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An Evaluation of the Effect of Treatment with Mixture of Cadmium and Lead on the Growth of Okra [*Abelmoschus esculentus* (L.) Moench]

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ABSTRACT: *Abelmoschus esculentus* (L.) Moench was grown from seeds in soil polluted with a mixture of cadmium and lead in the ratio 1:1. The experiment consisted of control and four treatments (25, 50, 75 and 100 mg/kg) in three replicates. Data for growth parameters (seedling emergence, plant height, number of leaves, leaf loss, leaf area, and stem girth) were collected weekly for 3 months and number of flowers and fruits later in the experiment. The research lasted for 5 months. Physico-chemical and heavy metal analysis were carried out on soil samples at the end of the experiment. The leaf, root and fruit of plant samples were also analyzed for heavy metal content at this time. There was significant difference between control and the 75mg/kg and 100mg/kg treatments at day 6 with percentage emergence values of 77.1, 82.9, 65.7, 60 and 57.1 percent for control, the 25mg/kg, 50mg/kg, 75mg/kg and 100mg/kg respectively. There was no significant difference between control and other treated plants for the other growth parameters investigated. There was a decrease in the nutrient composition of samples analyzed as concentration of treatment increased. The uptake of the metals was in the order Pb > Cd, with the highest accumulation recorded in the root; 1.58ppm Pb and 0.25ppm Cd, and the least accumulation recorded in the fruit; 0.26ppm Pb and 0.13ppm Cd.

Keywords: Concentrations, treatment, cadmium, lead, *Abelmoschus esculentus*

Introduction

Heavy metals as a term is used to describe a group of metals linked to contamination and toxicity (Duffus, 2002) Heavy metals are present naturally in soils or as a result of human activities such as mining, electroplating, waste disposal and metal smelting (Ross, 1994). Heavy metal pollution poses a serious threat worldwide because these metals are toxic, non-biodegradable and are transferred along the food chain.

Plants absorb heavy metals when they are present in the soil or irrigation water (Fusconi *et al*., 2006). Some metals like manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn) are classified as plant essential metals. These metals are however required in specific amounts as their deficiency or elevated concentrations may result in toxic effects. For example manganese is involved in splitting water molecules during photosynthesis, too high concentrations can damage plants and inhibit their growth (Wang *et al*., 2009). Heavy metals such as Cadmium (Cd) and Lead (Pb) are toxic and adversely affect plant growth by affecting the growth of leaves and root, inhibiting enzymatic activities, and reducing plant productivity (Lai *et al*., 2012).

Cadmium is phytotoxic as it inhibits important processes like respiration, photosynthesis, and water and mineral uptake (Kuo *et al*., 2006). It also reduces the rate of new cell production and root growth (Liu *et al*., 2004), it inhibits anti-oxidative enzymes activities (Correa *et al*., 2006) and induces oxidative stress in cells (Sandalio *et al*., 2001). Cd induces changes in plants at biochemical, physical and genetic levels, resulting in the reduction of

plant growth (Nouariri *et al*., 2006), leaf chlorosis and necrosis (Baryla *et al*., 2001) and ultimately to the death of the plant (Toppi and Gabrielli., 1999). Like cadmium, lead is also phytotoxic. It affects photosynthesis in plants by reducing the chlorophyll content. This occurs as Pb reduces the uptake of chlorophyll essential elements such as Magnesium (Mg) and iron (Fe), affecting the chloroplast altering essential enzymatic processes of photosynthesis and disturbing the closing of the stomata (Sharma and Dubey, 2005). Lead has been seen to have significant impacts on seedling dry mass, shoot and root length, and weight (Farooqi *et al*., 2009). It also affects the process of respiration and metabolism of plants (Paolacci *et al*., 1997).

Heavy metals have gained attention in studies analyzing their effects on agricultural plants. Most of the studies however focus on the adverse effect of a single metal, only a few deal with mixtures of heavy metals. Since agricultural plants are exposed to pollutants such as heavy metals, pesticides, and fertilizers, the interaction of these pollutants is inevitable.

