PHYTO-REMEDIATION OF LEAD-CONTAMINATED SOIL USING AMARANTHUS CRUENTUS

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ABSTRACT
Previous studies have shown that some vegetables have the ability to absorb metals from soil. Since they are early maturity species, they possess the potential to be used as phytoremediating agents. Also, synthetic chelates have been found to induce lead desorption from soil matrix, thereby enhancing uptake into plant tissues. Therefore, a study was carried out to determine the potential of *Amaranthus cruentus* as a soil lead remediating plant. The experiment was carried out using a randomized block design. Soil samples were subjected to five levels of lead contamination namely control, 600ppm, 600ppm + EDTA, 1800ppm and 1800ppm + EDTA, each treatment had five replicates. Three plants each were carefully transplanted from nursery to experimental pots and grown for 32 days. Ethylenediaminetetra acetic(EDTA) (3.0 mmole EDTA/kg soil) was applied to EDTA amended treatment 8 days before harvesting. The result showed that EDTA has some effect on lead solubility in soil as well as lead absorption by *A.cruentus*. However, there were variable increases in lead uptake from the contaminated soil to the plants. Lead contamination did not have significant effect on growth and yield parameters of *A cruentus*. Since the transfer factor (TF) of the plant is greater than one, it may be a promising species for phytoremediation.

Keywords:- *Amaranthus cruentus*, Lead, *phytoremediation,* soil

INTRODUCTION

The increasing exploitation, production and consumption of the earth's raw materials (fossil fuel and minerals), coupled with the exponential growth, of the world's population over the past 200yrs have resulted in environmental build up of waste products of which heavy materials are of particular concern (Appel and Ma, 2002). Soils are an important sink for these metals due to its high metals retention capacities. However, important heavy metals posing threats to soil quality and human health include lead because it is being used for a wide variety of industrial, urban and agricultural applications and can be toxic to man (Appel and Ma, 2002). Conventional cleanup technology is generally expensive and often, harmful to desirable soil properties (such as texture and organic matter) for the restoration of contaminated sites.

Phytoextraction is the use of plants to remove contaminants from soil by accumulation in plant tissue and this is a promising clean-up technology for a variety of metal containing soil (Fuhrmann *et al*, 2002 Lasat, 2002). In the phytoremediating process, several sequential crops of selected plant species can be cultivated to reduce the concentration of heavy metals in contaminated soils to environmentally acceptable levels (Zhen *et al*, 2002). Heavy metals can be translocated to above ground plant parts. The metal-rich plant material may be safely harvested and removed from site without extensive excavation, disposal costs and loss of top soil associated with traditional remediation practices (Blaylock *et al,* 1997). However, successful phytoextraction require plants that are capable of producing high biomass while accumulating large amount of contaminants in the biomass from the soil (Tu *et al*, 2002, Shen *et al,* 2002).

Vegetables however have been found to absorb heavy metals from the soil as well as from surface deposits on part of vegetables exposed to polluted air. (Yusuf *et al*, 2002). *Amaranthus cruentus* is a robust annual herb which belong to the family Amaranthaceae. It is a popular plant and its leaves are edible and are of a good nutritional value (Burkil, 1985). Its protein, carbohydrate and lipid contents are quite high (Odegba and Sadiq, 2002). This plant was chosen for the study because it is widely cultivated and eaten as a vegetable in most parts of West Africa (Burkil, 1985).The objectives of the study therefore were:

(i) To assess the accumulation and distribution of lead in *A. cruentus* planted on lead-contaminated soil
(ii) To assess possible increase in lead uptake by *A. cruentus* from EDTA amended soils contaminated with lead.
(iii) To assess the potential of *A – cruentus* as a possible bioremediation plant.

MATERIALS AND METHODS

Experimental Plants Procurement

Seeds of *A. cruentus* were purchased from Osiele market in Abeokuta Ogun State, Nigeria. Enough seeds were purchased in a single batch for the study. The seeds were raised in a nursery for 21 days. Seedlings of equal height and vigour were selected and transplanted into pots, each treatment having 5 replicates. The plants height were measured from the soil level to the terminal bud using a meter rule at 7 days interval. Number of leaves were also counted weekly as the plant grew. The seedlings were subjected to the following five treatments.

