REMEDIATIVE POTENTIAL OF BAMBARA NUT ON LEAD AND ZINC POLLUTED SOILS

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ABSTRACT

The ability of Bambara nut (*Vigna subterranea*, Accession TVSU 102) in the phytoextraction and accumulation of lead and zinc, in polluted soils was investigated in a pot experiment. The soils were polluted with 100, 150 and 200 $mgkg^{-1}$ of lead and zinc nitrates respectively. Chelant Ethyldiaminetetraacetic acid (EDTA) and farmyard manure (cow dung) were added to increase the uptake of the metals to aerial biomass. Bambara nut extracted more Pb from the soil augmented with EDTA and manure. Plants sown in augmented soil had greater bioconcentration factor (1.25, 1.55, 1.62 and 1.65). Bambara nut was observed to accumulate more Zn within its tissues whether or not the soil was augmented or not, having bioconcentration and Plant soil co-efficient factor greater than one. When the plant was treated with lead nitrate at a concentration of 150 $mgkg^{-1}$, the amount of Pb removed and accumulated within plant tissues was 25.84 $mgkg^{-1}$. Farmyard manure enhanced metal uptake by bambara nut significantly more than EDTA. When the soil was treated with 150 $mgkg^{-1}$ of Pb but assisted with EDTA, the amount of Pb removed and accumulated within plant tissues was 84.20 $mgkg^{-1}$. However, when plants from soils treated with 150$mgkg^{-1}$ Pb was augmented with manure it accumulated 95.01 $mgkg^{-1}$. The same trend was observed for Zn bioaccumulation by the plants. Bambara nut (Accession TVSU 102) can stabilize Pb and Zn within its roots by immobilizing them. The treatment effects of the metal salt (Pb and Zn) were minimal on plant genomic DNA. Amendments (the use of farmyard manure and EDTA) generally improved Pb and Zn phytoextraction by the plant from the soils to a great extent. The results suggest that further Pb and Zn removal could be achieved by successive revegetation over a growing period.

Key words: Phytoextraction, metal excluder, phytostabilization, amendment, bioconcentration factor, co-efficient of similarity

INTRODUCTION

As a consequence of the industrial revolution, there is an enormous and increasing use of heavy metals that leads to high anthropogenic emission of heavy metals in the biosphere. Non-radioactive metals (Cadmium, Copper, Mercury, Lead and Zinc) and radioactive metals (Caesium and Uranium) are the most important metallic pollutants (Buckers, 2007). The presence of metals is an environmental concern when their concentrations begin to affect human health and the environment. Heavy metals are adsorbed by initial fast reactions (minutes, hours) followed by slow adsorption reactions (days) and are redistributed into different chemical forms with varying bioavailability, mobility and toxicity (Buckers, 2007). Phytoextraction may reduce the levels of heavy metals in sediments to acceptable levels with time, because metals are translocated to easily harvestable plant parts.

A few plants are able to survive and reproduce heavily on polluted soils or sediments with Pb, Cd, Cu and Zn (Baker, 1987). Bambara nut (*Vigna subterranea* (L.)Verdec.) is a legume crop that can withstand drought, resist pests and diseases and is able to thrive well in soils that are not chemically rich. In Botswana it is grown under semi-arid conditions by small farmers as a minor crop. Nwaichi *et al.* (2010) investigated the phytoextraction of copper and cadmium from crude oil-contaminated soils using Bambara nut.

Much work has not been carried out on the capability of Bambara nut in remediating Pb and Zn metal polluted soils. Presently, there are some exotic species available for phytoextraction application and within these species very few cultivars are available. It would therefore be imperative to identify new plant cultivars (local species) that can extract or accumulate heavy metals using amendments like farmyard manure

and helpful chemicals. The main objectives of the study were: (i) to investigate the ability of bambara nut to extract, stabilize or accumulate lead and zinc with or without amendments (ii) to investigate the effect of the metals (Pb and Zn) on the genomic DNA of treated plants.

Table 1: Selected Physico-Chemical Characteristics of Soil Samples Used for the Experiment.