The joint effect of mixtures of heavy metals can result in an additive, synergistic or antagonistic effect. Additive effect is seen when the components of the mixture affect the same target or have the same mode of action, and the measured mixture effect is the sum of the expected effects for the individual toxicants. Synergistic effect may occur due to accelerated bioaccumulation, inhibition or detoxification of one of the component of the mixture, or due to increased bio activation of one of the component of the mixture. In synergistic effect, the mixture effect is higher than the sum of the predicted effects for the individual toxicants. In the case of an antagonistic effect, the observed mixture effect is lower than the sum of the predicted effects for the individual toxicants.This study investigates the effect of cadmium and lead mixture on the agricultural plant okra (*A. esculentus*).

Materials and methods

Study area: The study was carried out in the experimental plot of the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria which lies within the humid Tropical vegetation. Latitude 6º 30′ 0″N and longitude 6º 0′ 0″ E

Collection of plant materials and soil samples: *Seed:* Seeds of *A. esculentus* were obtained from the Bioresource Centre, Bayelsa.

Soil*:* Soil samples were collected from capitol, University of Benin, Edo State - a site which had remained undisturbed for over fifteen (15) years. Top soil (0 - 10 cm), of known physicochemical property was collected and dried. Thereafter, 5kg soil each was placed into 35 pieces of bottom – perforated planting bags.

Preparation of site*:* The site used for the experimental layout was properly weeded and the surface covered with black cellophane to confine the roots to the soil within the planting bags.

Methodology: The already perforated planting bags were properly identified and laid out on the prepared site in a completely randomized design. Five (5) seeds were sown in each bag containing 5kg soil and later thinned down to one (1) after fourteen (14) days of sprouting. The soil was polluted with cadmium and lead mixture before the seeds were sown. There were four (4) concentrations (25, 50, 75, 100mg/kg) in three (3) replicates and control. Both metals were measured and dissolved in distilled water and dispensed. After the soil treatment, data were collected on a weekly basis for 5 months. Soil and plant analyses were done at the end of the 5 month period.

Field data collection

Plant height: For plant height measurements, metre rule was used to measure the height of the plant.

Number of leaves: The total number of leaves of *A. esculentus* was taken by visual counting of the leaves on the plants.

Leaf area: Leaf area measurements of the study plants were obtained from the third leaf from the top and determinations done using the proportional method according to (Eze, 1965).

Girth: Girth of *A. esculentus* was taken weekly from the 5^{th} week. The diameter of the shoot was obtained using the Esal vernier caliper. (Girth = πd).

Soil Physicochemical Analyses: Soils were dried at ambient temperature (22-25ºC), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried and less than 2mm samples were stored in polythene bags for subsequent analysis. The fraction was used for the determination of selected soil physicochemical properties and the heavy metal fractions.

pH and Electrical conductivity: 20 g of fine soil was placed in a container and 50 ml of distilled water added. The suspension was shaken for 30 mins and allowed to settle. Electrical conductivity and pH of the solution were measured using a pH meter (Model 215) and conductivity meter. The pH meter was first standardized using a buffer solution.

Organic carbon: 1.0g of the soil sample was placed in a 250ml conical flask. Then 10ml of $K_2Cr_2O_7$ and 20ml concentrated H_2SO_4 were added and the mixture was hand shaken for minutes. Distilled water was then added to

make the volume up to 150ml. 10ml of phosphoric acid and 8 drops of diphenylamine solution were then added. A blank determination was done using 10ml $K_2Cr_2O_7$ and 20ml concentrated H_2SO_4 solution and titrated to a green colour with ferrous ammonium sulphate solution.
The total organic carbon (TOC) was calculated as:

$$\% \text{ TOC} = \frac{\text{Titre value of blank} - \text{Titre value of sample X 0.3 X M1.33}}{\text{Weight of sample}}$$

