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Screening of Pearl Millet for Phytoextraction Potential in Soil Contaminated with Cadmium and Lead

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ABSTRACT

Pot experiments were designed to assess the potential of pearl millet (*Pennisetum glaucum*) in the phytoextraction of cadmium (Cd) and lead (Pb) from contaminated soil. A moderately contaminated clayey loam was loaded singly with 0 – 150 mg $kg^{-1}$of Cd and Pb, followed by ethylenediaminetetraacetic acid (EDTA) and/or poultry manure treatments. Pearl millet was grown and observed weekly for changes in height, leaf breadth; and post-harvest shoot wet/dry biomass and Cd/Pb concentrations. Growth profiles were essentially sigmoid with growth rates appearing to decelerate with metal dose. Based on the potting media, growth rates and millet biomass followed the order: soil-metal-manure > soil-metal-EDTA-manure > soil-metal > soil-metal-EDTA and were less in Cd- than Pb-loaded soils. Tissue metal concentrations in the various potting media were: soil-metal (22.2 – 83.5 mg Cd/kg; 16.2 – 63.5 mg Pb/kg); soil-metal-EDTA (46.5 – 105.2 mg Cd/kg; 35.3 – 86.6 mg Pb/kg); soil-metal-manure (4.6 – 38.2 mg Cd/kg; 5.0 – 25.6 mg Pb/kg); soil-metal-EDTA-manure (6.3 – 45.7 mg Cd/kg; 6.0 – 35.2 mg Pb/kg). Soil-to-millet transfer factors, f (%) showed that cadmium ($8.8 \le f(\%) \le 89.1$) was more phytoavailable to millet than Pb ($5.0 \le f(\%) \le 59.4$). The findings may be useful in the phytoremediation of soils moderately contaminated by Cd and Pb.

Keywords: *Cadmium, lead, contaminated soil, phytoavailability, phytoremediation, biomass, pearl millet, chelant, poultry manure*

1. INTRODUCTION

Aside the natural sources (soil parent material, windblown dusts, volcanic eruptions, marine aerosols, and forest fires), most other possible sources of toxic metals in contaminated soils and growth media are traceable to uncontrolled anthropogenic activities from agriculture (fertilizers, sewage sludges and animal wastes used as amendments, pesticides and irrigation water); energy production (emissions from power stations); mining and smelting (metal tailings, smelting, refining and transportation); secondary metal production and recycling operations (melting of scrap, refining, plating alloying); urban-industrial complexes (incineration of wastes and waste disposal) and automobile emissions (combustion of petroleum fuels) (Reichman, 2002; Basta *et al*., 2005; Wuana and Okieimen, 2011). The toxic metals most commonly found in contaminated soils are lead, chromium, arsenic, zinc, cadmium, copper, mercury and nickel (GWRTAC, 1997). Soils are naturally able to limit the toxic effects of these metals, however, at elevated metal concentrations, this natural ability weakens and the toxic metals may be mobilised resulting in contamination of groundwater, agricultural products and other environmental media (Gismera *et al*., 2005; Fawzy, 2008). Depending on the reactions of the various metals with soils and the uptake/translocation rates by plants, metals can be categorised as those posing little risk to agriculture, those posing food chain risks, and those posing risks to plant or animal productivity (Chaney *et al*., 1996). In order to reduce the associated risks, the search for low-cost and ecologically sustainable technologies to remediate soils contaminated by the toxic metals has continued to receive much attention.

In recent times, less-invasive *in situ* technologies, such as phytoremediation, have been considered as primary remedies to minimise soil disturbance and restore soil quality and functionality (Pedron and Petruzzelli, 2011). Phytoremediation is considered as a publicly appealing (green) remediation technology that uses vegetation and associated microbiota, soil amendments and agronomic techniques to remove, contain, or render the toxic metals harmless in the soil (Vysloužilová *et al*., 2003; Helmisaari *et al*., 2007). A general rule in phytoremediation is that, native species are preferred to exotic plants, which can be invasive and endanger the balance of the ecosystem (Kimenyu *et al*., 2009). Apart from the well-known metal hyperaccumulating crops such as *Thlaspi caerulescens*, *Ipomea alpine*, *Haumaniastrum robertii*, *Astragalus racemosus* and *Sebertia acuminate* (Lasat, 2000), high metal-accumulating ability has also been reported for cereal crops such as maize (*Zea mays*), sorghum (*Sorghum bicolor*) and alfalfa (*Medicago sativa*)

(Vijayarengan, 2005; Zhuang *et al*., 2009). Recently, there are indications that these crops could become useful in the phytoremediation of moderately contaminated soils and as bioenergy precursors (Pinto *et al*., 2004; Kimenyu *et al*., 2009; Zhuang *et al*., 2009; Wuana and Okieimen, 2010; Bareen *et al*., 2012). Pearl millet (*Pennisetum glaucum*) is among the common cereals that are frequently grown and consumed by humans as food and animals as fodder. A study relating to its phytoremediation potential under the influence of plant growth regulators and a saprobic fungus has, however, only been reported recently (Bareen *et al*., 2012). More research is, therefore, required to understand issues concerning the effects of soil metals on millet growth and phytoavailability in response to soil amendments such as chelants and manures in order to screen for its phytoremediation potential.

