerance index of S. jacquemontii: The tolerance index of *S. jacquemontii* was decreasing with increasing metal concentrations in the soil (Figure 12). The reduction in biomass production of the plants changed under heavy metal stress conditions in *S. jacquemontii.* Plants grown in Pb polluted soils showed a lower tolerance index as compared to plants grown in Cu-polluted soil. The highest tolerance index was shown by plants grown in Zn-polluted soils. This shows that Zn exerted less toxic effects on the biomass production in *S. jacquemontii* as compared to the stresses exerted by Cu and Pb.

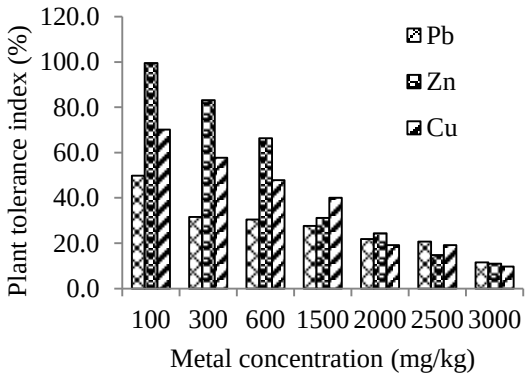

Fig 12 Effect of metal on tolerance Index of *S. jacquemontii*

The order of toxicity showed the following pattern: Pb > Cu > Zn. These findings are similar to what Athar and Ahmad (2002) observed: that Zn exerted less toxic effects on wheat as compared to Cu and Pb. For roots, biomass production was highly affected in all concentrations, with the exception of Cu metal at 100 mg/kg DW. The reason for this is that metal induced changes in metabolic processes and root morphological properties are possible reasons for the substantial decrease in the metal tolerance ability of the plants (Mahmood *et al.*, 2007). The order of toxicity of heavy metals was as follows: Pb > Cu > Zn. The findings of this study are similar to those obtained by Branzini and Zubillaga, (2010) who observed that root growth was the most sensitive phytotoxicity parameter as compared to other parameters like shoot growth.

Conclusions: The toxicity of Cu, Pb, and Zn in both plants showed the following order: Pb>Cu>Zn. At metal concentrations above 1,500 mg/kg, both *L. cornuta* and *S. jacquemontii* failed to grow properly, suggesting that amendments may be required to reduce the toxicity of the metals to these plants if phytoremediation has to be applied in heavily polluted sites. Pb exerted higher toxicity effects on both experimental plants as compared to Zn and Cu.

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REFERENCES

Alkorta, I; Hernandez-Allica, JJM; Becerril, I; Amezaga, I; Albizu, M; Garbisu, C (2004). Chelate-enhanced phytoremediation of soils polluted with heavy metals. *J. Environ. Sci. Bio-Technol.* 3, 55-70.

Athar, R; Ahmad, M (2002). Heavy Metal Toxicity: Effect on Plant Growth and Metal Uptake by Wheat, and on Free Living Azotobacter. *J. Water. Air. Soil Pollut.* 138, 165–180.

Branzini, A; Zubillaga, M.S (2010). Assessing Phytotoxicity of Heavy Metals in Remediated Soil. *Int. J. Phytoremed.* 12: 4, 335- 342.

Chintey, R; Prakash, P; Kumar, K (2022). Evaluation of Germination Attributes, Metal Tolerance Index and Phytotoxicity Index in Green Gram Cultivars [Vigna radiata (L) Wilczek] under Arsenic Toxicity. *Res. J. Agric. Sci.* 13(4): 1289–1292

Eun, SO; Youn, HS; Lee, Y (2000). Lead disturbs microtubule organization in the root meristem of *Zea mays*. *J. Physiol. Plant.* 110, 357–365.

Garba, ST; Barminas, JT (2010). Phytoremediation and the Desert towards as sustainable Environmental Development. *J. World Rural Observa.* 2 (2) 15-20.

Gill, M (2014). Heavy metal stress in plants: a review. *Intern. J. Adv. Res.* 2, (6): 1043-1055.

