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Effects of Ethylenediaminetetraacetic acid (EDTA) Concentration on Extraction of Added Lead in Soil and its Uptake by Cowpea

[1]Dagari, M. S., [2]Jimoh, W. L. O. and [2*]Musa, M. S.
[1]Department of Chemistry, Federal University, Gashua, Nigeria
[2]Department of Pure and Industrial Chemistry, Bayero University, P.M.B. 3011, BUK, Kano, Nigeria
***Correspondence Email:** msmusa.chm@buk.edu.ng; m_smusa@yahoo.com

ABSTRACT
The effects of EDTA on extraction of added lead in soil and its uptake by cowpea were investigated in a growth chamber experiment. Cowpea seeds were planted and harvested in soil samples treated with Pb^{+2} added as lead nitrate at the concentrations of 0, 1000, 2000, 3000 and 4000 mg/kg with EDTA concentrations of 0, 10, 50 and 250 mg/kg. EDTA solubilized soil Pb^{+2} generated by forming a Pb-EDTA complex in a slow reversible process between pH 5.2 and pH 7.7 was highly significant ($Pr< 0.01$). This facilitated Pb uptake by cowpea leading to significant accumulation in all parts of the plant and decrease in weights of plants harvested compared to the control. From this investigation, EDTA was found to remove considerable amounts of added Pb in soil and hence could be used as remediator of Pb contaminated soils.

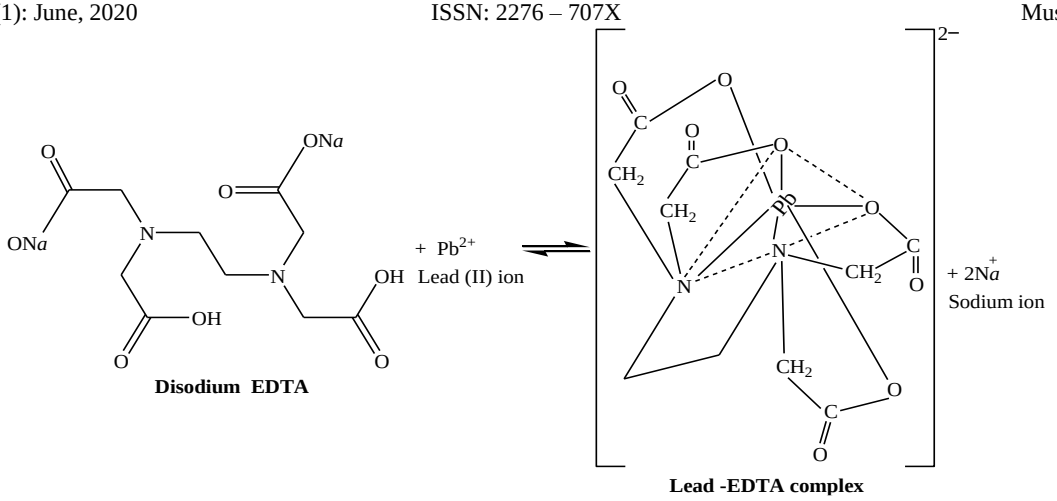
Keywords: Cowpea, EDTA, Growth chamber, Lead, Soil

INTRODUCTION

Soil quality which depends on the degree of pollution is highly associated to human health (Romic and Romic, 2003; Velea *et al.*, 2009). Soil behaves as a source of pollution that transfers pollutants to food chain as well as groundwater, and finally to humans and other animals (Sardar *et al.*, 2010). A lot of agricultural crops can be accumulated with trace elements, thereby potentially resulting in increase in the metal contents of agricultural products (Dziubanek *et al.*, 2015; Ran *et al.*, 2016; Obiora *et al.*, 2016). Plants especially vegetables which is an important part of people's diets, contain a wide range of both toxic and essential metals (Xu *et al.*, 2013; Rodrigueziruretagoiena *et al.*, 2015). Contaminated plants can (cause serious clinical and physiological problems for humans, especially when consumed in large quantities (Sharma *et al.*, 2007).

Translocation of trace elements from soil to plant is a key factor leading to contamination of these potentially toxic substances into the food chain (Ali *et al.*, 2013). Trace elements which are available for plant uptake are those that mostly exist in insoluble forms in soil solutions (Elless *et al.*, 2000). Among the common trace elements, Pb has attracted particular attention. This is because of its widespread use in industrial processes and wide distribution in the earth's crust. Lead is considered to be a nonessential element to animals and plants, but high levels cause problems to the environment and human health. Lead adsorption onto roots has been documented to occur in several plant species: Vigna unguiculata (Kopittke *et al.*, 2007), Festuca rubra (Ginn *et al.*, 2008), Brassica juncea (Meyers *et al.*, 2008), Lactuca sativa (Uzu *et al.*, 2009), and Funaria hygrometrica (Krzesłowska *et al.*, 2009, 2010). Once lead has penetrated into the root system, it may accumulate there or may be translocated to aerial plant parts. For most plant species, the majority of absorbed lead (approximately 95% or more) is accumulated in the roots, and only a small fraction is translocated to aerial plant parts, as has been reported in Vicia faba, Pisum sativum, and Phaseolus vulgaris (Shahid *et al.*, 2011). Translocation of lead to aerial plant parts increases in the presence of organic chelators like ethylenediaminetetraacetic acid (EDTA) (Zaier *et al.*, 2010; Barrutia *et al.*, 2010).