Consequently, the specific objectives of this study were to: (i) load a parent soil with increasing doses of Cd and Pb followed by ethylenediaminetetraacetic acid (EDTA) chelant and/or poultry manure amendments, and (ii) design pot experiments to assess the phytoavailability of Cd and Pb to millet by monitoring changes in plant height, leaf breadth and post-harvest wet/dry biomass yield as well as shoot metal concentrations. The study was conducted on a composite soil sampled in the vicinity of the Benue Industrial Layout sited in Makurdi (a rapidly growing city located at 7.44ºN, 8.33ºE in the Lower Benue River Basin, a major agricultural zone in north-central Nigeria) from the months of April – October, 2012.

2. MATERIALS AND METHODS

2.1 Chemicals, Reagents and Apparatus

The disodium salt of ethylenediaminetetraacetatic acid [$Na_2EDTA.2H_2O$] used as chelant, cadmium nitrate [$Cd(NO_3)_2{\cdot}4H_2O$] and lead nitrate [$Pb(NO_3)_2$] used as artificial sources of Cd and Pb in soil were all supplied by Kermel, China. 1.0 M HNO_3 and 1.0 M NaOH solution were used for pH adjustments as the case may be. The following apparatuses were used: hand trowel, polythene bags**,** mortar and pestle, 2mm sieve, glassware (borosilicate, Pyrex), weighing balance (Gallenkamp 80), pH meter (Fisher Hydrus 300 model), shaker (Model TT 12F, Techmel and Techmel, Texas, US), an electric heater, centrifuge (Model TGL-16G, Shanghai, China), atomic absorption spectrophotometer, AAS (Buck Scientific Model 200A, Norwalk, Connecticut, US).

2.2 Soil Sampling, Artificial Contamination and Treatment with Chelant and/or Manure

Top (0 – 20 cm) soil samples were collected from a cultivated field in the vicinity of the Benue Industrial Layout, Makurdi (7.44ºN, 8.33ºE), north-central Nigeria using a chrome-plated trowel at ten different locations and composited as the parent soil. Raw poultry manure was collected from a poultry farm in Makurdi, and kept for two weeks to age. The physicochemical properties of the parent soil and the raw poultry manure used as an amendment were determined as described by Wuana *et al*. (2012a).

Table1. Some properties of parent soil and poultry manure used for the study

Property	Parent soil	Poultry manure
pH	6.8±0.1	7.2±0.0
Sand (%)	45.8±0.2	–
Silt (%)	23.0±0.1	–
Clay (%)	31.2±0.3	–
Organic matter, OM (%)	5.9±0.2	31.5±1.0
Pseudototal Cd, $[Cd]_T$ (mg kg^{-1})	2.20±2.1	0.9±0.02
Pseudototal Pb, $[Pb]_T$ (mg kg^{-1})	51.0±2.3	5.0±0.1

Mean of triplicate determinations ± standard deviation

In order to study the effect of metal dose on millet growth, the parent soil was singly spiked with increasing doses (0, 50, 100 and 150 mg kg^{-1}) of Cd and Pb according to the method of Saifullah *et al*., (2010) with some modifications. Consequently, standard metal solutions were made by dissolving 0.14, 0.27 and 0.41 g of $Cd(NO_3)_2.4H_2O$ and 0.08, 0.16 and 0.24 g of $Pb(NO_3)_2$ separately in 20-mL portions of deionised water in a beaker. The metal solutions were sprayed over a thin layer of soil, thoroughly mixed to achieve uniformity in metal spiking, placed in large polythene containers, and allowed to equilibrate with periodic mixing for 2 weeks. The soil was air-dried, and the whole procedure repeated for two additional equilibrium periods. At the end of the third equilibrium cycle, the spiked soils were sub-sampled; digested and pseudototal concentrations of Cd and Pb determined by AAS analysis.

The effects of EDTA chelant and/or poultry manure on metal phytoavailability in contaminated soil were assessed by adding to 1.0-kg portions of the metal-spiked soils, 0.05 g/kg of EDTA chelant (0.14 mmol/kg) and 52.63 g of poultry manure (5 % w/w) separately or in combination to furnish soil-metal; soil-metal-EDTA; soil-metal-manure and soil-metal-EDTA-manure potting media. Each treatment was performed in triplicate and incubated for 2 weeks at ambient temperature to simulate field conditions. Sub-samples of the amended soils were digested and pseudototal Cd and Pb contents determined by AAS analysis.

