



Removal of Pb and Zn from Soil using cowpea (*Vigna unguiculata*) and maize (*Zea mays*) Plants

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ABSTRACT: This study investigated the potential of cowpea (*Vigna unguiculata*) and maize (*Zea mays*) plants to remove Pb and Zn from soil. The crops were exposed to three concentrations (100, 150 and 200 mgkg⁻¹) of each metal salts during the study. When the plants were treated with lead nitrate at a concentration of 150 mgkg⁻¹, the amount and percentage of Pb removed and accumulated within plants' tissues were 65.68 mgkg⁻¹/kg (44.79%) and 78.93 mgkg⁻¹ (53.0%) for cowpea and maize with bioconcentration factors 0.80 and 0.78 respectively. However, when the plants were assisted they had greater bioconcentration factors. Farmyard manure enhanced metal uptake by cowpea and maize significantly than EDTA. Maize extracted more Pb into its roots and translocated to shoots when assisted with EDTA than cowpea. Maize was able to translocate more Pb while cowpea translocated more Zn through the vascular system, thereby acting as phytoextractors for the different metals respectively.

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There has been continuous concern about the effect of accumulated metals such as lead (Pb), Mercury (Hg) and Chromium (Cr) especially on humans and the environment (Gardea-Torresdey *et al.*, 2004). Among heavy metals, Pb and Zn are the major contaminants found in soil, sediments, air and water. Heavy metals, unlike organic pollutants, cannot be chemically degraded or biodegraded by microorganisms. An alternative biological approach to deal with this problem is phytoremediation, that is, the use of plants to clean up polluted waters and soils (Salt *et al.*, 1995). Phytoremediation is quite effective metal removal and it is environmentally-friendly Kalay *et al.*, (1999). This cost-effective plant-based approach to remediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. Cowpea is more tolerant to drought, infertile soils and acid stress than common beans (Islam *et al.*, 2006). According to Omoloye, (2009), Maize (*Zea mays*) is the most important staple cereal in Nigeria after sorghum and millet and it has the widest geographical spread in terms of production and utilization among cereals. Maize is an annual cereal that produces extensive fibrous root system with large biomass, withstands adverse conditions and produces abundant seeds with ease of cultivation

under repeated cropping (Garbisu and Alkorta, (2001); Zhang *et al.*, (2007). Hence, this study investigated the level of removal of lead (Pb) and zinc (Zn) by two crops: Cowpea (*Vigna unguiculata*) and Maize (*Zea mays*). Three approaches were used for phytoremediation of heavy metals in this study namely: natural phytoextraction (without amendment), soil organic amendment using farm yard manure and chemical enhancement using Ethylene diamine tetra-acetate acid (EDTA).

MATERIALS AND METHODS

Dry seeds of Cowpea (*Vigna unguiculata*) accession: Tvu 3788 and Maize (*Zea mays*) accession: ACR.91SUWANI-SRC1 were collected from the International Institute of Tropical Agriculture (I.I.T.A) Ibadan. The farmyard manure (cow dung) was obtained from a local abattoir along LASU Road, Igando, Lagos. The metal salts lead nitrate and zinc nitrate as well as the chelate: ethylene diamine acetate (EDTA) was purchased from Finlab, Nigeria Ltd, Anthony, Lagos and Labio Scientific, Mushin, Lagos. The phytoremediation study was carried out at a garden along Lagos State University (LASU) Road, Akesan, Lagos. The soil used was sandy loam (silt 28.58%, clay 30.93% and sand 49.81%) with pH 6.42, Moisture content 8.18%, Total Nitrogen content 0.16 mgkg⁻¹, Phosphorus content 0.026 mgkg⁻¹, Lead

content 1.4220 mgkg^{-1} and Zinc content 0.7800 mgkg^{-1} . Three kilogram soil was mixed thoroughly and then filled into 80 black cellophane bags. The bags were perforated at the sides and bases to avoid water logging and also to increase the soil aeration. The EDTA (Chelate) and manure were added to the soils and left to stabilize for 5 days before the introduction of metal (Wu *et al.*, (2000)). The soils were watered during this period. The Eighty pots were arranged in four major groups as follows: control (untreated soil), soil with metals, soil with metals and augmented with manure and soils with metals and augmented with chelate respectively. The EDTA (Chelate) and manure were added to the soils in about 46 bags and left to stabilize for 5 days before the introduction of metal salt. The experiment was carried out under a period of 60 days (Wu *et al.*, (2000)).