Ethylenediaminenetetraacetic acid (EDTA) continues to be explored extensively for soil treatment because of its ability to mobilize metal cations efficiently coupled with only a minor impact on the physical and chemical properties of the soil matrix. Huang *et al.* (1997) reported that EDTA is the most effective Pb ion chelator (Scheme 1).



Scheme 1: Structures of Disodium EDTA and Lead – EDTA Complex

Considering the environmental and health hazards caused by lead, it would be important to investigate its isolation in soil and accumulation in plant grown on the soil. This is important because information would be provided as to the levels of lead especially in plant parts grown in the considered area. Therefore, the present work is aimed at evaluating the effects of EDTA on the isolation of Pb^{2+} in soil and its subsequent accumulation in plant parts (cowpea).

Materials and Methods

Reagents/Apparatus

All reagents and all metal salts used in this study were of analytical grade purity and used without further purification. Deionized water used was prepared in a Milli-Di Millipore machine (SAS 67120 MOLSHEM, France). Glassware and plastic containers were washed with detergent, rinsed with distilled water and then soaked in 10% HNO_3 for 24 hr. Finally, the materials were washed with deionized water and dried in an oven at 80°C for 24 hr.

A growth chamber which regulates the temperature at 26 ± 2°C during a daily 14-hr photoperiod and at 18 ± 2°C during darkness was used to provide uniform atmospheric and climatic conditions for the plant growth.

Sampling

The soil sample and cowpea seeds (V*ignaunguiculata*) used for this study were collected from International Institute of Tropical Agriculture (IITA) farm in Wasai village, Minjibir local government area of Kano state. Figures 1 to 3 show the locations of the sampling and planting sites.