2.3 Pot Experiments with Pearl Millet

Pearl millet was chosen as a common cereal crop grown and consumed in the study area. The seeds were cold-treated (10ºC) for 3 days to break dormancy and synchronize germination prior to pot experiments (Revathi *et al*., 2010). Pot experiments adopted the procedure of Wuana *et al*., (2012a). Five seeds of millet were sown in perforated cylindrical polyvinyl chloride pots (volume = 1500 mL) each containing 1.0 kg of soil. The seedlings were thinned to three after emergence. Surface irrigation with deionised water was adopted to water the plants during growth. Night and day cycles were naturally obtained by maintaining the pots in an open area. The plants were monitored for 8 weeks for changes in height, leaf breadth, and wet/dry biomass. Prior to harvest, pots were left without watering for 1 day. The shoot biomass was harvested from the soils after the 56th day, rinsed with deionised water, and dried at 110°C for 72 h. The concentrations of Cd and Pb (mg kg^{-1} dw of millet biomass) was determined by HNO_3 – H_2O_2 digestion (Nolan *et al*., 2005) followed by AAS analysis.

2.4 Digestion of Millet Biomass

One gram of dried and ground millet biomass was digested with a mixture of 4.0 mL of 65 % v/v HNO_3 and 2.0 mL of 35 % v/v H_2O_2. The mixture was heated almost to dryness. After evaporation, 4.0 mL of concentrated HNO_3 and 2.0 mL of concentrated H_2O_2 was added to the residue and heated until a clear digest appeared. The total digestion time was about 3 h at 130ºC. The digest was made up to the 5.0 mL with 1.0 M HNO_3.

2.5 Analysis of Cd and Pb in Soil and Plant Digest

Buck Scientific standard AA Grade Cd and Pb solutions were used to calibrate the AAS machine. Calibration and measurement of elements were done on atomic absorption spectrophotometer at 228.8 nm for Cd and 217.0 nm for Pb. The instrument settings and operational conditions were done in accordance with the manufacturer's specifications.

2.6 Quality Control and Statistical Analysis of Data

Analytical grade chemicals were used to prepare standard solutions and reagents. All glassware and plastics were washed with deionised water, rinsed with (1 + 1) HNO_3 and finally with deionised water. Procedural blank samples were subjected to similar treatments using the same amounts of reagents. In all cases, measurements were performed in triplicate. By applying SPSS 17.0 Statistics (SPSS, Chicago, Ill.), one-sample T-tests were used to test the significance of differences within individual treatments; while analysis of variance (ANOVA) was used to test differences for all investigated variables during the experiment between treatments and controls at the 5 % probability level ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Assessment of Changes in Growth Parameters and Wet/Dry Biomass of Millet

The most widespread visual evidence of metal phytotoxicity is attenuation in plant growth parameters with increasing metal dose in soil (Reichmann, 2002). In this study, changes in the growth attributes (heights and leaf breadths), post-harvest wet and dry shoot biomass and metal concentrations in millet were investigated by pot experiments (figure 1) under conditions of Cd and Pb stress as well as chelant and/or poultry manure treatments.

Figure 1. Some millet plants growing in soils contaminated with Cd or Pb

Millet plants were monitored weekly for changes in height and leaf breadth with time spanning a total period of 8 weeks and results illustrated in figure 2a – b. Post-harvest shoot wet and dry biomass yields for millet determined after 8 weeks of growth in soils stressed with increasing doses of Cd/Pb and/or amended with EDTA chelant or poultry manure are presented in figure 3.