Plant and Soil Sample Preparation and Digestion: After treatment, in order to check the level of metal removal, the plants and soil samples were taken to the laboratory for heavy metal analysis. Each plant sample was separated into leaves, roots, and stems and then dried at 50°C for 8 hours in an oven (Roy *et al.*, 2005). The several methods used for the metal analysis followed the procedures employed by Oladele *et al.*, (2016)

Translocation Factor (TF): To evaluate the potential of cowpea and maize for phytoextraction, the translocation factor was calculated according to Yan *et al.*, (2005). This ratio indicates the ability of these plants to translocate metals from the roots to the aerial parts of the plant according to Marchiol *et al.*, (2004).

$$TF = \frac{\text{Metal conc in aerial parts } (\mu\text{g/g})}{\text{Metal conc in roots } (\mu\text{g/g})}$$

Translocation factor (TF) values < 1 , indicated that the plant has accumulated metals and stored it largely within the roots. While Translocation factor (TF) values > 1 (greater than one) indicates translocation of accumulated metals to plants' aerial parts.

The Multiplication Coefficient (MC)/Bioconcentration Factor (BCF) was used to determine the amount of heavy metals accumulated by the plants with respects to its concentration within the soil. The BCF according to Yoon *et al.*, (2006) was also calculated using the equation:

$$MC/BCF = \frac{\text{Metal conc in roots } (\mu\text{g/g})}{\text{Metal conc in soil } (\mu\text{g/g})}$$

Where metal concentration in R is the concentration of heavy metal within the roots and metal concentration in S is the concentration of heavy metal in the soil.

The higher the BCF value the more appropriate is the plant for phytoextraction Blaylock *et al.*, (1997). BCF values greater than 2 ($BCF > 2$) were considered as high values (Mellem *et al.*, 2012).

The Plant-Soil Coefficient (PSC) was also calculated (Stoltz and Greger, 2002). It was used to determine the amount of heavy metals accumulated by the plants with respects to its concentration within the soil. This is the ratio of metal within whole plant / metal within the soil.

$$PSC = \frac{\text{Metal conc in whole plant } (\mu\text{g/g})}{\text{Metal conc in roots } (\mu\text{g/g})}$$

PCR Analysis and Gel Scoring: Genomic DNA isolation from young seedlings was by modified CTAB method and PCR Analysis followed the procedure described by Khan *et al.*, (2007); Ogundipe and Ogunkanmi (2010).

Statistical Analysis: All the experiments were conducted in triplicates. All data collected were analyzed using standard deviation, t-test and analysis of variance (ANOVA) for statistical significance at 95% confidence interval. For each measured variable the means and standard errors (SE) were calculated. Descriptive statistics were calculated using the Microcal origin 5.0 and Microsoft Excel. Graphical illustrations were done for vivid representation of the data obtained.

RESULTS AND DISCUSSION

Tables 1 to 4 show that maize translocated and extracted more lead into its tissues than cowpea whether on amended soil or not. Cowpea showed more zinc tolerance and uptake whether assisted or not than for maize following its high translocation factor, found to be greater than 1 in most cases. However, maize extracted more lead into its roots and translocated to shoots when assisted with EDTA and manure than cowpea, having its translocation factors being more than 1. However, both plants had bioaccumulation factor/plant-soil co-efficient above 1. When the plants were treated with lead nitrate at a concentration of 150 mgkg^{-1} , the amount and percentage of lead removed and accumulated within plants' tissues were 65.68 mgkg^{-1} (44.79%) and 78.93 mgkg^{-1} (53.0%) for cowpea and maize respectively (Tables 1 and 3). However, when the plants were