Cation exchange capacity: 5 g of soil were placed into sterile conical flask and 20 ml of extracting solution (NH_4OAc) was added into the 250 ml volumetric flask containing the soil samples. Whatman filter paper was then used to filter the solution. Also 0.1 ml of the filtrate was transferred to a test tube and diluted with 10 ml 0.015 % strontium chloride solution. The sample was analyzed for sodium (Na) and potassium (K) by flame emission and for Ca and Mg by Atomic Absorption Spectrophotometry (AAS).
***Sample preparation for analysis of metals*:** Both plant and soil samples were ground into fine powder. 2 g portions of the samples were weighed accurately and 10ml concentrated HNO_3 was added to each. The samples were digested on a hot plate for 15 min. The digest was cooled and 5 ml of concentrated nitric acid was added and heated for additional 30 min. The latter step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 5ml of concentrated hydrochloric acid and 10ml of distilled water was added and the sample was heated for additional 15 min without boiling. The sample was cooled and filtered through a Whatman No. 42 ash less filter paper and diluted to 60ml with distilled water. Cadmium and Lead content in the digested samples were analyzed for using the Atomic Absorption Spectrophotometer.
Statistics: Statistical analysis was carried using the SPSS software package version 16.0 and Microsoft Excel 2010. One-way analysis of variance to confirm the variability and validity of results was performed. The Duncan's multiple range tests was applied to determine significant differences among treatments at a significance level of $p < 0.05$.

Results

The percentage seedling emergence of *A. esculentus* is shown in Figure 1. The 25mg/kg treatment enhanced seedling emergence while higher concentrations had adverse effects on the seedling emergence of *A. esculentus.* There was significant difference ($p<0.05$) between the control and 75 and 100 mg/kg treatments at day 6 and 7 after planting, with seedling emergence values of 77.1±5.22, 60.0±9.76 and 57.1±10.71 percent respectively.
The height of *A. esculentus* is shown in Figure 2. There was no significant difference in height between the control and all the treatments. At 9 WAP, plant height recorded was 36.8±3.61, 37.5±4.19, 34±3.22, 33.17±5.50 and 37.8±2.46 cm for control, 25, 50, 75 and 100 mg/kg treatments respectively. There was no pattern of effect along the concentration gradient.
The result for number of leaves and leaf loss recorded for *A. esculentus* are shown in Tables 1 and 2 respectively. There was no significant difference in the number of leaves between control and all the treated plants, with control and the 100 mg/kg treatment having values of $5.00^a \pm 1.00$ and $4.33^a \pm 0.33$ respectively. There was significant difference at weeks 9 and 11 in the number of leaves lost recorded for control and the 100 mg/kg treated plants with values $3.33^a \pm 0.67$ and $5.33^b \pm 0.33$ for control and the 100 mg/kg treatment respectively. Control however recorded the least number of leaves lost.

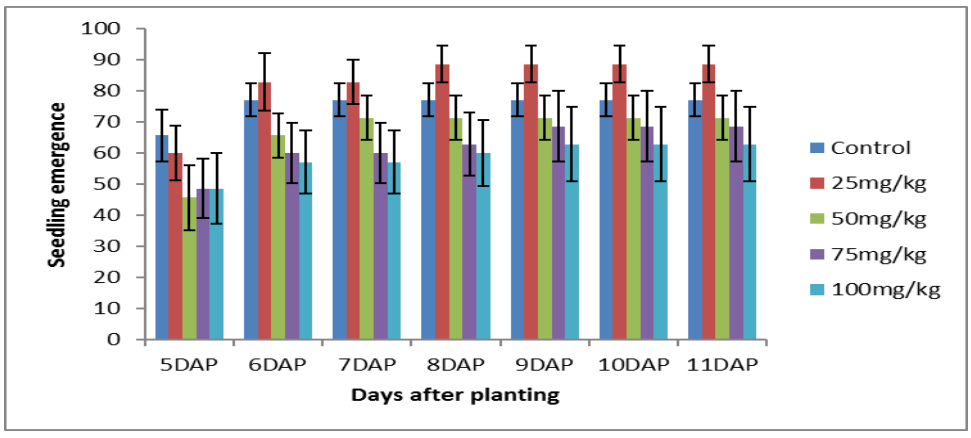

Figure 1: Effect of treatment with mixture of Cd and Pb on the percentage seedling emergence of *A. esculentus*