Soil parameters	Values Obtained
Soil type	**Sandy loam (silts 28.58%, clay 30.93% and sand 49.81%).**
Soil pH	**6.42**
Organic matter	**3.28 %**
Total Organic carbon	**3.2 %**
Moisture content	**8.18%**
Total Nitrogen content	**0.16** $mgkg^{-1}$
Phosphorus content	**0.026** $mgkg^{-1}$
Lead content	**1.4220** $mgkg^{-1}$
Zinc content	**0.7800** $mgkg^{-1}$

MATERIALS AND METHODS

Dry seeds of Bambara nut (*Vigna subterranea*) accession Tvsu 102 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 Lagos State University (LASU) Road, Igando, Lagos. The metal salts (lead nitrate and zinc nitrate) and chelant; ethylene diamine acetate (EDTA) were purchased from Finlab, Nigeria Limited, Anthony, Lagos and Labio Scientific, Mushin, Lagos. The concentrations of the metals (Pb and Zn) were determined by the root growth inhibition test as described by Jorge and Arruda (1997) and considering the certified reporting regulatory limits by US EPA (2001) and DPR-EGASPIN (2002), the concentrations of the metals (100 $mgkg^{-1}$, 150 $mgkg^{-1}$ and 200 $mgkg^{-1}$) were of acceptable levels. The concentrations of Ethylene diamine acetate (EDTA) were determined following the method by Nascimento *et al.* (2006).

Soil Analysis Before Planting

The soil type, pH and Total Organic Matter were determined following the method by White (2006) (Table 1). The Total Nitrogen, Available Phosphorus and Moisture Content were estimated according to the procedure by AOAC (1990). The amount of Pb and Zn present in the soil was determined according to the procedure by Lone *et al.* (2008).

Experimental Description

The soil was mixed thoroughly and then filled into 60 black cellophane bags. Each cellophane bag has a radius 13 cm and height 17 cm. Three thousand grams (3 kg) of soil were placed in each bag. Within each bag, a depth of 4 cm above the soil surface was maintained for the addition of manure and water. The bags were arranged in four (4) rows designated as control (untreated soil), soil with metals, soil with metals and augmented with manure and soils with metals and augmented with chelant respectively. The EDTA (Chelant) and manure were added to the soils in about 18 bags and left to stabilize for five days before the introduction of metal salt. The experiment was carried out under a period of 60 days (Wu *et al.*, 2000).

Analysis of Plant and Soil for Heavy Metal Content

The treated plant and soil samples were digested with concentrated HNO_3 + $HClO_4$ following a modified method described by Lone *et al.,* (2008). The plant and soil samples were analyzed for lead (Pb) and zinc (Zn) accumulation ($mgkg^{-1}$ DW) using Atomic Absorption Spectrometer (AAS) (Lone *et al.,* 2008).

Determination of the Translocation Factor/ Bioconcentration Factor and the Plant-Soil Coefficient

Multiplication Coefficient (MC) /Bioconcentration Factor (BCF) according to Stoltz and Greger (2002) were also calculated using the equation:

$$MC/BCF = \frac{\text{Concentration of R } (\mu g/g)}{\text{Concentration in S } (\mu g/g)}$$

where R is the concentration of heavy metal in the roots and S is the concentration of heavy metal in soil).

The Plant-Soil Coefficient (PSC): This is the ratio of metal concentration (whole plant) / metal concentration (soil) according to Stoltz and Greger (2002) was also calculated:

$$PSF = \frac{\text{Concentration of P}(\mu g/g)}{\text{Concentration in S } (\mu g/g)}$$

where P is the concentration of heavy metal in the plants and S is the concentration of heavy metal in soil.

The Translocation Factor (TF):

The translocation factor for metals within a plant was expressed by the ratio of metal concentration (shoot) / metal concentration (root) (Stoltz and Greger, 2002).

$$TF = \frac{\text{Concentration of S } (\mu g/g)}{\text{Concentration in R } (\mu g/g)}$$

where S is the concentration of heavy metal in the shoot and R is the concentration of heavy metal in soil.