assisted they had greater bioconcentration factor. Farmyard manure (cow dung) enhanced metal uptake by cowpea and maize significantly than EDTA. Maize extracted more lead into its roots and translocated to shoots when assisted with EDTA than cowpea. Also, in plants treated with 200 mgkg⁻¹ of Pb and assisted with EDTA, the amount and percentage of lead removed and accumulated within plants' tissues were 67.67 mgkg⁻¹ (34.0%) and 107.03 mgkg⁻¹ (53.14%) for cowpea and maize respectively. However, in the plants treated with 100m mgkg⁻¹ of lead and augmented with farmyard manure, the amount and percentage of lead removed and accumulated within plants' tissues were 49.88 mgkg⁻¹ (49.24%) and 82.89 mgkg⁻¹ (82.14%) for cowpea and maize respectively. This was probably through mechanisms such as uptake, sequestration in roots and metal redistribution to various tissues in the shoot through phloem and xylem transport.

Table 1: Concentration of Lead (Pb) (mgkg⁻¹) found in Cowpea and the Soil after Metal Treatment and Augmentation

| Treatment concentration | Zn concentration (mgkg ⁻¹) in plant parts, Soil and Bio concentration factor (BCF) | Zn concentration (mgkg ⁻¹) in plant parts, Soil and Translocation factor (TF) | Zn concentration (mgkg ⁻¹) in plant parts, Soil and Plant-Soil Coefficient (PSCF) |
|------------------------------------|--|---|--|
| 100 mgkg ⁻¹ Zn | Root=32.70 mgkg ⁻¹ Soil = 21.23 mgkg ⁻¹ BCF=1.54 | Shoot=38.42 mgkg ⁻¹ Root=32.70 mgkg ⁻¹ TF= 1.18 | Shoot=38.42 mgkg ⁻¹ Root=32.70 mgkg ⁻¹ Soil=21.23mgkg ⁻¹ PSCF = 3.35 |
| 150 mgkg ⁻¹ Zn | Root=44.90 mgkg ⁻¹ Soil = 35.08 mgkg ⁻¹ BCF=1.28 | Shoot=59.21 mgkg ⁻¹ Root=44.90 mgkg ⁻¹ TF= 1.34 | Shoot= 59.21 mgkg ⁻¹ Root=44.90 mgkg ⁻¹ Soil=35.08mgkg ⁻¹ PSCF = 2.96 |
| 200 mgkg ⁻¹ Zn | Root=64.13mgkg ⁻¹ Soil = 49.33 mgkg ⁻¹ BCF=1.30 | Shoot=57.22 mgkg ⁻¹ Root=64.13 mgkg ⁻¹ TF= 0.89 | Shoot=24.5 mgkg ⁻¹ Root = 64.13 mgkg ⁻¹ Soil=49.33 mgkg ⁻¹ PSCF = 2.47 |
| 100 mgkg ⁻¹ Zn + EDTA | Root=21.72mgkg ⁻¹ Soil = 36.20mgkg ⁻¹ BCF=0.60 | Shoot=38.42 mgkg ⁻¹ Root=21.72 mgkg ⁻¹ TF= 1.77 | Shoot=38.42 mgkg ⁻¹ Root = 21.72 mgkg ⁻¹ Soil=36.20mgkg ⁻¹ PSCF = 1.66 |
| 150 mgkg ⁻¹ Zn + EDTA | Root=85.79 mgkg ⁻¹ Soil = 70.32mgkg ⁻¹ BCF=1.22 | Shoot=13.33 mgkg ⁻¹ Root=85.79 mgkg ⁻¹ TF= 0.81 | Shoot=13.33 mgkg ⁻¹ Root = 85.79mgkg ⁻¹ Soil=70.32 mgkg ⁻¹ PSCF = 2.20 |
| 200 mgkg ⁻¹ Zn + EDTA | Root=74.50mgkg ⁻¹ Soil =56.02mgkg ⁻¹ BCF=1.33 | Shoot=44.24 mgkg ⁻¹ Root=74.50mgkg ⁻¹ TF= 0.59 | Shoot=44.24 mgkg ⁻¹ Root=74.50mgkg ⁻¹ Soil=56.02 mgkg ⁻¹ PSCF = 2.12 |
| 100 mgkg ⁻¹ Zn + manure | Root=37.53mgkg ⁻¹ Soil =25.19 mgkg ⁻¹ BCF=1.49 | Shoot=35.02 mgkg ⁻¹ Root=37.53mgkg ⁻¹ TF= 0.93 | Shoot=35.02 mgkg ⁻¹ Root=37.53mgkg ⁻¹ Soil=25.19 mgkg ⁻¹ PSCF = 2.88 |
| 150 mgkg ⁻¹ Zn + manure | Root=56.15mgkg ⁻¹ Soil =36.44mgkg ⁻¹ BCF=1.03 | Shoot=38.13 mgkg ⁻¹ Root=56.15mgkg ⁻¹ TF= 0.67 | Shoot=38.13 mgkg ⁻¹ Root=56.15mgkg ⁻¹ Soil=36.44mgkg ⁻¹ PSCF = 1.73 |
| 200 mgkg ⁻¹ Zn + manure | Root=92.79mgkg ⁻¹ Soil =67.24mgkg ⁻¹ BCF=1.38 | Shoot=35.42 mgkg ⁻¹ Root=92.79mgkg ⁻¹ TF=0.38 | Shoot=35.42mgkg ⁻¹ Root=92.79mgkg ⁻¹ Soil=67.24 mgkg ⁻¹ PSCF = 1.91 |