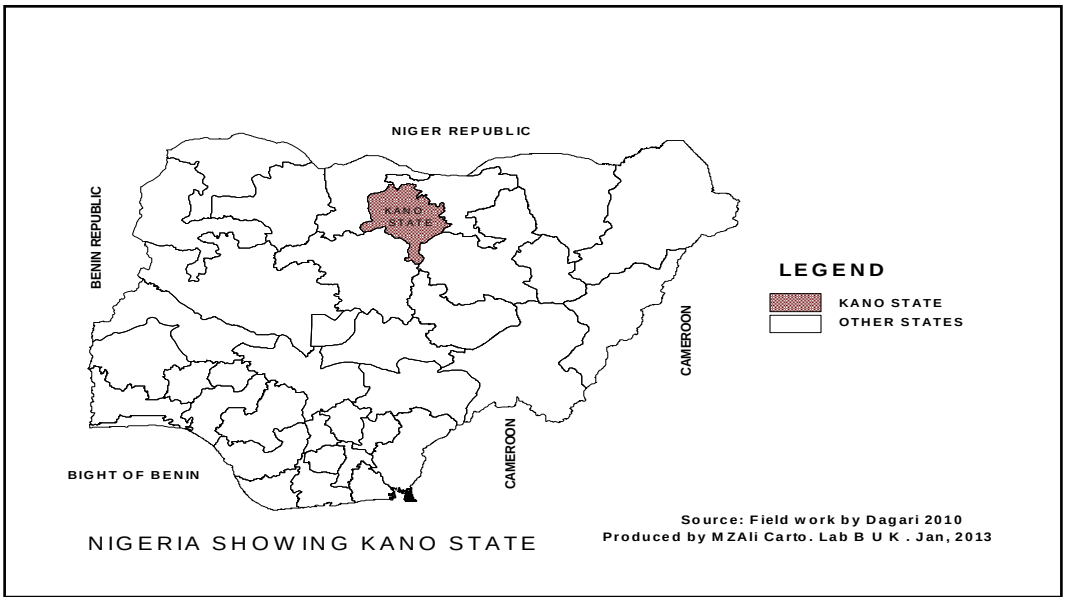


Figure 1: Map of Nigeria Showing Kano State

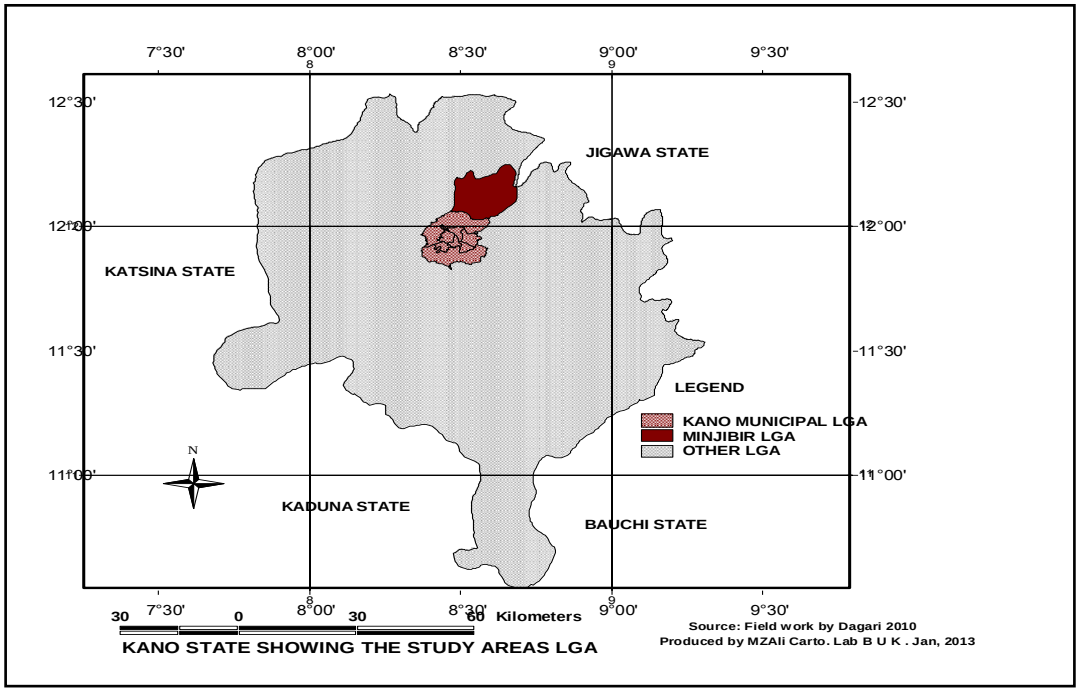


Figure 2: Map of Kano State Showing the Study Local Government Area

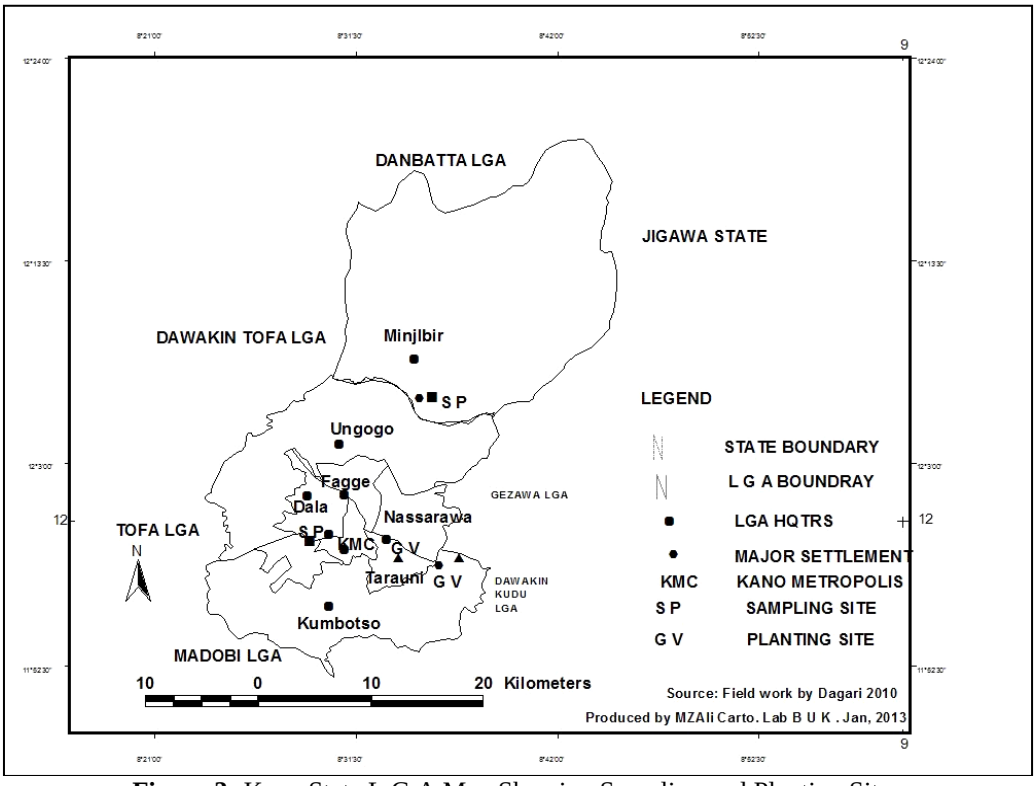


Figure 3: Kano State L.G.A Map Showing Sampling and Planting Sites

Plant Growing/Harvesting

In the factorial experiment with 5 rates of soil Pb^{+2} treatment (0, 1000, 2000, 3000, 4000 mg Pb/kg soil applied as $Pb(NO_3)_2$ and 4 rates of EDTA concentration (0,10,50,250) arranged in randomized blocks with 4 replications, plants were grown in 4-litre plastic pots each containing 3 kg oven dried soil. Macronutrient fertilization was done with the following: 0.91 g/kg of urea, 2.86 g/kg of super simple, 0.62 g/kg of K-chloride and

0.33 g/kg of Mg-sulphate. The micronutrients were prepared in a 1L solution containing 11.54 g/L of Cu-sulphate, 0.51 g/L of Na-molybdate, 25 g/L of Zn-sulphate and 2.94 g/L of boric acid. One millilitre of this stock solution per kg of soil was applied. Both liming and fertilization were performed with high-solubility analytical grade salts (Epstein *et al.*, 1999).

The surviving plants including the control (at zero Pb^{2+} treatment) were harvested and washed with tap water until no soil particles were visible. They were then soaked in 1% nitric acid for 30 s, rinsed with deionised water and dried in the oven. The weight of each plant harvested was recorded. The plants were then separated into roots, stems, leaves and seeds (Wong and Lau, 1985).