I - Uncontaminated with lead (control)

II - Soil contaminated with 600ppm lead
III - Soil contaminated with 600ppm lead + 50mMole EDTA
IV - Soil contaminated with 1800pmm lead
V - Soil contaminated with 1800ppm lead +50mMole EDTA

EDTA was applied as a solution to the soil surface. The plants were harvested 8days after the first application of EDTA (Zhen et al, 2002).

Soil Sample Analysis

The physico-chemical parameters of the soil were determined prior to planting and after harvest. The soil samples were air-dried, ground and sieved and parameters determined include pH, particle size distribution, organic carbon, cation exchange capacity and total lead and soluble lead.

Soil pH determination: 20g of air-dried, sieved soil was weighed into 50ml beaker. 20ml of distilled – deionized water was added and allowed to stand for 30 minutes; and stirred occasionally with a glass rod. The pH meter electrode was inserted into the partly settled suspension and the pH value measured.

Particle size Distribution Analysis: - The hydrometer method (Juo, 1988) was adopted. 100g of air-dried soil which has been passed through a 2mm sieve was weighed and transferred to a conical flask. 100 ml of calgon solution was added, stirred and left overnight. The soil suspension was made up to 1000 ml mark in a 1 litre measuring cylinder. The cylinder was covered and inverted several times until all soil was in suspension .The cylinder was placed on a flat surface and time noted . At 40 seconds, the hydrometer was inserted into the soil suspension immediately and the first reading on the hydrometer was recorded. The hydrometer was removed and the temperature of the suspension taken using a thermometer. After the first hydrometer reading, the suspension was left to stand for 3 hours and a second reading taken; the temperature of the suspension was taken again.

Calculation: -

$$\text{Sand} = 100 - \frac{\text{Corrected 40 seconds hydrometer reads} \times 100}{\text{Weight of sample}}$$

$$\text{Clay} = \frac{\text{Corrected 3 hours hydrometer reading} \times 100}{\text{Weight of sample}}$$

Silt = 100 – (%Sand + % Clay).

Determination of Organic Carbon: - Walkley – Black Method was used (Tu *et al*, 2002). 0.1g of soil sample was weighed into a conical flask in duplicate. 10ml of 1N $k_2Cr_20_7$ was pipetted into each flask and swirled gently to disperse the soil; 20ml of conc. H_2SO_4 was added rapidly. The flask was immediately swirled gently until soil and reagents were mixed. The mixture was then swirled vigorously and allowed to stand for about 30minutes. 100ml of distilled water was then added after 30minutes. 4 drops of indicator was added to the mixture and titrated with 0.5N ferrous sulphate solution. The colour changed at the end point from orange to light green and then green.

$$\% \text{ Organic carbon in soil} = \frac{\text{Me } K_2Cr_2O_7 - \text{Me } FeSO_4) \times 0.003 \times 100 \times f}{\text{Weight of air-dried soil}}$$

Where Me = Normality of solution x volume of solution used
F = correction factor = 1.33
% organic matter in soil = % organic carbon x 1.729

Determination of Total Lead in Soil: 1.0g of soil sample (passed through 0.5mm sieve) was weighed into crucibles in duplicate. 10ml of conc. H_2SO_4, 10ml of conc. $HClO_4$ and 5ml of conc. HNO_3 were added. The mixture was swirled gently and heated at low to medium heat on a hot plate. The heating was continued until the solution dried off and the crucible was allowed to cool. 50ml of distilled-deionized water was added to rinse the crucible gradually and then filtered. The filtrate was then analyzed for lead using AAS.

Determination of Cation Exchange Capacity (CEC)

Determination of Exchangeable bases (Ca, Mg, K, Na, Mn) Neutral ammonium acetate method (Tu *et al,* 2002, Juo, 1988) was used. 5g of air-dried soil was weighed into sample bottle, 60ml of 1N ammonium acetate solution was added and shaken using an orbital shaker for $2^1/_2$hours. The clear supernatant was filtered into a 100ml measuring cylinder. Another 30ml of ammonium acetate solution was added to the soil and shook for 30 minutes. The supernatant was also filtered into the same cylinder. Ammonium acetate was added to make up to 100ml. K, Ca and sodium were determined using a flame photometer while Mg and Mn were determined using Atomic Absorption Spectrophotometry