These plants being able to tolerate these metals might also be due to accumulation within the cell wall without penetration into the protoplast through a kind of mechanism (Blaylock *et al.*, 1997). All treated plants showed the trend of metal accumulation in this

order: Root > Stem > Leaf. The amount of Pb removed by plants in soils treated with Pb and augmented with EDTA, was greater when compared with the amount of Zn, removed by plants in soils treated with Zn and augmented with EDTA. The maximum bioaccumulation coefficient was observed in maize plant. The soil-plant transfer factor or bioaccumulation factor, *f*, expressed as the ratio of plant metal concentration divided by the total metal concentration in soil, may be used as indicators of the plant accumulation behavior (Kimenyu *et al.*, 2009). Maize is capable of continuous phytoextraction of metals from contaminated soils by translocating them from roots to shoots. The higher the *f* factor, the more effective is the phytoextractor (Nascimento *et al.*, 2006). Based on its ability to uptake heavy metal and sensitivity to high metal pollution, maize accumulated significant amounts of heavy metals when induced through the addition of metal chelates like EDTA.

This study therefore shows that EDTA supported the plants more in Pb uptake especially in maize. This agrees with the findings of exposing plants to EDTA for a longer period (40 to 70 days) could improve metal translocation in plant tissue as well as the overall phytoextraction performance (Mark and Ronald, 2006). Farmyard manure supported both cowpea and maize more in metal tolerance and uptake due to the fact that increase in organic matter content of the soil increases metal availability in the soil. This agrees with the findings in which farmyard manure assisted Bambara nut in metal (Oladele *et al.*, 2016). This observation however, disagreed with the study that organic amendments like compost, farmyard manure or biosolids may effectively reduce the bioavailability of heavy metals in soils due to its high content of organic matter (Brown *et al.*, 1994).

All treated plants experienced reduction in the quality of DNA extracted by having decrease/increase in their total number of bands compared to their control (Table 5). However, the close values obtained from their co-efficient of similarity showed the effects of metal treatment to be minimal for these concentrations tested. This may be due to the fact that the DNA of leaves of young seedlings with few days of exposure to lead and zinc nitrate were used for the investigation and little amount of metals were found within these leaves, when amended with manure, the total number of bands of treated plants was found to be more than when not amended. The decrease in the total number of bands for all treated plants compared to their control are however a great indication of toxicity on the DNA as well as the genetic makeup of the plants hence, its effect on reproduction and

genotypes/phenotypes of successful offspring. Samples augmented with manure were found to show little or no deviation from their control groups probably because of increased organic matter in the presence of manure.