Digestion of Plant Parts

The various plant parts were ground to fine powder. Based on availability, 0.125 or 0.25 g (root), 1.00 g (stem), 0.75 g (leaf) and 0.50 g (seed) were used for analysis. The parts were weighed into porcelain crucibles and ashed at 450°C in a muffle furnace to constant weight. The ash was dissolved in 0.1M nitric acid, filtered and made to mark in a 25 cm^3 volumetric flask. Lead in the plant extracts were determined by atomic absorption spectrophotometry and expressed on a dry weight basis (Wong and Lau, 1985).

RESULTS AND DISCUSSION

The physicochemical properties of the soil were determined using standard methods and instruments. Results show that the soil had 1.16% oxidizable organic matter, 3.44 meq/100g of cation exchange capacity, 4.37% exchangeable sodium, 0.02 mS/cm electrical conductivity, 2.60 µg/g water soluble phosphate and pH 7.08

Synthetic chelates were found to desorb heavy metals from soil matrix into soil solution, facilitate metal transport into the xylem and increased metal translocation from roots to shoots (Blaylock *et al.*, 1997; Begonia *et al.*, 2005; Huang *et al.*, 1997; Epstein *et al.*, 1999; Antosiewicz, 2004; Marmiroli *et al.*, 2005). Figures 4 to 7 show the variations of Pb^{+2} concentration in soil fractions at 1000 to 4000 mg/kg Pb^{+2} against 0 to 250 mg/kg EDTA soil. The water soluble Pb^{+2} before planting and after harvest changed significantly ($Pr< 0.01$) at all levels. Addition of 0 – 250 mg EDTA to soils amended with 1000 Pb^{+2}/kg soil increased the water soluble Pb^{+2} before planting from a control value of 2.941 to 6.618 mg/kg. The corresponding increase for water soluble Pb^{+2} after harvest was 3.309 to 6.618 mg/kg.