Calculation:

$$\text{Exchangeable bases} = \frac{\text{Concentration of base X } 10^2}{\text{atomic mass per charge x mass of soil}}$$

Determination of Exchangeable Acidity (Al and H) Titrimetry was used (Juo, 1988). The soil was first extracted with 1N KCl. This was carried out by weighing 5g of the air- dried soil (passed through 2mm sieve) into sample bottle, 90ml of 1N KCl was added and shaken for 2hours using an orbital shaker. The supernatant was filtered. 25ml of extract was pipetted into a conical flask and 100ml of distilled water was added 4 drops of phenolphthalein indicator was added and the solution was titrated with 0.05N HCl was added to bring the colour back to the colourless state and 10ml of NaF solution was added to observe any colour change and the reading taken.

Calculation

Exchangeable acidity (c mol ckg-1) = (ml of NaoH) N x 102 where
Sample weight (g)

N = normality of HCl

Exchangeable H = Exchangeable acidity - Exchangeable Al

Cation Exchange capacity, CEC = Total exchangeable bases +
Total exchangeable acidity

Plant sample Analysis: The vegetables were harvested separately according to soil treatment. The 5 replicates of each treatment were pooled together to give composite sample of each treatment. The plants were then washed in water to eliminate dust, dirt, possible parasites or their eggs and they were again washed with deionized water (Yusuf *et al,* 2002). The leaves, stems and roots of each composite sample were then separated as sub-samples. Each sub-sample was oven-dried at 90^0c for 24 hours. The wet digestion method was used (Yusuf et al 2002). 1g of dry matter was weighed into 50ml beakers, followed by addition of 10ml mixture of analytical grade acids: $HN0_3$; H_2S0_4; $HCl0_4$ in the ratio 1:1:1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at a temperature of about 90^0C until about 4ml was left in the beaker. Then, a further 10ml of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4ml. After cooling, of solution was filtered to remove small quantities of waxy solids and made up to a final volume (50ml) with distilled water.

Lead concentrations were determined using Atomic Absorption spectrophotometry.

Analysis of soluble lead after Harvest:- After plants were harvested 8 day post-application of EDTA, the soil at 3cm above the experimental pots were collected for soluble lead analysis. Soluble lead was extracted by deionised water with 1:5 soil to water ratio and centrifuged. The supernatant solution was filtered through 0.4μm filter paper and lead concentration determined using Atomic Absorption Spectrophotometry.

RESULTS AND DISCUSSION

Soil properties were determined before treating the soil with lead and with or without EDTA. The lead level in the uncontaminated soil sample was found to be 8.5mg/kg. Textural triangle was used to determine the class name of the soil. The experimental soil was sandy soil. The physico-chemical properties of the soil are shown in Table 1.

Table 1: Physico-chemical properties of experimental soil

Properties	Value
Clay (%)	7.35 ± 0.07
Silt, %	5.15 ± 0.64
Sand, %	87.50 ± 0.70
pH	6.79 ± 0.17
Organic carbon, %	18.35± 0.28
Cation Exchange capacity (ECO) ($molckg^{-1}$	11.80 ± 0.37
Total Lead, mg/kg	8.50 ± 0.10

The control plants quickly outgrew those on contaminated soils. Plant height decreased in the exposed plants. Also, plant height decreased in the EDTA amended soils than the unamended polluted soils. This may be attributed to more Lead mobilization to the plants leading to increased lead toxicity to plants. The analysis of variance however showed that there were no significant differences in plant height amongst the treatments. So, EDTA application had no significant effect on the height of *A cruentus* .

Also, the number of leaves in the control plants were greater than those planted on contaminated soils. though, the analysis of variance showed no significant difference at 95% confidence level. The dry matter yield of leaves, stems and roots was highest for control and lowest in the treated plants. For the leaves and stems, the lowest was 1800ppm + EDTA plants, while it was the 600ppm + EDTA treatment for the roots. It was observed that the dry matter yields per pot correspond to the concentration of lead in the shoots of *A cruentus*. Dry matter yields decreased with increased lead concentration in shoots (Table 2).