Table 2: Concentration of Lead (Zn) (mgkg^{-1}) found in Cowpea and the Soil after Metal Treatment and Augmentation

| Treatment concentration | Pb concentration (mgkg^{-1}) in plant parts, Soil and Bio concentration factor (BCF) | Pb concentration (mgkg^{-1}) in plant parts, Soil and Translocation factor (TF) | Pb concentration (mgkg^{-1}) in plant parts, Soil and Plant-Soil Coefficient (PSCF) |
|------------------------------------|---|--|--|
| 100 mgkg^{-1} Pb | Root=31.58 mgkg^{-1} Soil = 49.26 mgkg^{-1} BCF=0.73 | Shoot=23.43 mgkg^{-1} Root=31.58 mgkg^{-1} TF= 0.74 | Shoot= 23.43 mgkg^{-1} Root= 31.58 mgkg^{-1} PSCF = 1.27 |
| 150 mgkg^{-1} Pb | Root=36.58 mgkg^{-1} Soil = 45.73 mgkg^{-1} BCF=0.80 | Shoot=29.1 mgkg^{-1} Root=36.58 mgkg^{-1} TF= 0.80 | Shoot= 29.1 mgkg^{-1} Root= 36.58 mgkg^{-1} PSCF = 0.74 |
| 200 mgkg^{-1} Pb | Root=32.91 mgkg^{-1} Soil = 88.95 mgkg^{-1} BCF=0.37 | Shoot=24.5 mgkg^{-1} Root=32.91 mgkg^{-1} TF= 0.75 | Shoot=24.5 mgkg^{-1} Root= 32.91 mgkg^{-1} PSCF = 0.65 |
| 100 mgkg^{-1} Pb + EDTA | Root=47.68 mgkg^{-1} Soil = 34.30 mgkg^{-1} BCF=1.39 | Shoot=18.52 mgkg^{-1} Root=47.68 mgkg^{-1} TF= 0.38 | Shoot=18.52 mgkg^{-1} Root= 47.68 mgkg^{-1} PSCF = 1.93 |
| 150 mgkg^{-1} Pb + EDTA | Root=70.97 mgkg^{-1} Soil = 38.12 mgkg^{-1} BCF=1.22 | Shoot=13.33 mgkg^{-1} Root=70.97 mgkg^{-1} TF= 0.19 | Shoot=13.33 mgkg^{-1} Root= 70.97 mgkg^{-1} PSCF = 2.20 |
| 200 mgkg^{-1} Pb + EDTA | Root=47.25 mgkg^{-1} Soil = 109.88 mgkg^{-1} BCF=0.43 | Shoot=20.12 mgkg^{-1} Root=47.25 mgkg^{-1} TF= 0.43 | Shoot=20.12 mgkg^{-1} Root= 47.25 mgkg^{-1} PSCF = 0.61 |
| 100 mgkg^{-1} Pb + manure | Root=24.30 mgkg^{-1} Soil = 43.39 mgkg^{-1} BCF=0.56 | Shoot=25.59 mgkg^{-1} Root=24.30 mgkg^{-1} TF= 1.05 | Shoot=20.12 mgkg^{-1} Root= 47.25 mgkg^{-1} PSCF = 1.55 |
| 150 mgkg^{-1} Pb + manure | Root=56.15 mgkg^{-1} Soil = 54.51 mgkg^{-1} BCF=1.03 | Shoot=38.13 mgkg^{-1} Root=56.15 mgkg^{-1} TF= 0.67 | Shoot=38.13 mgkg^{-1} Root= 56.15 mgkg^{-1} PSCF = 1.73 |
| 200 mgkg^{-1} Pb + manure | Root=92.79 mgkg^{-1} Soil = 92.79 mgkg^{-1} BCF=1.38 | Shoot=35.42 mgkg^{-1} Root=92.79 mgkg^{-1} TF=0.38 | Shoot=35.42 mgkg^{-1} Root= 92.79 mgkg^{-1} PSCF = 1.91 |