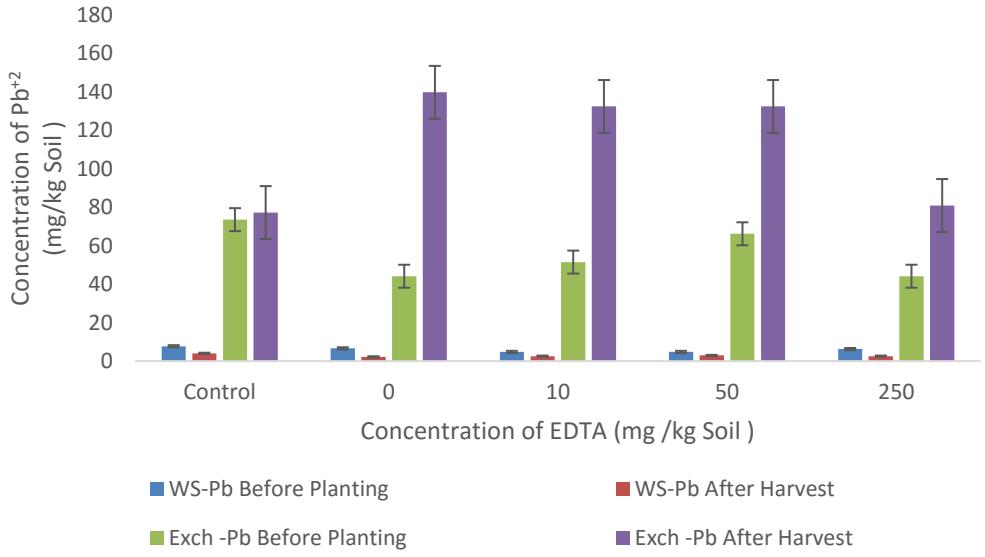


Figure 4: Variation of Pb^{+2} in soil fractions at 1000 mg Pb^{+2}

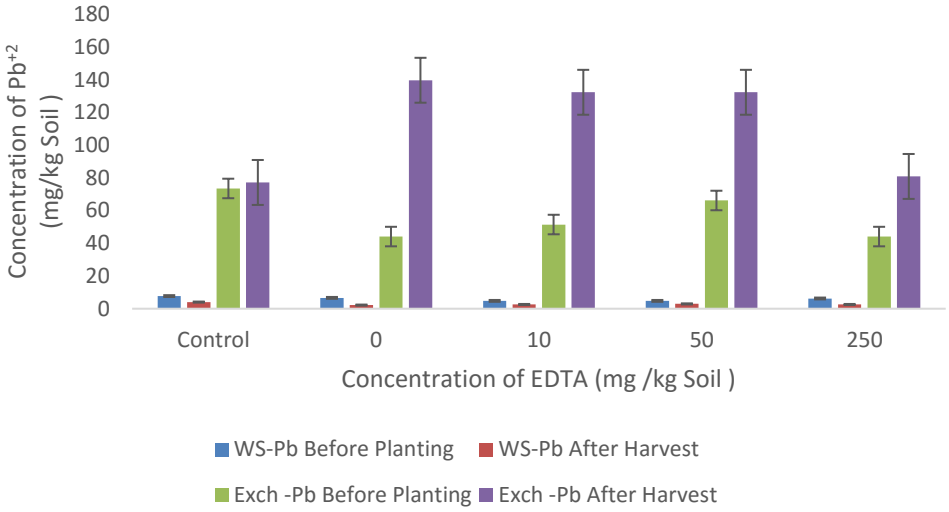


Figure 5: Variation of Pb^{+2} in soil fractions at 2000 mg Pb^{+2}

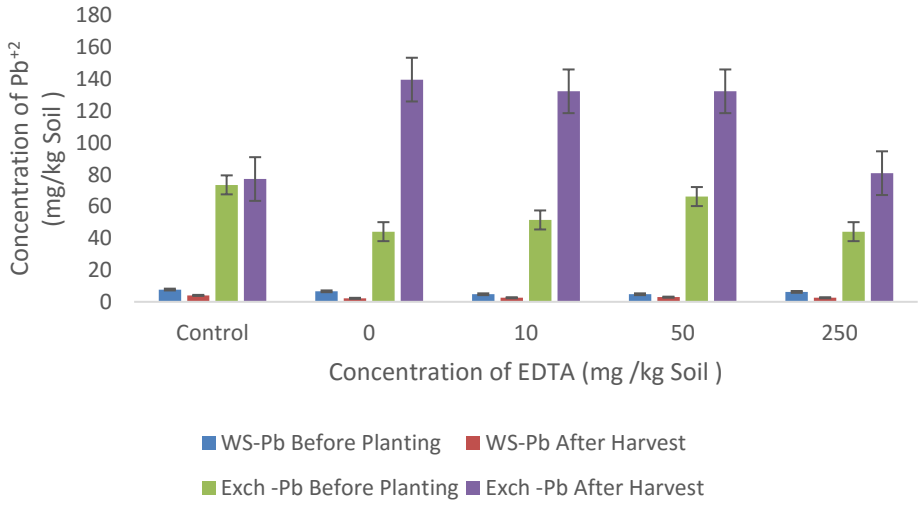


Figure 6: Variation of Pb^{+2} in soil fractions at 3000 mg Pb^{+2}

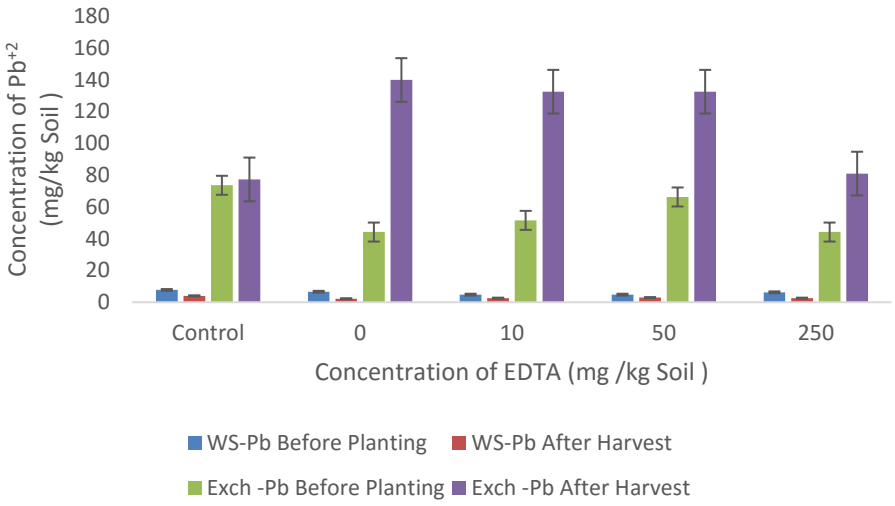


Figure 7: Variation of Pb^{+2} in soil fractions at 4000mg Pb^{+2}

Addition of 0 to 250 mg EDTA to soils amended with 2000, 3000 and 4000 Pb^{+2}/kg soil decreased the water soluble Pb^{+2} before planting from 6.985 to 4.779, 6.618 to 6.250 and 7.721 to 4.779 mg/kg respectively. The corresponding values for water soluble Pb^{+2} after harvest were 5.882 to 3.676, 5.147 to 2.574, 4.044 to 2.941 mg/kg respectively. The increase in water soluble lead with increase in amount of EDTA added is supported by the report of Blaylock *et al.* (1997) in which addition of 0.1, 1.0, 5.0 and 10mmol EDTA/kg of soil resulted in extraction of 3, 22, 64 and 73% of the total soil Pb respectively. According to Laurie *et al.* (1991), addition of EDTA, a chelating agent, to soil forms a Pb-EDTA complex with the soluble Pb in the soil solution in a slow reversible process between pH 5.2 and pH 7.7. Increasing the concentration of EDTA favoured the formation of the complex. The process continued until the chelate became saturated.

$$EDTA^{4-} + Pb^{2+} \rightleftharpoons [EDTAPb]^{2-}$$

$$\text{where } \beta = \frac{[EDTAPb]^{2-}}{[EDTAPb^{4-}][Pb^{2+}]} = 9.12\,10^{-19}\,dm^3mol^{-1}$$

There were insignificant changes in exchangeable lead before planting (Pr=0.99>0.05) and after harvest (Pr = 0.73 > 0.05). Addition of 0 – 250 mg EDTA to 1000 mg/kg soil increased the exchangeable Pb^{+2} before planting from a control value of 6.250 to 14.706 mg/kg. The corresponding increase in exchangeable Pb^{+2} after harvest was 6.985 to 33.088 mg/kg. Addition of 0 to 250 mg EDTA to soils amended with 2000, 3000 and 4000 Pb^{+2}/kg soil decreased the exchangeable Pb^{+2} before planting from 55.147 to 25.735, 55.147 to 44.118 and 73.529 to 66.176 mg/kg respectively. The corresponding values for exchangeable Pb^{+2} after harvest were 102.941 to 44.118, 77.206 to 80.882 and 77.206 to 132.353 mg/kg respectively.