Hussain, A; Abbas, N; Arshad, F; Akram, M; Khan ZI; Ahmad, K; Mansha, M; Mirzaei, F (2013). Effects of diverse doses of Lead (Pb) on different growth attributes of *Zea-Mays* L. *J. Agric. Sci.* 4, (5):262-265.

Isak, RS; Parveen, RS; Rafique AS; Alamgir, AS (2013). Phytotoxic effects of Heavy Metals (Cr, Cd, Mn and Zn) on Wheat (Triticum *aestivum* L.) Seed Germination and Seedlings growth in Black Cotton Soil of Nanded, India. *Res. J. Chem. Sci.* 3, (6) 14-23.

Jaishankar, M; Tseten, T; Anbalagan, N; Mathew, BB; Beeregowda, KN (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol.* 7(2):60-72. doi: 10.2478/intox-2014-0009.

Machiwa, JF (2010). Heavy metal levels in paddy soils and rice (*oryza sativa* (l)) from wetlands of Lake Victoria basin, Tanzania. *Tanz. J. Sci.* 36, 59-72.

Islam, KR; Mahmood, T; Muhammad, S (2007). Toxic effects of heavy metals on early growth and tolerance of cereal crops. *Pakistan J. Bot.* 39(2): 451- 462.

Mkumbo, S; Mwegoha, WJ; Renman, G (2012). Assessment of the phytoremediation potential for Pb, Zn and Cu of indigenous plants growing in a gold mining area in Tanzania. *Intern. J. Environ. Sci. 2*(4), 2425–2434.

Mousan, SR (2011) Zinc in crop production and interaction with phosphorus. *Australian J. Basic. Appl. Sci*. 5 (9): 1503-1509.

Munzuroglu, O; Geckil, H (2002). Effects of metals on seed germination, root elongation and coleoptiles and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Arch. Environ. Contam. Toxicol*. 43: 203-213.

Mwegoha, WJ (2008). The Use of Phytoremediation Technology for Abatement Soil and Ground Water Pollution in Tanzania: Opportunities and Challenges. *J. of Sustainable Dev. Afr.* 10 (1):140-156

Nagajyoti, PC; Lee, KD; Sreekanth TVM (2010). Heavy metals, occurrence and toxicity for plants: A review. *J. of Environ Chem Lett* 8:199–216.

Opeolu, BO; Adenuga, OO; Ndakidemi, PA; Olujimi, OO (2010). Assessment of phyto-toxicity potential of lead on tomato (*Lycopersicon esculentum L*) planted on contaminated soils. *Intern. J. Phy. Sci.* 5 (2): 68-73

Pendias, KA (2010). Trace Elements in Soils and Plants. 4th ed. Boca Raton, FL: CRC Press.

Ren, Z; Tella, M; Bravin, MN; Comans, RNJ; Dai, J; Garnier, J; Sivry Y; Doelsch, E; Straathof, A; Benedetti, MF (2015). Effect of dissolved organic matter composition on metal speciation in soil solutions. *Chem. Geol.* 398 (2015) 61–69.

Rivelli, AR; De Maria, S; Puschenreiter, M; Gherbin, P (2012). Accumulation of Cadmium, Zinc and Copper by *Helianthus annuus L*.: impact on plant growth and uptake of nutritional elements. *Int. J. Phytoremed.* 14: 320-334.

Shao, HB; Chu, LY; Lu, ZH; Kang, CM (2008). Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *Inter. J. Biol. Sci.* 4, 8–14.

Sharma, P; Dubey, RS (2005). Lead Toxicity in Plants. *Brazilian J. Plant Physiol.* 17: 1-19.

Violante, A; Cozzolino, V; Perelomov, L; Caporale, AG; Pigna, M (2010). Mobility and Bioavailability of Heavy Metals and Metalloids in Soil Environments *J. Soil Sci. Plant Nutr.* 10 (3): 268 - 292

Usman, A; Kuzyakov, Y; Stahr, K (2005). Effect of Clay Minerals on Immobilization of Heavy Metals and Microbial Activity in a Sewage Sludge-Contaminated Soil. *J. Soil. Sed.* 5 (4) 245-252.

Yadav, SK (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African J. Bot.* 76, 167–179.