Genomic DNA Isolation

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 a t-test. The standard errors (SE) and Analysis of Variance (ANOVA) performed were used for statistical significance at 95% confidence interval. Descriptive statistics were calculated using the Microcal origin 5.0 and Microsoft Excel.

RESULTS AND DISCUSSION

The results below on Tables 2 and 3 show that without assistance, bambara nut extracted less Pb compared to when augmented with EDTA and Manure. However, Bambara nut was observed to accumulate a lot of Zn within its tissues whether augmented or not, having bioconcentration and Plant Soil Co-efficient factor greater than one. When the plants were treated with lead nitrate at a concentration of 200 $mgkg^{-1}$, the amount of lead removed and accumulated within plant tissues was 91.19 $mgkg^{-1}$ (Table 2). However, when the soils were augmented the plant had greater bioconcentration factor. When treated with 200 $mgkg^{-1}$ of Pb but assisted with EDTA, the amount of Pb removed and accumulated within plants' tissues was 124.85 $mgkg^{-1}$. However, when the plant was treated with 200 $mgkg^{-1}$ of Pb and augmented with manure, the amount of Pb removed and accumulated within plants' tissues was 128.21 $mgkg^{-1}$. Farmyard manure (cow dung) enhanced metal uptake by bambara nut more significantly than EDTA. The same trend was observed for Zn bioaccumulation by the plants (Table 3). Bambara nut showed the trend of metal accumulation in this order: Root > Stem > Leaf. It was also observed that plants assisted with EDTA removed greater amount of lead than zinc (Tables 2 and 3). Lead and zinc translocation in Bambara nut from roots to shoots whether on amended soil or not were minimal because most metals remained at the root zone. Roots can actively exude substances like organic acids and nitrogenous compounds which improve soil nutrients and enhance metal bioavailability, tolerance and uptake. Bambara nut displayed the property of an "excluder" having a translocation factor well below 1 and little or no metals actually got translocated to its stem and

leaves, especially when the soil was not augmented. This is confirmed by Kimenyu *et al.* (2009), that 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. According to Stoltz and Greger (2002) metal excluder species have translocation factor to be typically lower than 1. Bambara nut was able to retain these metals especially zinc at its root zone possibly with the help of some plant exudates and manure which increased the soil organic matter. It was able to stabilize/immobilize these metals at the root zones possibly through mechanisms such as accumulation in roots by vacuole sequestration, cell wall binding, complex formation by root exudates, precipitation within root zone due to little or no xylem loading and translocation. According to Ryan *et al.* (2001) roots can actively exude protons and other substances like organic acids and nitrogenous compounds. This has to do with the nutrient acquisition of the plant, especially that of P, Mn, Fe and Zn and is also influenced by other factors like Ph. According to Baker (1987), Pb accumulated mostly in the roots of *Vicia faba*. Baker (1987) also grouped plants into accumulators or excluders and observed that excluder plants reduces uptake of elements.

Table 2: Mean Concentration of Lead (Pb) ($mgkg^{-1}$) Uptake in the Roots and Shoots of Bambara Nut