Figures 8 to 11 show the variations of lead in plant parts against 0 to 250 mg EDTA/kg soil. Lead accumulation in all plant parts is highly significant (Pr< 0.01) at all levels of added Pb^{+2}. Addition of 0 to 250 mg EDTA to 1000 mg/kg soil increased the root Pb^{+2} from a control value of 44.118 to 66.176 mg/kg dry matter. Addition of 0 – 250 mg EDTA to 2000, 3000 and 4000 mg/kg soil increased the root Pb^{+2} from 66.176 to 360.294, 73.529 to 125.000, 73.529 to 58.824 mg/kg dry matter respectively.

Addition of 0 to 250 mg EDTA to 1000 mg/kg soil increased the stem Pb^{+2} from a control value of 11.029 to 20.221 mg/kg dry matter. When the Pb^{+2} levels were increased to 2000, 3000 and 4000 mg/kg soil at 0 to 250 mg EDTA/kg soil, the stem Pb^{+2} changed from 22.059 to 60.662, 36.765 to 31.250 and 16.544 to 14.706 mg/kg dry matter respectively.

Addition of 0 to 250 mg EDTA to 1000 mg/kg soil increased the leaf Pb^{+2} from a control value of 14.706 to 19.608 mg/kg dry matter. When the Pb^{+2} levels were increased to 2000, 3000 and 4000 mg/kg soil at 0 to 250 mg EDTA/kg soil, the leaf Pb^{+2} changed from 41.667 to 22.059, 51.471 to 19.608 and 31.863 to 14.706 mg/kg dry matter respectively.

Addition of 0 to 250 mg EDTA to 1000 mg/kg soil increased the seed Pb^{+2} from a control value of 0.000 to 14.706 mg/kg dry matter at 200 mg/kg soil. When the Pb^{+2} levels were increased to 2000, 3000 and 4000 mg/kg soil at 0 to 250 mg EDTA/kg soil, the seed Pb^{+2} changed from 14.706 to 0.000, 14.706 to 3.676 and 22.059 to 29.412 mg/kg dry matter respectively.