Table 3: Concentration of lead (Pb) (mgkg^{-1}) found in Maize and the Soil after Metal Treatment and Augmentation

| Treatment concentration | Zn concentration (mg/kg) in plant parts, Soil and Bioconcentration factor (BCF) | Zn concentration (mg/kg) in plant parts, Soil and Translocation factor (TF) | Zn concentration (mg/kg) in plant parts, Soil and Plant-Soil Coefficient (PSCF) |
|--------------------------------|--|--|--|
| 100 mgkg^{-1} Zn | Root=15.25 mgkg^{-1} Soil = 33.11 mgkg^{-1} BCF= 0.46 | Shoot=49.31 mgkg^{-1} Root= 15.25 mgkg^{-1} TF= 1.95 | Shoot=49.31 mgkg^{-1} Root= 15.25 mgkg^{-1} PSCF= 3.23 |
| 150 mg/kg Zn | Root= 42.03 mgkg^{-1} Soil = 43.12 mgkg^{-1} BCF= 0.97 | Shoot= 59.41 mgkg^{-1} Root= 42.03 mgkg^{-1} TF= 1.41 | Shoot= 59.41 mgkg^{-1} Root= 42.03 mgkg^{-1} PSCF= 2.35 |
| 200 mg/kg Zn | Root= 65.43 mgkg^{-1} Soil = 42.42 mgkg^{-1} BCF= 1.54 | Shoot= 54.33 mgkg^{-1} Root= 65.43 mgkg^{-1} TF= 0.83 | Shoot= 54.33 mgkg^{-1} Root= 65.43 mgkg^{-1} PSCF= 2.82 |
| 100 mg/kg Zn + EDTA | Root= 24.69 mgkg^{-1} Soil = 39.14 mgkg^{-1} BCF= 0.63 | Shoot= 32.03 mgkg^{-1} Root= 24.69 mgkg^{-1} TF= 1.30 | Shoot= 32.03 mgkg^{-1} Root= 24.69 mgkg^{-1} PSCF= 1.45 |
| 150 mg/kg Zn + EDTA | Root= 62.47 mgkg^{-1} Soil = 32.12 mgkg^{-1} BCF= 1.95 | Shoot= 37.95 mgkg^{-1} Root= 62.47 mgkg^{-1} TF= 0.61 | Shoot= 37.95 mgkg^{-1} Root= 62.47 mgkg^{-1} PSCF= 3.13 |
| 200 mg/kg Zn + EDTA | Root= 104.41 mgkg^{-1} Soil = 40.08 mgkg^{-1} BCF= 2.61 | Shoot= 39.81 mgkg^{-1} Root= 104.41 mgkg^{-1} TF= 0.38 | Shoot= 39.81 mgkg^{-1} Root= 104.41 mgkg^{-1} PSCF= 3.59 |
| 100 mg/kg Zn + manure | Root= 35.39 mgkg^{-1} Soil = 16.92 mgkg^{-1} BCF= 2.09 | Shoot= 45.48 mgkg^{-1} Root= 35.39 mgkg^{-1} TF= 1.29 | Shoot= 45.48 mgkg^{-1} Root= 35.39 mgkg^{-1} PSCF= 4.78 |
| 150 mg/kg Zn + manure | Root= 52.09 mgkg^{-1} Soil = 29.35 mgkg^{-1} BCF= 1.77 | Shoot= 59.12 mgkg^{-1} Root= 52.09 mgkg^{-1} TF= 1.14 | Shoot= 59.12 mgkg^{-1} Root= 52.09 mgkg^{-1} PSCF= 3.79 |
| 200 mg/kg Zn + manure | Root= 70.12 mgkg^{-1} Soil = 32.48 mgkg^{-1} BCF= 2.16 | Shoot= 80.31 mgkg^{-1} Root= 70.12 mgkg^{-1} TF= 1.15 | Shoot= 80.31 mgkg^{-1} Root= 70.12 mgkg^{-1} PSCF= 4.68 |