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 = 48.67 $mgkg^{-1}$ Soil = 49.32 $mgkg^{-1}$ BCF = 0.99	Shoot=1.33 $mgkg^{-1}$ Root =48.67 $mgkg^{-1}$ TF = 0.03	Shoot=1.33 $mgkg^{-1}$ Root = 48.67 $mgkg^{-1}$ Soil = 49.32 $mgkg^{-1}$ PSCF = 1.01
150 $mgkg^{-1}$ Pb	Root = 25.63 $mgkg^{-1}$ Soil = 108.84 $mgkg^{-1}$ BCF = 0.24	Shoot = 0.204 $mgkg^{-1}$ Root = 25.63 $mgkg^{-1}$ TF = 0.01	Shoot = 0.204 $mgkg^{-1}$ Root = 25.63 $mgkg^{-1}$ Soil = 108.84 $mgkg^{-1}$ PSCF = 0.24
200 $mgkg^{-1}$ Pb	Root = 87.91 $mgkg^{-1}$ Soil = 106.33 $mgkg^{-1}$ BCF = 0.83	Shoot = 3.28 $mgkg^{-1}$ Root = 87.91 $mgkg^{-1}$ TF = 0.04	Shoot = 3.28 $mgkg^{-1}$ Root = 87.91 $mgkg^{-1}$ Soil = 106.33 $mgkg^{-1}$ PSCF = 0.86
100 $mgkg^{-1}$ Pb + EDTA	Root = 33.79 $mgkg^{-1}$ Soil = 40.51 $mgkg^{-1}$ BCF = 0.83	Shoot = 25.52 $mgkg^{-1}$ Root = 33.79 $mgkg^{-1}$ TF = 0.76	Shoot = 25.52 $mgkg^{-1}$ Root = 33.79 $mgkg^{-1}$ Soil = 40.51 $mgkg^{-1}$ PSCF = 1.46
150 $mgkg^{-1}$ Pb + EDTA	Root = 70.97 $mgkg^{-1}$ Soil = 51.44 $mgkg^{-1}$ BCF = 1.25	Shoot = 13.33 $mgkg^{-1}$ Root = 70.97 $mgkg^{-1}$ TF = 0.50	Shoot = 13.33 $mgkg^{-1}$ Root = 70.97 $mgkg^{-1}$ Soil = 51.44 $mgkg^{-1}$ PSCF = 1.86
200 $mgkg^{-1}$ Pb + EDTA	Root = 88.40 $mgkg^{-1}$ Soil = 56.90 $mgkg^{-1}$ BCF = 1.55	Shoot = 36.45 $mgkg^{-1}$ Root = 88.40 $mgkg^{-1}$ TF = 0.41	Shoot = 36.45 $mgkg^{-1}$ Root = 88.40 $mgkg^{-1}$ Soil = 56.90 $mgkg^{-1}$ PSCF = 2.19
100 $mgkg^{-1}$ Pb+ manure	Root = 30.72 $mgkg^{-1}$ Soil = 31.80 $mgkg^{-1}$ BCF = 0.97	Shoot = 0.28 $mgkg^{-1}$ Root = 30.72 $mgkg^{-1}$ TF = 0.001	Shoot = 0.28 $mgkg^{-1}$ Root = 30.72 $mgkg^{-1}$ Soil = 31.80 $mgkg^{-1}$ PSCF = 0.98
150 $mgkg^{-1}$ Pb + manure	Root = 61.25 $mgkg^{-1}$ Soil = 56.12 $mgkg^{-1}$ BCF = 1.62	Shoot = 33.76 $mgkg^{-1}$ Root = 61.25 $mgkg^{-1}$ TF = 0.03	Shoot = 33.76 $mgkg^{-1}$ Root = 61.25 $mgkg^{-1}$ Soil = 56.12 $mgkg^{-1}$ PSCF = 1.67
200 $mgkg^{-1}$ Pb + manure	Root = 92.79 $mgkg^{-1}$ Soil = 70.10 $mgkg^{-1}$ BCF = 1.65	Shoot = 35.42 $mgkg^{-1}$ Root = 92.79 $mgkg^{-1}$ TF = 0.04	Shoot = 35.42 $mgkg^{-1}$ Root = 92.79 $mgkg^{-1}$ Soil = 70.10 $mgkg^{-1}$ PSCF = 1.72

Table 3: Mean Concentration of Zinc (Zn) ($mgkg^{-1}$) Uptake in the Roots and Shoots of Bambara Nut