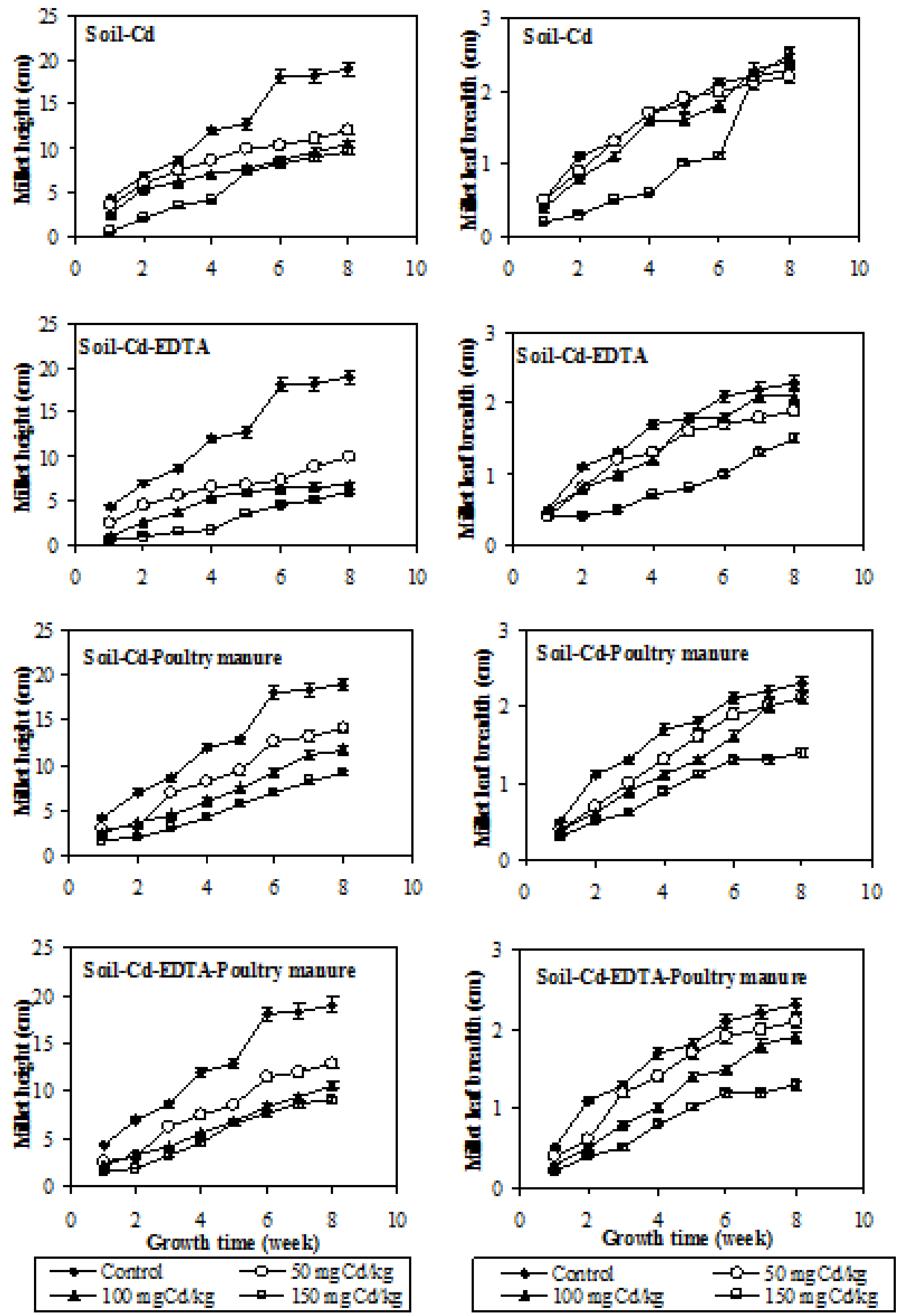


Figure 2a. Growth profiles of millet in soils loaded with increasing doses of Cd, followed by EDTA and/or poultry manure treatment