Table 4: Concentration of Zinc (Zn) (mg/kg) found in the Roots and Shoots of Maize after Metal Treatment and Augmentation

| Treatment concentration | Pb concentration (mgkg^{-1}) in plant parts, Soil and Bio concentration factor (BCF) | Pb concentration (mgkg^{-1}) in plant parts, Soil and Translocation factor (TF) | Pb concentration (mgkg^{-1}) in plant parts, Soil and Plant-Soil Coefficient (PSCF) |
|------------------------------------|---|--|--|
| 100 mgkg^{-1} Pb | Root=34.43 mgkg^{-1} Soil = 35.87 mgkg^{-1} BCF=0.96 | Shoot=28.68 mgkg^{-1} Root=35.87 mgkg^{-1} TF= 0.74 | Shoot=28.68 mgkg^{-1} Root=34.43 mgkg^{-1} PSCF = 1.76 |
| 150 mgkg^{-1} Pb | Root=36.58 mgkg^{-1} Soil = 62.0 mgkg^{-1} BCF=0.59 | Shoot=32.26 mgkg^{-1} Root=36.58 mgkg^{-1} TF= 0.88 | Shoot= 32.26 mgkg^{-1} Root=36.58 mgkg^{-1} PSCF = 1.11 |
| 200 mgkg^{-1} Pb | Root=63.85 mgkg^{-1} Soil = 81.86 mgkg^{-1} BCF=0.78 | Shoot=53.26 mgkg^{-1} Root=63.85 mgkg^{-1} TF= 0.83 | Shoot=53.26 mgkg^{-1} Root= 64.13 mgkg^{-1} PSCF = 1.44 |
| 100 mgkg^{-1} Pb + EDTA | Root=30.80 mgkg^{-1} Soil = 51.33 mgkg^{-1} BCF=1.06 | Shoot=40.84 mgkg^{-1} Root=30.80 mgkg^{-1} TF= 1.33 | Shoot=40.84 mgkg^{-1} Root= 30.80 mgkg^{-1} PSCF = 2.46 |
| 150 mgkg^{-1} Pb + EDTA | Root=58.61 mgkg^{-1} Soil = 42.47 mgkg^{-1} BCF=1.38 | Shoot=45.62 mgkg^{-1} Root=58.61 mgkg^{-1} TF= 0.78 | Shoot=45.62 mgkg^{-1} Root= 58.61 mgkg^{-1} PSCF = 2.50 |
| 200 mgkg^{-1} Pb + EDTA | Root=49.22 mgkg^{-1} Soil = 52.92 mgkg^{-1} BCF=0.93 | Shoot=57.80 mgkg^{-1} Root=49.22 mgkg^{-1} TF= 1.17 | Shoot=57.80 mgkg^{-1} Root= 49.22 mgkg^{-1} PSCF = 2.01 |
| 100 mgkg^{-1} Pb + manure | Root=56.38 mgkg^{-1} Soil = 15.28 mgkg^{-1} BCF=3.69 | Shoot=26.52 mgkg^{-1} Root=56.38 mgkg^{-1} TF= 0.47 | Shoot=26.52 mgkg^{-1} Root=56.38 mgkg^{-1} PSCF = 5.43 |
| 150 mgkg^{-1} Pb + manure | Root=56.15 mgkg^{-1} Soil = 25.64 mgkg^{-1} BCF=2.19 | Shoot=38.13 mgkg^{-1} Root=56.15 mgkg^{-1} TF= 0.65 | Shoot=38.13 mgkg^{-1} Root=56.15 mgkg^{-1} PSCF = 3.61 |
| 200 mgkg^{-1} Pb + manure | Root=83.62 mgkg^{-1} Soil = 56.50 mgkg^{-1} BCF=1.48 | Shoot=57.32 mgkg^{-1} Root=83.62 mgkg^{-1} TF=0.69 | Shoot=57.32 mgkg^{-1} Root=56.50 mgkg^{-1} PSCF = 2.50 |