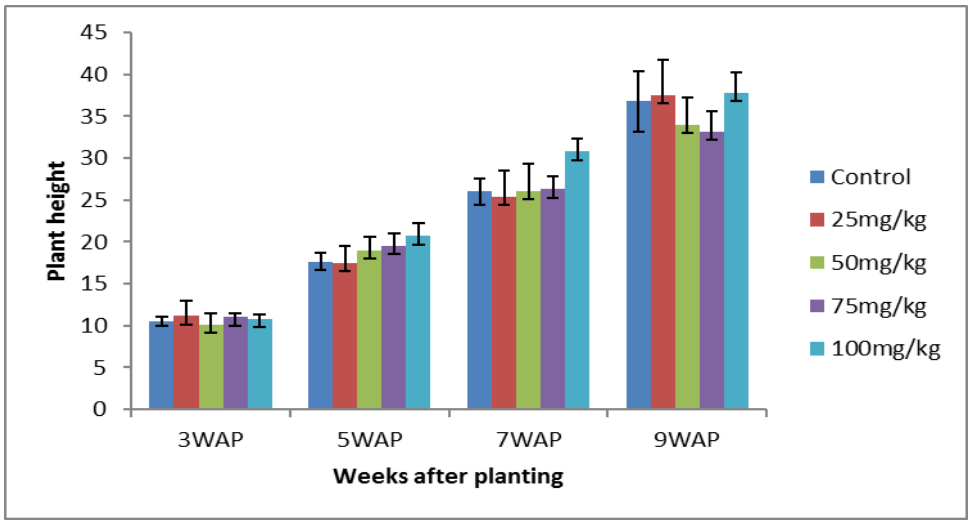

Figure 2: Effect of treatment with mixture of Cd and Pb on the height (cm) of *A. esculentus*

Table 1: Effect of treatment with mixture of Cd and Pb on the number of leaves of *A. esculentus*

Treatments	Weeks After Planting			
	3	**5**	**7**	**9**
Control	$5.00^{a} \pm 0.00$	$5.33^{a} \pm 0.33$	$5.33^{a} \pm 0.67$	$5.00^{a} \pm 1.00$
25mg/kg	$4.67^{a} \pm 0.33$	$5.67^{a} \pm 0.67$	$5.67^{a} \pm 0.33$	$4.67^{a} \pm 0.88$
50mg/kg	$4.33^{a} \pm 0.33$	$6.33^{a} \pm 0.88$	$5.67^{a} \pm 0.88$	$4.67^{a} \pm 0.33$
75mg/kg	$4.67^{a} \pm 0.33$	$5.00^{a} \pm 0.00$	$4.67^{a} \pm 0.67$	$5.33^{a} \pm 0.67$
100mg/kg	$5.00^{a} \pm 0.00$	$6.33^{a} \pm 0.33$	$6.33^{a} \pm 0.33$	$4.33^{a} \pm 0.33$

*Means with the same superscript have no significant difference.