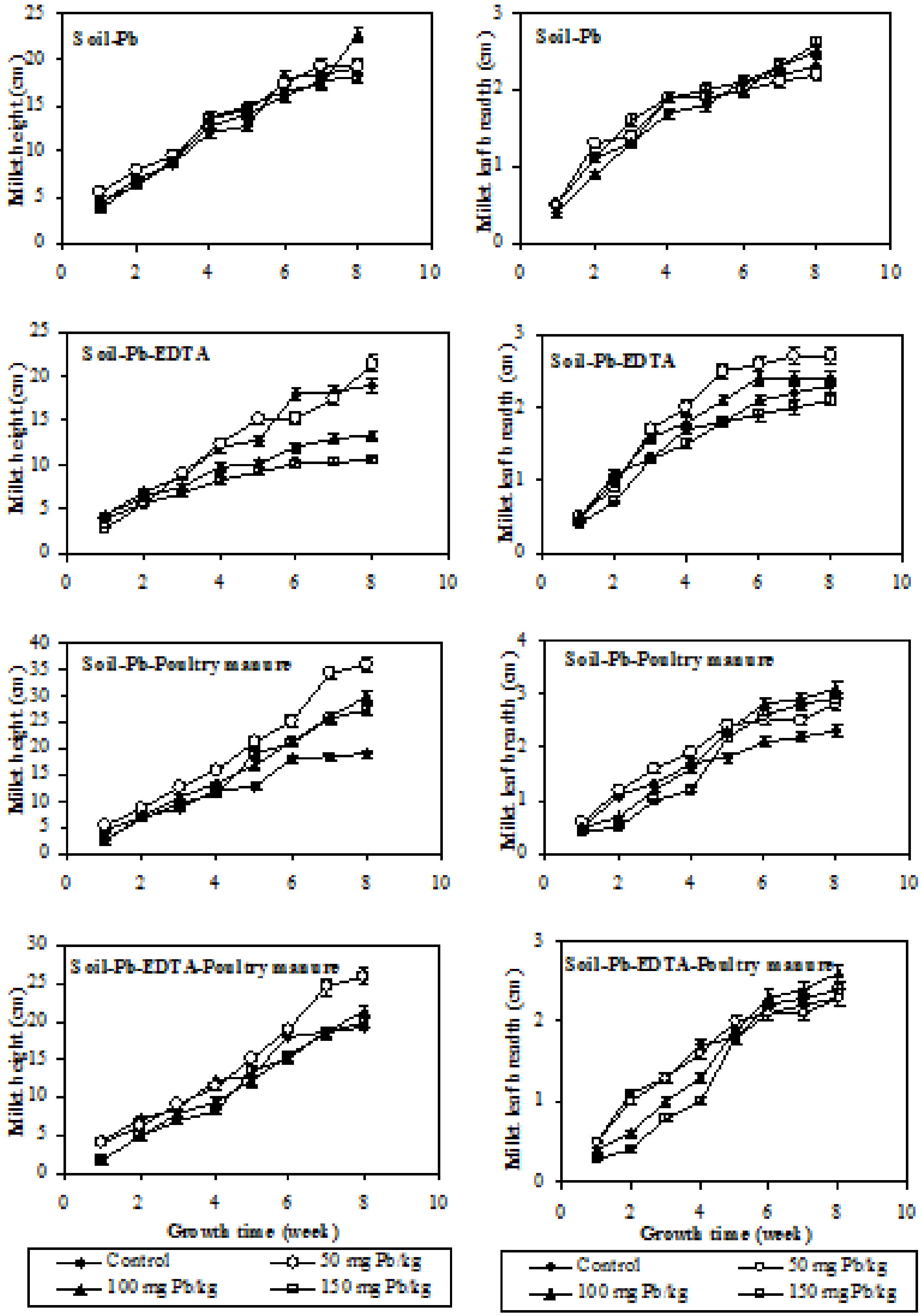


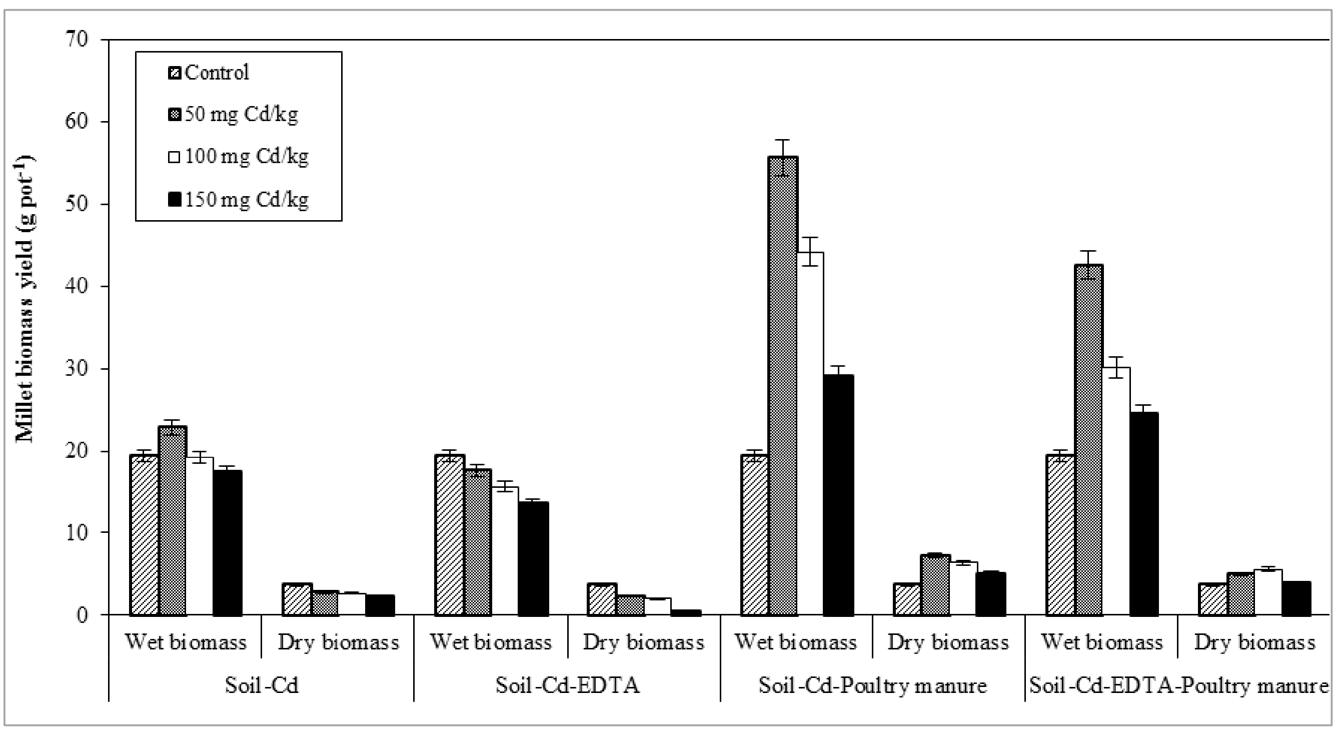
Figure 2b. Growth profiles of millet in soils stressed with increasing doses of Pb, followed by EDTA and/or poultry manure treatment

In the soil-metal potting media, growth curves of millet were essentially sigmoid (S-shape); i.e. plant heights and leaf breadths increased slowly in the first two weeks, followed by a sharp increase up to the sixth week and then retardation by the eighth week. Changes in the growth parameters with time were statistically significant ($p < 0.05$) for all the treatments. Growth rates were fastest in the control set up (parent soil) and slowed down with increasing metal stress in the soil in most potting media. Depending on metal dose in the soil, growth rates appeared to follow the order parent soil > 50 mg kg^{-1} > 100 mg kg^{-1} > 150 mg kg^{-1}. In the Cd-loaded soils, maximum millet height and leaf breadth were 19.0 and 2.5 cm, respectively (figure 2a); while in the Pb-loaded soils, maximum height and leaf breadth were 22.7 and 2.6 cm, respectively (figure 2b). The normal height of pearl millet plant may range from 50 – 400 cm.