Treatment concentration	Zn concentration ($mgkg^{-1}$) in plant parts, Soil and Bioconcentration 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 = 55.92 $mgkg^{-1}$ Soil = 39.10 $mgkg^{-1}$ BCF = 1.49	Shoot=2.20 $mgkg^{-1}$ Root = 55.92 $mgkg^{-1}$ TF = 0.04	Shoot=2.20 $mgkg^{-1}$ Root = 55.92 $mgkg^{-1}$ Soil = 39.10 $mgkg^{-1}$ PSCF = 1.49
150 $mgkg^{-1}$ Zn	Root = 94.94 $mgkg^{-1}$ Soil = 48.71 $mgkg^{-1}$ BCF = 1.95	Shoot = 1.39 $mgkg^{-1}$ Root = 94.94 $mgkg^{-1}$ TF = 0.02	Shoot = 1.39 $mgkg^{-1}$ Root = 94.94 $mgkg^{-1}$ Soil = 48.71 $mgkg^{-1}$ PSCF = 1.98
200 $mgkg^{-1}$ Zn	Root = 84.88 $mgkg^{-1}$ Soil = 75.0 $mgkg^{-1}$ BCF = 1.13	Shoot = 1.03 $mgkg^{-1}$ Root = 84.88 $mgkg^{-1}$ TF = 0.01	Shoot = 1.03 $mgkg^{-1}$ Root = 84.88 $mgkg^{-1}$ Soil = 75.0 $mgkg^{-1}$ PSCF = 1.15
100 $mgkg^{-1}$ Zn + EDTA	Root = 43.17 $mgkg^{-1}$ Soil = 48.17 $mgkg^{-1}$ BCF = 0.90	Shoot = 1.04 $mgkg^{-1}$ Root = 43.17 $mgkg^{-1}$ TF = 0.05	Shoot = 1.04 $mgkg^{-1}$ Root = 43.17 $mgkg^{-1}$ Soil = 48.17 $mgkg^{-1}$ PSCF = 0.92
150 $mgkg^{-1}$ Zn + EDTA	Root = 87.21 $mgkg^{-1}$ Soil = 52.18 $mgkg^{-1}$ BCF = 1.69	Shoot = 1.00 $mgkg^{-1}$ Root = 87.21 $mgkg^{-1}$ TF = 0.01	Shoot = 1.00 $mgkg^{-1}$ Root = 87.21 $mgkg^{-1}$ Soil = 52.18 $mgkg^{-1}$ PSCF = 1.69
200 $mgkg^{-1}$ Zn + EDTA	Root = 61.63 $mgkg^{-1}$ Soil = 93.12 $mgkg^{-1}$ BCF = 0.66	Shoot = 0.83 $mgkg^{-1}$ Root = 61.63 $mgkg^{-1}$ TF = 0.01	Shoot = 0.83 $mgkg^{-1}$ Root = 61.63 $mgkg^{-1}$ Soil = 93.12 $mgkg^{-1}$ PSCF = 0.67
100 $mgkg^{-1}$ Zn + manure	Root = 40.62 $mgkg^{-1}$ Soil = 42.88 $mgkg^{-1}$ BCF = 0.95	Shoot = 5.00 $mgkg^{-1}$ Root = 40.62 $mgkg^{-1}$ TF = 0.12	Shoot = 5.00 $mgkg^{-1}$ Root = 40.62 $mgkg^{-1}$ Soil = 42.88 $mgkg^{-1}$ PSCF = 1.06
150 $mgkg^{-1}$ Zn + manure	Root = 74.20 $mgkg^{-1}$ Soil = 58.44 $mgkg^{-1}$ BCF = 1.27	Shoot = 11.94 $mgkg^{-1}$ Root = 74.20 $mgkg^{-1}$ TF = 0.16	Shoot = 11.94 $mgkg^{-1}$ Root = 74.20 $mgkg^{-1}$ Soil = 58.44 $mgkg^{-1}$ PSCF = 1.47
200 $mgkg^{-1}$ Zn + manure	Root = 71.79 $mgkg^{-1}$ Soil = 46.41 $mgkg^{-1}$ BCF = 1.86	Shoot = 66.62 $mgkg^{-1}$ Root = 71.79 $mgkg^{-1}$ TF = 0.15	Shoot = 66.62 $mgkg^{-1}$ Root = 71.79 $mgkg^{-1}$ Soil = 46.41 $mgkg^{-1}$ PSCF = 2.15

Proteins such as channel type in the root plasma membrane of tobacco have been identified to mediate cross-membrane movement of Pb (Arazi *et al*., 1999). Zn is an essential micronutrient and is mobile in plants. Vacuolar zinc trafficking in *Silene vulgaris* has been observed (Chardonnes *et al*., 1999). Also, Bambara nut being able to tolerate these metals might also be due to accumulation within the cell wall without penetration into the protoplast; a mechanism described by Abdul (2010). The chelant (EDTA) was also observed to remove more lead than zinc. This agrees with the findings of Mark and Ronald (2006) that 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. To the best of our knowledge, this is the first report on the use of bambara nut in removing Pb and Zn directly from heavy metal polluted soils and the effect of these metals on its DNA.