Table 2: Effect of treatment with mixture of Cd and Pb on the number of leaves lost by *A. esculentus*

Treatments	**Weeks After Planting**			
	5	**7**	**9**	**11**
Control	$0.67^{a} \pm 0.33$	$1.33^{a} \pm 0.33$	$3.33^{a} \pm 0.67$	$3.33^{a} \pm 0.67$
25mg/kg	$0.33^{a} \pm 0.33$	$0.67^{a} \pm 0.33$	$4.33^{ab} \pm 0.33$	$5.00^{ab} \pm 0.58$
50mg/kg	$0.00^{a} \pm 0.00$	$1.33^{a} \pm 0.67$	$4.67^{ab} \pm 0.33$	$5.00^{ab} \pm 0.00$
75mg/kg	$0.67^{a} \pm 0.33$	$1.33^{a} \pm 0.33$	$4.00^{ab} \pm 0.57$	$4.00^{ab} \pm 0.57$
100mg/kg	$0.33^{a} \pm 0.33$	$1.67^{a} \pm 0.33$	$5.00^{b} \pm 0.00$	$5.33^{b} \pm 0.33$

*Means with the same superscript have no significant difference.

Leaf area results (Table 3) showed that the treatment enhanced leaf area though inconsistent with the levels of treatment. There was however no significant difference between the treatments except at 7WAP where the 100mg/kg treatment recorded a significantly higher value than control, with values $57.16^{a} \pm 5.46$ and $90.26^{b} \pm 2.34$ for control and the 100 mg/kg treatment.

Results obtained for stem girth Table 4 show a significant difference between control ($2.93^{a} \pm 0.23$ cm) and the 100 mg/kg treatment ($3.53^{b} \pm 0.18$cm) only at 9WAP. Growth in girth was significantly enhanced by the treatment. There was a significant reduction in the number of flowers produced as recorded for the treated plants compared to the control (Table 5). Control plants had $3.00^{b} \pm 0.58$ flowers while the 100 mg/kg treatment had $1.00^{a} \pm 0.00$ respectively. The number of fruits produced by the plants was however not significantly different between control and all the treatments. The control and 100mg/kg treatment had values of $1.00^{a} \pm 0.58$ and $0.67^{a} \pm 0.67$ number of fruits respectively.

Results on soil physicochemical properties (Table 6) show that the amount of essential elements in the soil decreased with increasing concentration of heavy metals treatment. The amount of Cd and Pb present in the soil increased with increasing concentration of treatment. Soil pH, conductivity and organic carbon content however did not follow a consistent pattern.

Table 3: Effect of treatment with mixture of Cd and Pb on the leaf area (cm^2) of *A. esculentus*

Treatments	Weeks After Planting			
	5	**7**	**9**	**11**
0	$29.89^{ab} \pm 2.73$	$57.16^{a} \pm 5.46$	$64.36^{a} \pm 6.79$	$62.80^{a} \pm 1.96$
25	$30.89^{ab} \pm 2.29$	$71.96^{ab} \pm 7.18$	$65.79^{a} \pm 8.62$	$52.87^{a} \pm 16.09$
50	$27.77^{ab} \pm 3.22$	$73.36^{ab} \pm 12.91$	$67.28^{a} \pm 4.91$	$54.33^{a} \pm 18.29$
75	$26.44^{a} \pm 7.26$	$59.42^{a} \pm 10.91$	$76.98^{a} \pm 19.57$	$65.66^{a} \pm 21.70$
100	$40.35^{b} \pm 1.46$	$90.26^{b} \pm 2.34$	$72.79^{a} \pm 5.07$	$72.96^{a} \pm 5.07$

*Means with the same superscript have no significant difference.

Table 4: Effect of treatment with mixture of cd and pb on the Stem girth of *A. esculentus*

Treatments	**Weeks after planting**			
	7	**8**	**9**	**11**
Control	$2.67^{a} \pm 0.10$	$2.88^{ab} \pm 0.15$	$2.93^{a} \pm 0.23$	$3.43^{a} \pm 0.23$
25mg/kg	$2.77^{a} \pm 0.03$	$3.00^{ab} \pm 0.12$	$3.03^{ab} \pm 0.03$	$3.47^{a} \pm 0.03$
50mg/kg	$2.70^{a} \pm 0.17$	$2.83^{ab} \pm 0.09$	$3.13^{ab} \pm 0.12$	$3.47^{a} \pm 0.15$
75mg/kg	$2.40^{a} \pm 0.26$	$2.60^{a} \pm 0.06$	$2.87^{a} \pm 0.12$	$3.63^{a} \pm 0.56$
100mg/kg	$3.00^{a} \pm 0.29$	$3.17^{b} \pm 0.22$	$3.53^{b} \pm 0.18$	$3.97^{a} \pm 0.09$

*Means with the same superscript have no significant difference.