In the soil-metal-EDTA potting media, growth profiles were still sigmoid with rates decreasing in the order of metal dose in soil. In Cd-loaded soils, maximum height and leaf breadth were 10.0 and 2.1 cm (figure 2a); while for Pb-loaded soils, corresponding growth parameters were 21.5 and 2.7 cm (figure 2b). Growth rates were slower in the presence of EDTA chelant in the soil. This is possibly due to enhanced metal solubilisation occasioned by formation of stable metal-EDTA chelates, with corresponding increase in metal phytoavailability. This effect was seemingly more pronounced in the Cd- than Pb-loaded soils explicably due to differences in the metal-EDTA conditional formation constants (Cd-EDTA; log K_f = 16.4 and Pb-EDTA; log K_f = 17.9 at 25ºC, 0.1 M ionic solution) (Neilson *et al*., 2003).

In the soil-metal-manure pots, maximum millet height and leaf breadth were 14.1 and 2.1 cm under Cd stress (figure 2a); while in Pb-stressed soils corresponding attributes were 36 and 3.1 cm, respectively (figure 2b). In the soil-metal-EDTA-manure media, maximum millet height and leaf breadth were 13.3 and 2.3 cm under Cd stress (figure 2a); while in Pb-stressed soils, corresponding growth parameters were 25.8 and 2.6 cm (figure 2b). It appeared that improved growth rates were observed relative to the control set-up and other potting media that did not receive manure. Overall the soil-metal-manure media recorded the greatest heights and leaf breadths. Observed differences in millet heights and leaf breadths were significant ($p < 0.05$) within and between individual potting media. Generally, the separation between individual growth curves appeared to be more distinct in Cd- than Pb-stressed soils signifying a greater response of millet to phytotoxicity from Cd than Pb. Additionally slower growth rates observed under Cd- than Pb-stress may suggest that millet is more tolerant to Pb- than Cd-phytotoxicity.

From figure 3 it can be seen that in the soil-metal potting media, wet and dry millet biomass yields ranged from (17.4 – 22.9) g pot^{-1} and (2.3 – 2.8) g pot^{-1}, respectively under Cd stress; while corresponding yields under Pb stress were (21.2 – 41.4) g pot^{-1} and (2.5 – 3.3) g pot^{-1}. In the soil-metal-EDTA potting media, millet wet and dry biomass yields ranged from (13.6 – 17.5) g pot^{-1} and (0.5 – 2.3) g pot^{-1}, respectively in response to Cd stress; while corresponding yields in Pb-stressed soils were (22.4 – 32.4) g pot^{-1} and (1.0 – 2.4) g pot^{-1}, respectively.