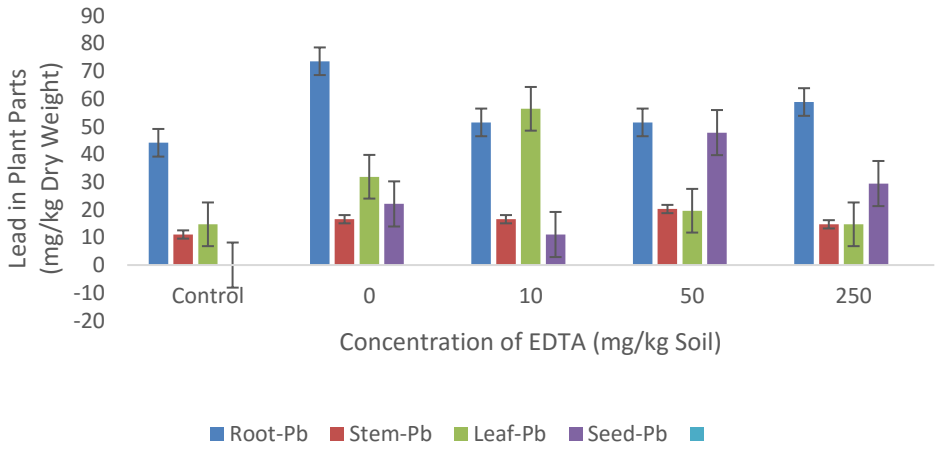


Figure 8: Variation of lead in plant parts at 1000 mg Pb^{+2}

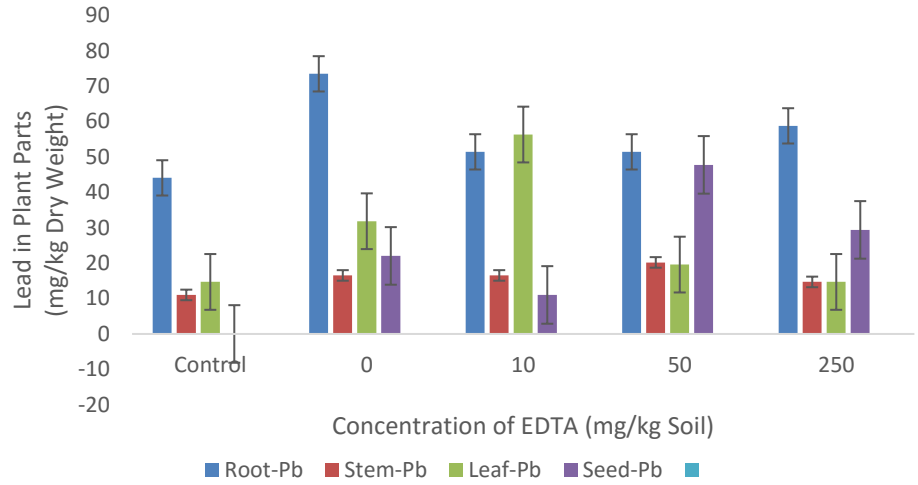


Figure 9: Variation of lead in plant parts at 2000 mg Pb^{+2}

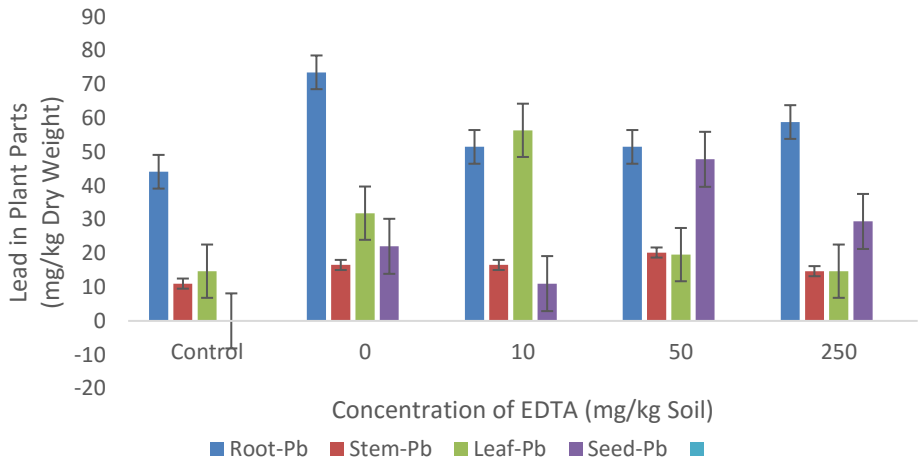


Figure 10: Variation of lead in plant parts at 3000 mg Pb^{+2}

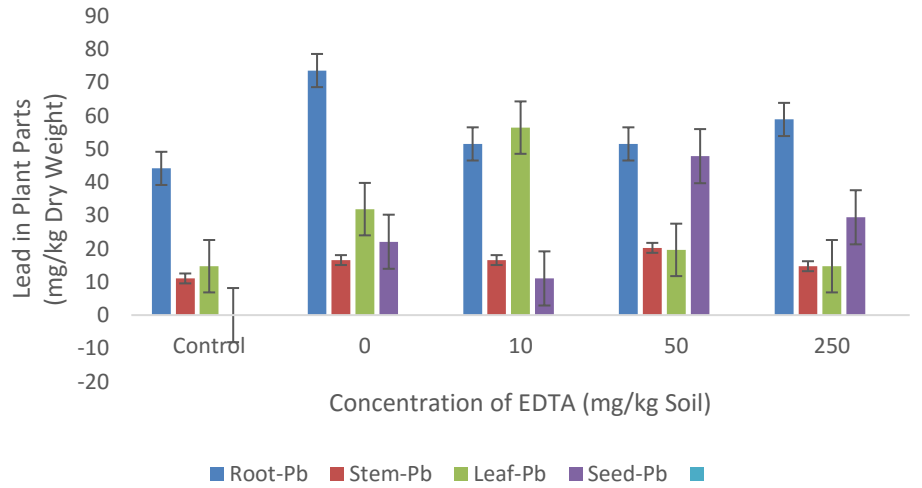


Figure 11: Variation of lead in plant parts at 4000 mg Pb^{+2}

Figure 12 gives the variation of Pb^{+2} TF against weight of EDTA at various levels Pb^{+2} /kg of soil. The translocation factor (TF) measures quantitatively the translocation of Pb^{+2} to aerial parts of plant (Wong and Lau, 1985; Macpherson and Martin, 1994; Blaylock *et al.*, 1997). For soils amended with 1000 mg Pb^{+2}/kg, addition of 0 to 250 mg EDTA/kg soil increased TF from a control value of 0.2813 to 0.3177. Addition of 0 to 250 mg EDTA/kg soil at 2000, 3000 and 4000 mg Pb^{+2}/kg soil changed the TF from 0.4688 to 0.1510, 0.7240 to 0.3750, 0.2396 to 0.6250 respectively The change in Pb TF with increase in amount of EDTA at a given value of added Pb^{+2} agreed with work of Blaylock *et al.* (1997). They studied the mechanism of translocation of Pb^{+2} to the shoots of Indian mustard. They added ^{14}C EDTA-Pb to the solution in which the plant was grown. This resulted in accumulation of ^{14}C labeled compounds in the shoots. The HPLC retention time of the ^{14}C labeled compound was found to be identical to an authentic standard. The amount of EDTA detected in the shoots was sufficient to chelate most of the Pb^{+2} accumulated in the tissue. It was therefore concluded that Pb^{+2} entered the plant and was transported to the shoot as an EDTA complex. In a similar report Laurie *et al.* (1991), proposed two possible pathways for metal uptake under the influence of complexes. One pathway involves the dissociation of a metal complex (ML) in the diffusion layer (solution phase) after which the released free metal ion (M) may be transported to the root cell across the plasma lemma. Another possible pathway involves the absorption of the metal complex by the root cell membrane. The complex then either undergoes dissociation in the cell membrane, with free metal ion being transported to the cell while the ligand (L) goes back to the solution phase, or the metal is transported to root cells across the plasma lemma in the form of a complex. Lead retention in roots is based on its binding to ion exchangeable sites on the cell wall and extracellular precipitation, mainly in the form of lead carbonates deposited in the cell wall (Dushenkov *et al.*, 1995). EDTA in combination with low pH effectively prevents cell wall retention of Pb^{+2}, thereby making it available for translocation to the shoots.