Table 5: Effect of treatment with mixture of Cd and Pb on the number of flowers and fruits of *A.esculentus* at the end of the experiment

Treatment (mg/kg)	**Number of flowers**	**Number of fruits**
Control	$3.00^{b} \pm 0.58$	$1.00^{a} \pm 0.58$
25	$1.00^{a} \pm 0.00$	$0.67^{a} \pm 0.67$
50	$1.00^{a} \pm 0.58$	$0.67^{a} \pm 0.67$
75	$1.00^{a} \pm 0.00$	$0.67^{a} \pm 0.67$
100	$1.00^{a} \pm 0.00$	$0.67^{a} \pm 0.67$

Table 6: Soil physicochemical properties

Treatments (mg/kg)	**pH**	**Conductivity**	**Pb**	**Cd**	**K**	**Na**	**Ca**	**Mg**	**Organic Carbon**
Control	7.00	160.67	0.09	0.03	482.67	11	37	7.22	1.23
25	6.00	170.67	6.85	2.54	992.33	15.33	19.33	6.25	0.59
50	7.67	160.00	9.31	2.90	694.00	8.67	17.67	4.94	1.91
75	5.87	171.00	19.95	10.00	715.67	9.67	19.33	4.09	1.19
100	6.70	141.00	30.48	15.75	799.33	5.33	25.00	3.76	2.28

Results on microbial analysis (Tables 7 and 8) show an increase in total bacterial and fungal count in the heavy metal treated soils, with the 75mg/kg treated recording the highest values. The bacteria *Bacillus subtilis* and *Staphylococcus aureus* recorded the highest occurrence in the treated soils, while *Pseudomonas aeroginosa* had the highest occurrence in control soils than all the heavy metal treated soils.

Table 7: Total microbial count of soil samples

Treatments (mg/kg)	**Total Bacteria Count** (cfu/g)	**Total Fungi Count** (cfu/g)
Control	13×10^3	12×10^3
25	10×10^3	13×10^3
50	20×10^3	9×10^3
75	26×10^3	26×10^3
100	20×10^3	10×10^3

Table 8: Total occurrence of bacteria and fungi in soil samples

Treatments (mg/kg)	*Aspergillus niger*	*Penicillium notatum*	*Mucor mucedor*	*Bacillus subtilis*	*Pseudomonas aeroginosa*	*Staphylococcus aureus*
Control	+	+	+	+	++	+
25	-	+	+	++	+	+
50	++	+	++	+	+	+
75	+	+	+	+	+	+
100	+	++	+	++	-	++

Key: ++=High; +=Present; -=Absent

Heavy metal analysis of the plant (Table 9) show that the uptake of the metals increased as the concentration of treatment increased. Uptake of Pb was higher than the uptake of Cd in the leaf, root and fruit. The highest uptake of the metal was however seen to be in the root of the plant.

Table 9: Heavy metals analysis of leaf, root and fruit of *A. esculentus* at the end of the experiment.

Treatments(mg/kg)	**Leaf**		**Root**		**Fruit**	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	0.08	0.05	0.13	0.07	BDL	BDL
25	0.46	0.15	0.88	0.27	0.65	0.07
50	0.57	0.25	1.44	0.22	0.11	0.11
75	0.91	0.31	1.58	0.25	0.26	0.13
100	-	-	-	-	-	-

BDL- below detection limit

Discussion

Data for seedling emergence of *A. esculentus* showed that the 25mg/kg treatment enhanced seedling emergence while higher concentrations had adverse effects on the seedling emergence of *A. esculentus.* This is in conformity with the findings of Houshmandfer and Moraghebi (2011) that at their highest concentration of heavy metal mixture (180mg/kg), there was a significant decrease in seed germination as compared to control. Germination and seedling establishment are vulnerable stages in the life cycle of a plant (Vange *et al*., 2004). Decrease in seed germination of a plant can be attributed to accelerated breakdown of stored food materials in seed, and by the application of heavy metal mixture (Shafiq *et al*., 2008). Abedin and Meharg (2002) also stated that the seedling and seed germination stage of a plant's life are sensitive to environmental factors such as heavy metal pollution.