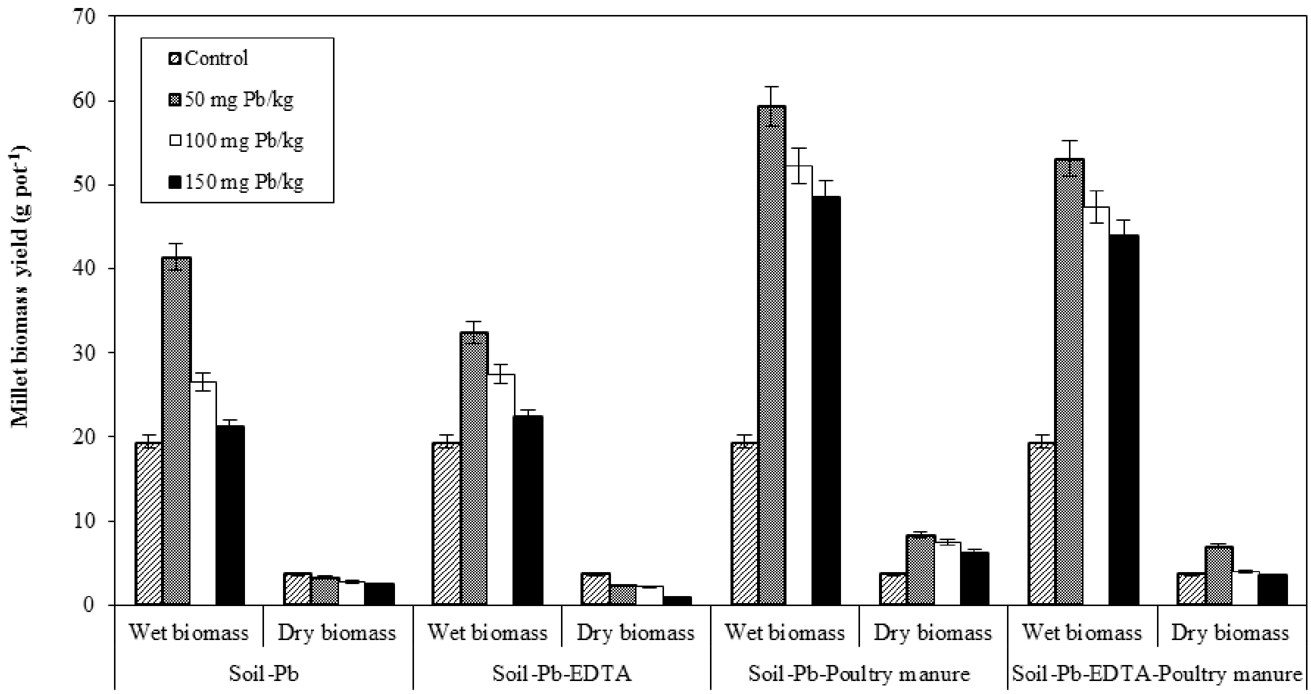


Figure 3. Post-harvest shoot biomass of millet in soils loaded separately with increasing doses of Cd and Pb, followed by EDTA and/or poultry manure treatment

The soil-metal-manure potting media gave wet and dry millet biomass yields as (29.1 – 55.6) g pot^{-1} and (5.1 – 7.2) g pot^{-1}, respectively in response to Cd dose in soil. Corresponding yields under Pb stress were (48.5 – 59.3) g pot^{-1} and (6.3 – 8.4) g pot^{-1}. In the soil-metal-EDTA-manure media, wet and dry millet biomass were (24.5 – 42.6) g pot^{-1} and (4.0 – 5.0) g pot^{-1}, respectively in Cd-loaded soils; while under Pb stress, corresponding yields were (44.0 – 53.1) g pot^{-1} and (3.6 – 7.0) g pot^{-1}.

In all cases, wet biomass yields were generally higher than corresponding dry yields. On a whole, wet and dry millet biomass decreased significantly ($0.01 \leq p \leq 0.05$) with increase in metal dose. Observed differences between the control and other potting media were also significant ($p < 0.01$). Decreased millet biomass was quite pronounced in the presence of EDTA chelant. The inclusion of manure as an amendment gave an overall enhancement in millet biomass. Depending upon the potting medium, biomass yields varied in the order: soil-metal-manure > soil-metal-EDTA-manure > soil metal > soil-metal-EDTA. Lower biomass yields were observed under Cd stress than in the case of Pb.

Overall, the foregoing observations may become useful whenever phytoextraction is the remedial option for Cd and Pb-contaminated soils. The growth of high biomass crops facilitated by optimum agronomic practices has been considered as an alternative to phytoremediation of soils contaminated by toxic metals (Zhuang *et al*., 2009).

3.2 Assessment of Changes in Metal Phytoavailability to Millet

Post-harvest shoot concentrations of Cd and Pb in millet, $[M]_p$ (figure 4) were measured to assess the response of the plant to possible phytotoxic effects. A measure of Cd and Pb transferability to millet, the soil-to-millet *transfer factor*, *f (%)* was calculated using the relationship:

$$f(\%) = ([M]_p/[M]_T) \times 100 \qquad (1)$$

where $[M]_T$ is the pseudototal metal concentration in soil.

In the soil-metal potting scenario, the ranges of $[Cd]_p$ and $[Pb]_p$ in millet were 22.4 – 83.5 mg kg^{-1} ($42.5 \leq f(\%) \leq 53.2$) and 16.2 – 63.5 mg kg^{-1} ($16.0 \leq f(\%) \leq 31.6$), respectively. In the case of the soil-metal-EDTA potting media, the ranges of $[Cd]_p$ and $[Pb]_p$ were 46.5 – 105.2 mg kg^{-1} ($63.1 \leq f(\%) \leq 89.1$) and 35.3 – 86.6 mg kg^{-1} ($35.0 \leq f(\%) \leq 59.4$), respectively. In soil-metal-manure media, $[Cd]_p$ and $[Pb]_p$ ranged from 4.6 – 38.2 mg kg^{-1} ($8.8 \leq f(\%) \leq 24.3$) and 5.0 – 25.6 mg kg^{-1} ($5.0 \leq f(\%) \leq 12.7$), respectively. The ranges of $[Cd]_p$ and $[Pb]_p$ observed in the soil-metal-EDTA-manure potting media were 6.3 – 45.7 mg kg^{-1} ($12.1 \leq f(\%) \leq 29.1$) and 6.0 – 35.2 mg kg^{-1} ($5.9 \leq f(\%) \leq 17.5$), respectively. With the exception of the soil-metal-manure media, $[M]_p$ increased linearly and significantly with metal dose in soil for Cd ($0.02 \leq p \leq 0.05$) and Pb ($0.04 \leq p \leq 0.05$). This observation which can be likened to the *salt effect* (since the metals were added as the salts, i.e. nitrates) was to be expected under the soil-metal and soil-metal-EDTA scenarios. Under normal circumstances, a curvi-linear response of $[M]_p$ to increasing metal dose, tagged the *plateau effect*, was to be expected upon the addition of poultry manure (Wuana *et al*., 2012b). The inclusion of EDTA chelant, however, nullified the so-called plateau effect in the soil-metal-EDTA media due to enhanced metal solubilisation in soil via chelation. This chelant-enhanced solubilisation, notwithstanding, manure addition still suppressed Cd and Pb uptake by millet.