The DNA analysis showed that the primer sequence involved four primers (OPJ-12, OPJ-13, OPO-08 and OPO-09) and the band range/sizes of the primers were between 1500bp-200bp. The banding patterns of the primers is shown on Figures 2 to 4. The treated plants experienced decrease in their total number of bands compared to their control. When assisted with manure, the plants had their total number of bands to be more than the treated plants. Infact, bambara nut treated with 200 $mgkg^{-1}$ of lead and assisted with manure had a total of 31 bands while the control had 23. 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 of plant samples following the results obtained from the dendogram (Figure 1). The dendogram showed similarity coefficient of both treated and control plant samples. All the plant samples fell in the same cluster. The similarity coefficients ranged from 0.51 to 0.80 .The lowest similarity index (0.51) was shown by bambara nut treated with 150 $mgkg^{-1}$ Pb. However, the similarity index of treated plant samples was found to vary differently from the control samples probably because of increased organic matter in the presence of manure (Table 4 and Figure 1). 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.

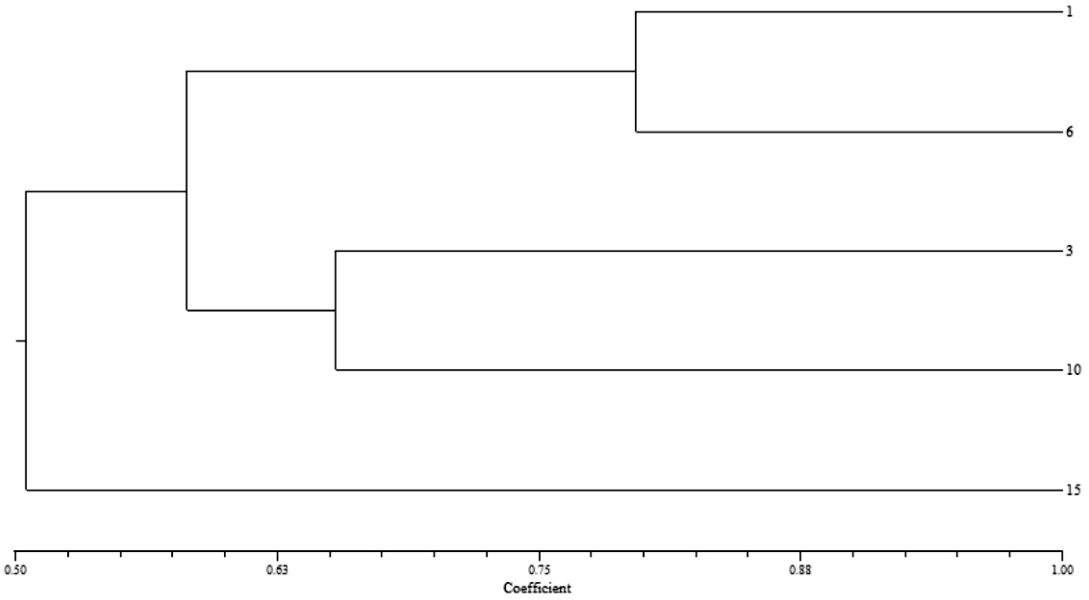


Figure 1: The Dendrogram of Coefficient of Similarities Among the Control and Treated Bambara Nut Plants Based on Band Polymorphisms Generated by PCR After Using the Primers.