Those augmented with EDTA and those without augmentation had similarity index showing great deviation from their control groups. This was probably due to reduced organic matter contents of the soil and the ability of the plants to uptake the metals. However, the metal treatment did not affect the quality of the DNA especially in cowpea treated samples. This may also be due to the short period of exposing cowpea and maize to lead and zinc required by the phytoremediation technique employed in this experiment. This work agrees with the findings that higher plants have been reported to produce varied response to heavy metals in their environment and these metals may interfere with the genetic constitution of plants (De wolf *et al.*, 2004). Similarly, the genotoxicity of heavy metals in kidney-bean (*Phaseolus vulgaris*) seedlings was studied and subjected to RAPD (Random Amplified Polymorphic DNA) analysis. Polymorphisms were evident in all treated plants as the presence and/or absence of DNA fragments in treated samples. A high number of both missing bands and new amplified fragment were observed. It was also found that missing bands and new fragments show the mutagenic effect of heavy metals on *P. vulgaris* (Enan, 2006). The unique bands (300bp, 405bp and 580 bp) obtained from this study which was common to most treated plants' genotypes especially maize plant clearly revealed the tolerance abilities of these plants and would act as marker for assessment of environmental doses of lead and zinc. These bands can be considered as potential markers to identify metal tolerant genotypes or may even be useful when converted into a simple-sequence PCR based marker that can be used for large-scale lead and zinc tolerance screening of genotypes. Markers related to drought tolerance in bread wheat genotypes using RAPD markers were also found (Pakniyat *et al.*, 2007). Molecular markers for new promising drought tolerant lines of rice under drought stress through RAPD-PCR and ISSR markers were also found (Youssef *et al.*, 2010). The identification of DNA markers diagnostic of Pb and Zn tolerance can accelerate the development of cultivars that can remain productive even under Pb and Zn stresses, and may be the starting point for identifying the specific genes responsible for differences in the response of plant genotypes to toxic lead and zinc levels.

It has been suggested that composting these plants and then applying the compost of the metals especially zinc to a Zn-deficient soil could be an effective technique for remediation of contaminated soils and redistribution of the zinc as a plant nutrient for Zn-deficient soils (Oladele *et al.*, 2016). The plants used for remediation can be incinerated to prevent transfer of metals through the food chain.

However, it has been suggested that the metals (Pb and Zn) can be removed chemically and the plants be used as fodder for animals. Also, after remediation, these plants can still be allowed to undergo recovery if replanted in clean (metal-free) soils with high organic matter content (nutrients) after remediation.

Table 5: Total number of bands by all the Primers and the Coefficient of Similarity among Control and Some Treated plants

| Lanes | Samples | Number of bands for all primers | Coefficient of Similarity |
|-------|---|---------------------------------|---------------------------|
| 5 | Control Cowpea | 29 | 0.76 |
| 2 | 100 mgkg ⁻¹ Zn + Manure Cowpea | 21 | 0.76 |
| 8 | 150 mgkg ⁻¹ Pb Cowpea | 22 | 0.80 |
| 9 | 200 mgkg ⁻¹ Pb + Manure Cowpea | 26 | 0.80 |
| 12 | 150 mgkg ⁻¹ Zn+ EDTA Cowpea | 19 | 0.70 |
| 14 | 150 mgkg ⁻¹ Pb + EDTA Cowpea | 18 | 0.58 |
| 4 | Control Maize | 24 | 0.53 |
| 7 | 200 mgkg ⁻¹ Pb + Manure Maize | 23 | 0.63 |
| 11 | 100 mgkg ⁻¹ Pb Maize | 18 | 0.73 |
| 13 | 200 mgkg ⁻¹ Zn Maize | 19 | 0.73 |

Conclusion: Farmyard manure enhanced metal uptake by cowpea and maize significantly than EDTA. Maize extracted more Pb into its roots and translocated to shoots when assisted with EDTA than cowpea. Maize was able to translocate more Pb while cowpea translocated more Zn thereby acting as phytoextractors for the different metals respectively. Generally, treatment effects of lead and zinc nitrates were minimal on the plants' DNA.

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