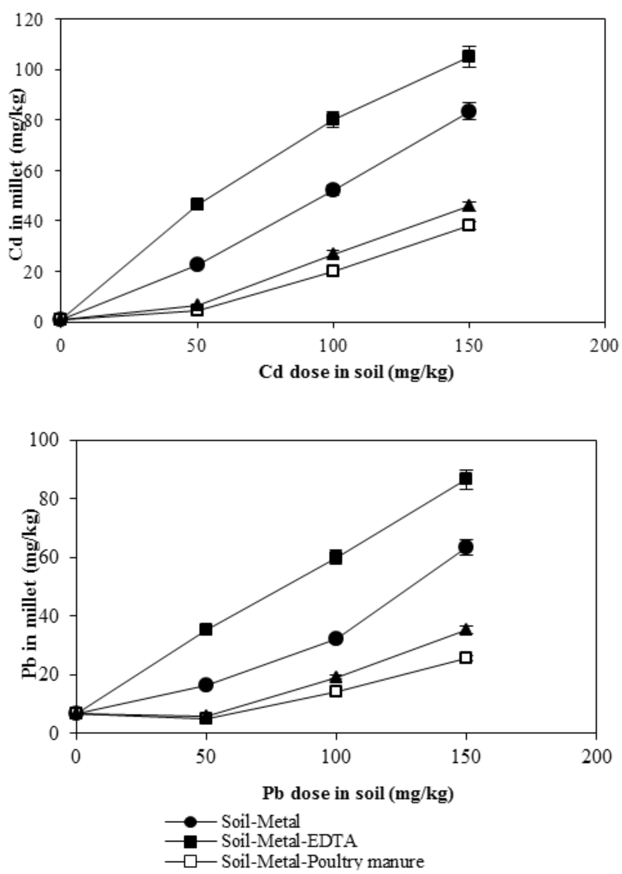


Figure 4. Post-harvest tissue Cd and Pb concentrations (mg/kg dw) in millet after 56 days of growth in soil loaded separately with increasing doses of the metals followed by EDTA and/or poultry manure treatment

Within individual potting media, only the soil-metal-EDTA scenario recorded statistically significant differences in the $[M]_p$ values for Cd ($p = 0.05$) and Pb ($p = 0.04$). Observed differences between the potting media were, however, not significant ($p > 0.05$). Depending on the potting media, $[M]_p$ and f varied in the order: Soil-metal-EDTA > soil-metal > soil-metal-EDTA > manure > soil-metal-manure.

Furthermore, $[M]_p$ and f values indicated that Cd was more phytoavailable to millet than Pb in all soils. This observation is corroborated by the lower plant biomass yields recorded in Cd- than Pb-stressed soils. Cd on one hand due to its lower affinity for metal sorbing phases (e.g., oxides, OM, silicate clays), is readily translocated to shoots at high levels before phytotoxicity is observed and has great potentials to escape the soil-plant barrier, resulting in transmission through in levels that have the potential to present risk to consumers (Chaney *et al*., 1999). Pb on the other hand is believed to be very strongly retained by the solid phase in most soils owing to its tendency to associate with insoluble weathering products formed by the oxidation of sulphides and insoluble soil fractions (Pueyo *et al*., 2008).

Tissue concentrations and soil-to-plant transfer factors also portrayed millet as a potential phytoextractor of Cd and Pb. The greater tendency of millet to accumulate more Cd than Pb may have induced phytotoxic responses like reduced growth rate and biomass yield. Since chelant and manure amendments have proved to respectively enhance and attenuate metal phytoavailability in the soil, alternate soil remediation techniques involving the application of inexpensive residues like poultry manure can reduce the solubility and environmental risk of metal toxicants.

4. CONCLUSION

The effects of increasing metal dose, EDTA chelant and poultry manure treatments on the growth and phytoextraction potential of pearl millet were studied. Growth profiles were essentially sigmoid with growth rates appearing to decelerate with metal dose. Based on the potting media, growth rates and millet biomass increased as soil-metal-manure > soil-metal-EDTA-manure > soil-metal > soil-metal-EDTA and were less in Cd- than Pb-loaded soils. Millet tissue concentrations and soil-to-millet transfer factors showed that cadmium was more phytoavailable to millet than Pb. The findings may be useful in the phytoremediation of soils moderately contaminated by Cd and Pb.

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