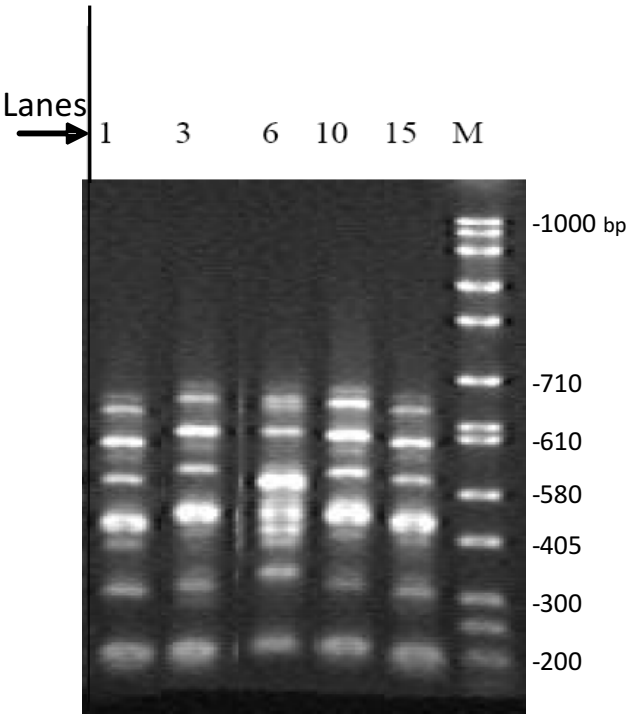


Figure 2: RAPD-PCR Amplification Based on the use of Primer OPJ-12.

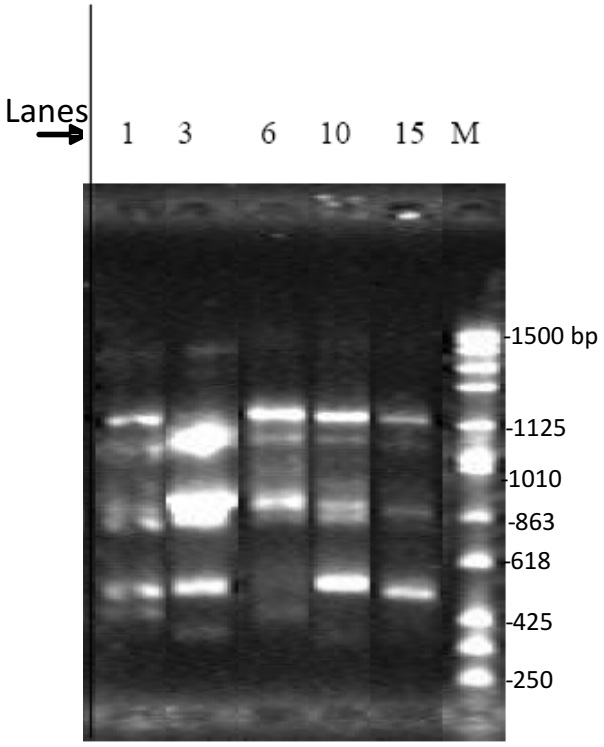
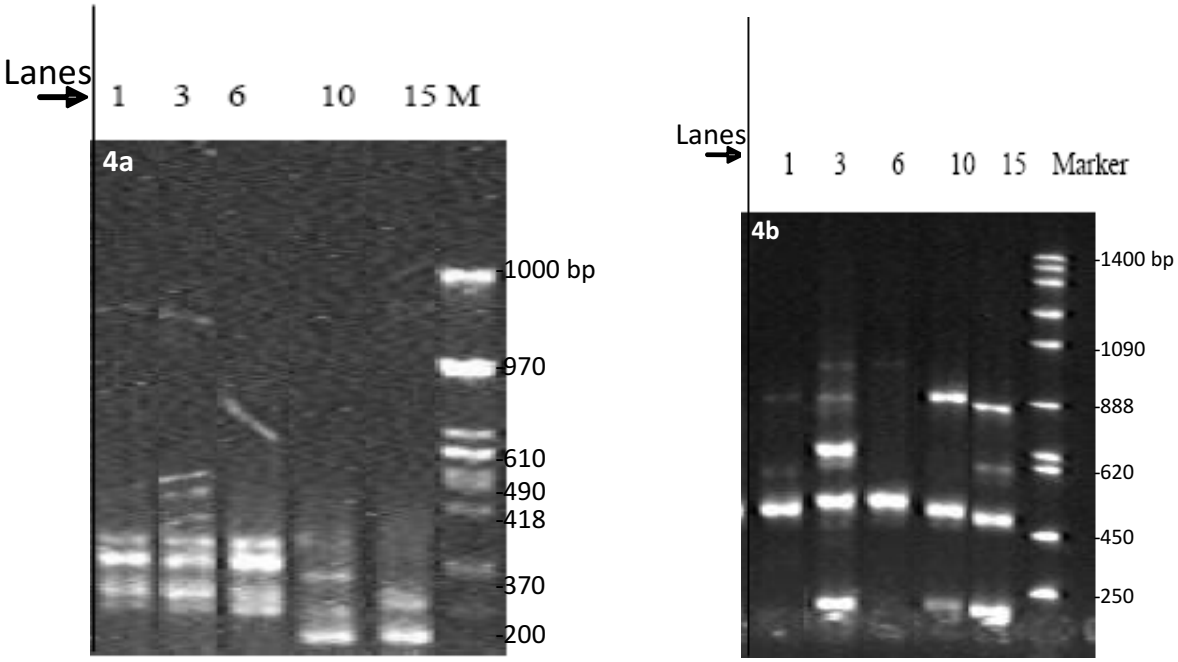