There was no significant difference in plant height and number of leaves recorded for control and all the treatments. There was however a significant difference in leaf loss as treated plants experienced higher values for leaf loss than control plants. Values for stem girth and leaf area were significantly different only between control and the 100mg/kg treatment. Edegbai and Anoliefo (2016a), observed decrease in leaf area when *Vernonia amygdalina* was treated with cadmium. However, there was no significant difference in leaf area between control and treated plants when the same plant was treated with lead alone (Edegbai and Anoliefo, 2016b). The presence of lead in this treatment appears to be antagonistic to cadmium.

There was an increase in the microbial count of treated soils; all treated soils also had same bacteria and fungi species as the control soil. Liu *et al*. (2007) suggested that under certain conditions, heavy metals can stimulate higher counts of microbial cells in the soil, which may be as a result of the succession of microorganisms.

There was no consistent pattern due to treatment in the result obtained for soil pH, conductivity, and organic carbon. Essential nutrients such as Na, Mg, and Ca decreased in all treated soils as compared to the control. K however was higher in the 50mg/kg and 75mg/kg treated soils than in the control soil. Alia (2015) discovered that increasing concentrations of heavy metal resulted in a decrease of Na, K, Ca, Fe, Mg, Mn, and Cu in spinach. The more heavy metal species in the soil, and the higher the concentration of the metals, the more they compete with the binding sites for these nutrient elements (Edegbai and Anoliefo, 2016a).

Plants exposed to the 100mg/kg treatment all died after a period of 3 months after planting. Edegbai and Anoliefo (2016a) recorded death of *Vernonia amygdalina* exposed to high concentrations of Cd treatment. Control plants recorded higher number of flowers than all the treated plants.

Results of heavy metal uptake by the plant revealed that the uptake of the metals increased with increasing concentration of treatments. Lead uptake was higher than Cd uptake in the leaf, root and fruit of the plant. The uptake of Pb in the plant parts was in the order root > leaf > fruit. Heavy metals interact with each other when they occur together in mixture. An antagonistic relationship can be suggested in this case where the presence of one metal may inhibit the uptake and activity of the other metal. The mobility and availability of heavy metals depend on the soil pH, content of the organic matter, grain size composition of soil and the type of metals (Guala *et al*., 2013).

The fruit of okra (*A. esculentus*) is the most consumed part of the plant in this part of the world. Although the highest mean accumulation of Pb and Cd recorded for the fruit were 0.26mg/kg and 0.13mg/kg which are lower than the recommended tolerable limit for these metals as proposed by FAO/WHO Expert Committee on Food additives (Cordex Alimentarius Commission, 1984) for leaves, stem and root of different vegetables (5.0 mg/kg Pb and O.3 mg/kg Cd), it is still a matter for concern as regular consumption of fruits exposed to these metals could lead to gradual accumulation of these metals in the body. The leaves, though not consumed as much as the fruit is also eaten as vegetable in some areas, and it was revealed in this study to also have taken up these metals.

In conclusion, man is at the risk of bioaccumulation of heavy metals through the food he consumes. Control measures should be put in place to limit the amount of heavy metal pollutants that penetrates soils and contaminate water. The plants were seen to have taken up these metals at higher amounts as the concentration of heavy metals increased. The fruit of the plant, which is the most consumed part of the plant, has been shown to bioaccumulate these metals, putting man at risk when plants that are grown in contaminated soils are consumed.

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