Table 2: Dry matter yield of *A. cruentus* in lead polluted soils with and without EDTA application

	Dry matter yield (g)			
Treatment	Leaves	Stem	Root	Total
Control	6.34	3.30	1.18	10.82
600ppm	4.70	2.46	0.96	7.12
600ppm + EDTA	3.97	2.38	0.74	6.09
1800ppm	3.06	2.02	0.74	6.03
1800ppm + EDTA	2.86	1.95	0.97	5.78

Lead levels in leaves and stems increased as the level of lead in the roots. Appreciable amount of lead was detected in the plants roots. Appreciable amount of lead was detected in the plants roots with respect to soluble lead in soil (Table 3). This is in agreement with the work of Jauert *et al*, 2002 who reported levels of cadmium in roots of strawberry.

Table 3: Total and soluble Lead levels in A. cruentus grown in lead contaminated solution.

	Lead Concentration mg/kg)				
Treatment	Leaves	Stem	Root	Soluble	Total
Control	87.0	2.0	82.0	3.5	171.0
600ppm	119.5	71.5	171.0	8.5	362.0
600ppm + EDTA	138.5	124.5	122.0	10.5	385.0
1800ppm	167.0	120.5	99.5	12.0	387.0
1800ppm + EDTA	174.5	195.0	83.5	156.0	453.0

The analysis of variance showed highly significant differences between treatments and lead absorption in *A. cruentus*. Hence treatments with EDTA application had higher lead levels in their tissues than Pb-contaminated soil without EDTA. Also the latter had high concentrations of lead in roots when compared with those grown on soils amended with EDTA. This is in agreement with observations of Zhen et al (2002) who reported lead concentrations in roots of plants without chelates applications. Lead concentration in soil significantly increased the shoot to root ratio of A.cruentus (Table4).The percentage of absorbed lead translocated from roots to shoots increased from 52% in the control sample to 82% in 1800ppm+ EDTA amended soils.

Lead distribution in leaves, stems and roots of *A. cruentus* was affected by EDTA application (Table 3) as there were increased levels in plants grown on EDTA amended soils, however the analysis of variance showed no significant difference.

Table 4: Lead translocation from roots to shoot A *cruentus*

Treatment	Shoot to Root ratio of Lead concentration (T.F)	Lead absorbed by shoot lead absorbed by whole plant (%)
Control	1.09	52.0
600ppm	1.12	52.8
600ppm+EDTA	2.16	68.3
1800PPM	2.89	74.3
18800PPM+EDTA	4.42	81.6

Shoot = Leaves + stems
T.F = Transfer factor

The identification of metal hyper-accumulators capable of accumulating high metal levels demonstrate that plant have genetic potential to clean up contaminated soils (Lasat, 2002). Hence bio-concentration factor (BF) may better characterize hyper-accumulators than concentration ratio (CR) (Tu *et al*, 2002). From the study, the BF which is based on water soluble lead reflect accurately plant accumulation of lead in the soil rather that on total soil lead (Table 5), as only a portion of total in the is soil is readily taken up by plant root as reported by Tu et al, 2002.

Table 5: Lead accumulation Characteristics of *A cruentus* grown in Lead- contaminated soils.

Treatment	CR-Shoot	CR-Root	BF-Shoot	BF-Root
Control	10.4	9.6	25.1	23.4
600ppm	0.0064	0.0057	22.5	20.4
600ppm+EDTA	0.0088	0.0041	25.0	11.6
1800ppm	0.0032	0.001	23.8	8.29
1800ppm+EDTA	0.0041	0.0009	2.37	0.58

CR = Concentration ratio
BF = Bio concentration factor

Apart from taking up large amount of contaminants from the soil, Phytoremediation spices should be able to transport most of the contaminants to the shoots which facilitates sequestering of pollutants (Tu,*et al* 2002). The transfer factor defined as ratio of metal concentration in shoots to that in roots is an index of translocation. Generally, the TF in all confirmed hyper accumulators are greater than one, where as it is usually below one in non – accumulators. From the study (Table 5) A cruentus can be said to be a hyper accumulator plant and can therefore be used as a bio -remediating plant.

CONCLUSION

A. Cruentus has the potential to translocate above average of lead from contaminated soils to shoot since the transfer factor was greater than one. Hence, it is a hyper accumulator and a promising plant for phyto-remediation. This may however; pose a health risk to humans if consumed since levels were highest in the leaves which is the plant part mostly consumed by humans. This also implies that plants meant for human consumption must not be planted on lead contaminated soils. However, to adopt the plant for remediation works, further studies on lead absorption, concentration, changes, nutrients qualities in the leaves and stems especially total and soluble lead in the soil should be carried out in details.