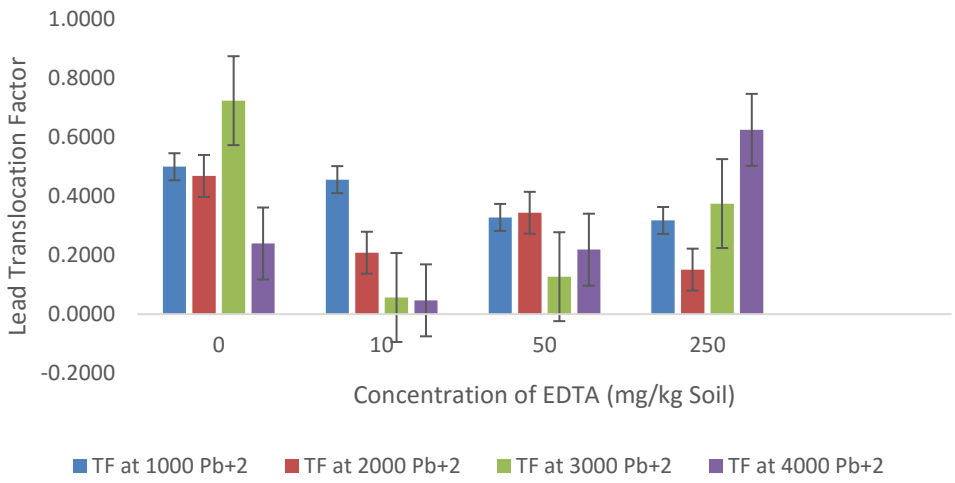


Figure 12: Variation of Lead Translocation Factor against Concentration of EDTA at various Levels of Added Pb^{+2}

Figure 13 shows the variation in weights of harvested plants (WHP) against concentration/level of EDTA at various levels of added Pb^{+2}. The weights of harvested plants changed insignificantly ($Pr = 0.71 > 0.05$) at all levels of added Pb^{+2}. Increasing the amount of EDTA from 0 to 250 mg/kg in soils amended with 1000 mg/kg Pb^{+2} decreased WHP from a control value of 11.5054 g to 4.7266 g dry weight. Addition of 0 – 250 mg/kg EDTA soil at 2000, 3000 and 4000 mg/kg soil changed the WHP from 4.6945 to 5.8680, 4.1943 to 5.0170 and 4.4526 to 4.3003 g dry weight respectively. The insignificant decrease in dry weight of plant is supported by the report of Vassil *et al.* (1998) that EDTA enhanced shoot accumulation of Pb^{+2} does not always appear to be related to physiological stress. Krobrukhv *et al.* (2004) reported that the decrease in dry weights of plants under Pb^{+2} treatment was due to impairment of physiological processes. This may lead to eventual cell death under severe treatment (Mishra and Choudhari, 1998).

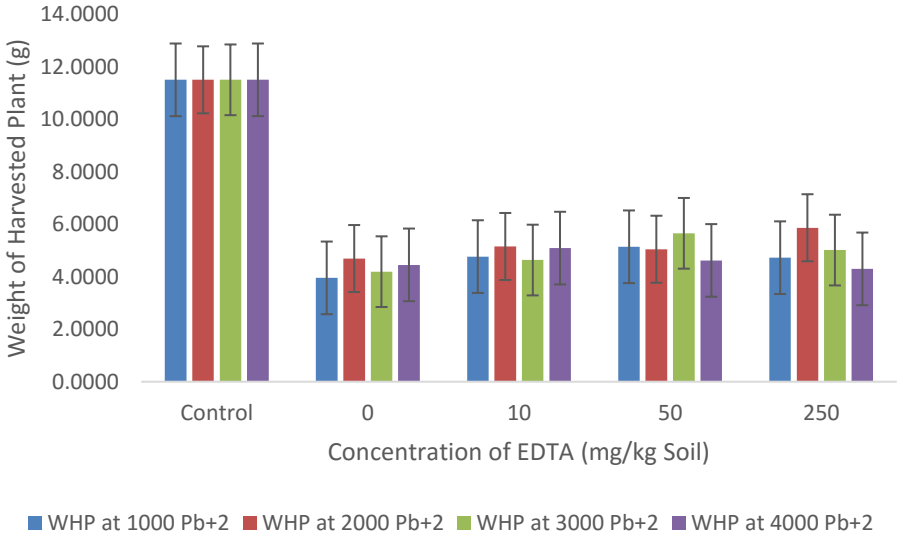


Figure 13: Variation of Weight of Harvested Plant against Concentration of EDTA at various Levels of Added Pb^{+2}

CONCLUSION

Addition of Pb^{+2} as lead nitrate at the rates of 0, 1000, 2000, 3000 and 4000 mg/kg soil and EDTA at 0, 10, 50 and 250 mg/kg soil increased significantly the water soluble Pb^{+2} before planting and after harvest. There were insignificant changes in exchangeable lead before planting and after harvest. EDTA, a chelating agent formed a Pb-EDTA complex with the soluble Pb^{+2} in the soil solution in a slow reversible process between pH 5.2 and pH 7.7. The Pb-EDTA complex was transported from the root to the shoot of the cowpea leading to significant accumulation in all parts of the plant. The weights of harvested plants changed insignificantly at all levels of added Pb^{+2} due to impairment of physiological processes (Kopittke *et al.*, 2007).

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