Figure 3: RAPD-PCR Amplification Based on the use of Primer OPJ-13.



Figures 4a & b: RAPD-PCR Amplification Based on the use of Primer OPO-08. .

Note: Lane 1: Control Bambara Nut. Lane 3: Manure + 200 mgkg^{-1} Pb Bambara Nut, Lane 6: Manure + 150 mgkg^{-1} Zn Bambara Nut, Lane 10: 100 mgkg^{-1} Zn + Edta Bambara Nut, Lane 15:150 mgkg^{-1} Pb Bambara Nut.

Table 4: 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
1	Control Bambara nut	23	0.79
3	200 mgkg^{-1} Pb + Manure Bambara nut	31	0.66
6	150 mgkg^{-1} Zn + Manure Bambara nut	27	0.79
10	100 mgkg^{-1} Zn + EDTA Bambara nut	22	0.66
15	150 mgkg^{-1} Pb Bambara nut	19	0.51

The findings of this study is at variance with the findings of De Wolf *et al.* (2004) 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. Fragment 300bp for lanes 1, 10 and 15 were visualized using primer OPJ-12. Fragment 405bp for lanes 1, 3, 6, 10 and 15 were also visualized using primer OPJ-12. Fragment 580bp for lanes 3, 10 and 15 were also visualized using primer OPJ-12. These unique bands (300bp, 405bp and 580 bp) were observed to be common to most treated plant genotypes. These clearly revealed the tolerance abilities of these plants and would act as marker for assessment of environmental doses of Pb and Zn. 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. Many researchers exploited DNA markers and detected some markers of abiotic stress. Youssef *et al.* (2010) found molecular markers for new promising drought-tolerant lines of rice under drought stress through RAPD-PCR and ISSR markers.

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 Pb and Zn levels. Bambara nut (Accession Tvsu 102) can stabilize Pb and Zn within its roots by immobilizing them. 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.

CONCLUSION

The study of remediative potential of Bambara nut on Pb and Zn polluted soils has shown that Bambara nuts sown in augmented soil had greater bioconcentration factor. Bambara nut was observed to accumulate more metals within its tissues whether or not the soil was augmented or not. Farmyard manure enhanced metal uptake by bambara nut significantly more than EDTA and that the treatment effects of the metal salts (Pb and Zn) were minimal on plant genomic DNA. Amendments (the use of farmyard manure and EDTA) generally improved Pb and Zn phytoextraction by the plant from the soils to a great extent. It was suggested that further Pb and Zn removal could be achieved by successive revegetation over a growing period. The plants used for remediation can be incinerated to prevent transfer of metals through the food chain. The systemic screening of plant species and genotypes for metal accumulation and resistance will enhance the spectra of genetic material available for optimization of phytoremediation technology and application on a commercial scale.

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