REFERENCES

Agbenin, J. O. (1995) – Laboratory manual for soil and plant analysis (selected) analysis (selected) methods and data analysis). Dept. of Soil Science, Faculty of Agric./Institute for Agric. Research, ABU, Zaria.

Alloway, B. J. and Ayres, D. C. (1993) – Chemical principle of Environmental Pollution 'Inorganic Pollutants'. Chapman and Hall, India, Pp.154 – 155.

Appel Chip and Ma Lena (2002) – heavy metals in the Environment – "Concentration, pH and surface charge effects on Cd and Pb sorption in three tropical soils "Journal of Environmental Quality (JEQ) vol.21 (March – April 2002/No. S Pp.589.

Blaylock, M.; Salt, D.; Dushenkov, S.; Zakharova, O.; Gussman, C., Ka pullnik, Y.; Ensley, B.; and Raskin, J (1997) – Enhanced accumulation of Pb in India mustard by soil- applied chelating agents. Environmental Science Technology, 81;860 – 865.

Burkil, H. M. (1985) – The useful plant of West Africa. 2ND Edition, vol. Families A-D Royal Botanic Gardens, Kew. Pp.51 – 52.

Fuhrmann M Mitch m, Lasat, Stephen D. Ebbs, Lern V. Kochian and Jay Cornish (2002); Plant and Environment interactions – Up take of Cesium – 137 and strontium – 90 from contaminated soil by three plant species – Application to phyto remediation' JEQ vol. 31 Pp. 904 – 909.

Jauert P.; Schumacher T. E.; Boe, A; and Reese R. N. (2002) – Pint and Environmental interactions – "Rhizopher Acidification and cadmium uptake by strawberry clever' JEQ vol. 31/March – April/No.2 JEVQ AA 31 (2) 373 – 704 (2002), Pp. 633.

Juo A.S.B. (ed), 1988 – Seledced Methods for Soil and Plant Analysis Manual Series No.1 International Institute of Tropical Agriculture, Ibadan, Nigeria.

Lasat M. M. (2002) – Reviews and analyses – Phyto extraction of Toxic metals; A review of biological mechanisms". Journal of Environmental; A review of biological mechnism'. Journal of Environments Quality (JEQ) Volume 31 Pp. 109 – 120.

Odegha V. J. and Sadiq A. O. (2002) – Effect of spent engine oil on the growth arameters, chlorophyll and protein level of Amaranthus hybridus. The Environmentalist, 22, 23 – 28.

Zhen – Guo S., Xiang – Dong L1, Chun – chun Wang, Huai – man Chen, and ung Chua, (2002). Heavy metals in the Environment – "Lead photo extraction from contaminated soil with high – Biomass plant species" JEQ Vol. 31 Pp. 1893 – 1900.

Tu Cong, Lena Q, Ma, and Bhaskar Boondade (2002) – Plant and Environment Interactions – Arsenic Accumulation in the hyper accumulator Chinese Brake and its utilization potential for phyto remediation" Journal of Environmental Quality (JEQ) Volume 31 Pp. 1671 – 1675.

Wegwu M. O., Obute G. C. and Osuji L. C. (2002) – 'Cadmium uptake by Telfairia Occidentalis Hoot F. (Cucurbitaceae) growth in Cd polluted soil". Global Journal o pure and applied sciences. Vol.8, No.4, Pp. 495 – 496.

Yusuf A. A., Arowolo T., and Bamgbose O. – 'Cd, Cu and Ni levels in vegetables from industrial and residential Areas of Lagos City, Nigeria.' Global Journal of Environmental Sciences Vol. No. 1 2002. Pp. 1- 3.

APPENDIX C: ANOVA Table for the effect of treatment on Pb absorption in A. hybridus

Sources of Variation	DF	SS	MS	Fcal	Ftab 10.05
Total	8	29,454.06			
Plant Parts	2	11,660.73	5,830.37	24.35**	6.94
Treatment	2	16,835.73	8,417.87	35.16**	
Error	4	957.60	239.4		

!** means highly significant