Growth and Development of Bitter Leaf (*Vernonia amygdalina* Del.) in Soils Treated with Mixture of Cadmium and Lead

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ABSTRACT: The effect of treatment with cadmium and lead (mixed in a 1:1 ratio) on the growth and development of *Vernonia amygdalina* Del. was investigated. The plant was grown from uniform green cuttings in buckets (each with 5kg dry soil) and allowed to stabilize for a month before being treated with the mixture of metals. The experiment was made up of control and four concentrations (25, 50, 75 and 100 mg/kg) of the metals in each mixture. Data were collected monthly for 12 months. Result on plant height, number of leaves, and number of branches and girth of stem revealed an adverse effect of treatment at the end of the experiment except leaf area which was enhanced. Values for number of leaves 35.67±7.53, 14.00±4.35, 12.33±0.88, 0.00 and 0.00 were recorded for control, the 25, 50, 75 and the 100 mg/kg treatments respectively,. There was decreased soil pH, microbial load and nutrients but an increase in soil carbon. The effect increased along the concentration gradient. There was more uptake of lead than cadmium.

Heavy metals are of great environmental concern due to their toxicity and cumulative behaviour (Yusuf *et al.*, 2002). Pollution of environment by toxic metal arises as a result of various industrial activities and has turned these metal ions into subjects of major health concern (Waisberg *et al.*, 2003). Heavy metals have become problematic in agricultural soils and crops as they are elements and as such they do not break down but rather persist in the environment endlessly (Jarup, 2003). Plants accumulate many metals and nutrients from the soil (Kachenko and Singh, 2006). As concentration of metals in soils increase, their uptake into plants also increases (Muchuweti *et al.*, 2006). Cadmium and lead are non-essential metals and they are often found in relatively large amounts in agricultural soils. The main sources of these metals are mining, industrial processes, atmospheric deposition, fertilizers and pesticides application (Zaltauskalte *et al.*, 2017). Of all toxic heavy metals, cadmium ranks the highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain (Shah and Dubey, 1998). Lead is one of the most abundant toxic metals in the earth crust. Elevated lead in soils may compromise soil productivity and even at very low concentration can inhibit some vital plant processes. *Vernonia amygdalina* is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes. In Nigeria, various ethnic groups boil the leaves in soups. Dry stems and branches provide fuel. Young twigs are used as toothpicks or chewing sticks. The plant is sometimes grown as a hedge. The branches are used as stakes to line fields. (Stevels, 1990; Mbinglo, 1998; Biholong, 1986) *Vernonia amygdalina* is also commonly used in traditional medicine. It is this versatility that has influenced its choice for this study. Heavy metal pollution is a multi-element problem in many areas (An *et al.*, 2004). Under these circumstances, synergistic and antagonistic interactions may be important, and predicted impact based on individual effects is likely to be erroneous (Ting *et al.*, 1991). There is therefore a clear need to understand the interactive effects produced by combinations of metal ions at different concentrations. The present study is aimed at determining the effect of the mixture of cadmium and lead on the growth and development of *Vernonia amygdalina*.

MATERIALS AND METHOD

Study Area: The study was carried out in the experiment plot of the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria which lies within the humid Tropical

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vegetation (latitude 6º 30′ 0″N and longitude 6° 0′ 0″ E).

Collection of Plant Materials and Soil Samples:
Stem: Stem cuttings of *V. amygdalina* used in the study were obtained from a hedge composed primarily of the plant within the Senior Staff Quarters of the University of Benin, Benin City, Edo State. The soils within the location had never been polluted with any known contaminant.

Soil: Soil samples were collected from the old Botanic Garden of the Department of Plant Biology and Biotechnology, University of Benin, Edo State – a site which had remained undisturbed for over fifteen (15) years. Top soil (0 – 10cm), of known physicochemical property was collected and dried. Thereafter, 5kg soil each was placed into 15 pieces of bottom – perforated 8 litres buckets.

Preparation of Stems: Uniform (30cm long, similar girth with 3-4 buds), young and freshly collected stem cuttings of *V. amygdalina* in preparation for planting were kept partially submerged in water for about one hour before planting. Three stems were subsequently planted in each bucket.

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 buckets.

The buckets earlier perforated and properly identified were laid out on the prepared site in a completely randomized design. Three stem cuttings of *V. amygdalina* were sown in each bucket containing 5kg soil and later thinned down to one (01) after fourteen (14) days of sprouting. The stands were allowed to stabilize for one (01) month before being exposed to treatment with cadmium and lead mixture. There were 4 concentrations (25, 50, 75 and 100 mg/kg) in 3 replicates and control. Cadmium and lead were measured and dissolved in distilled water and dispensed. After the soil treatment, data were collected on a monthly basis for 12 months (MAT – Months after Treatment). Soil and plant analyses were done at the end of 12 month period.

Field Data Collection:
Plant height: For plant height measurements, previously identified plant stands were tagged and growth monitored to ensure progressive evaluation and uniformity.

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

Leaf area: Leaf area measurements of the study plants were obtained from the previously tagged plants or their branches and determinations done using the proportional method according to (Eze, 1965).

Number of branches: The number of branches for *V. amygdalina* was taken by visual counting of branches on the tagged plants at given intervals.

Girth: Girth of *V. amygdalina* was taken monthly. The diameter of the shoot was obtained using the Esal vernier caliper. (Girth = πd).

Soil Physicochemical Analyses: The analyses were carried out following the methods outlined by Edegbai and Anoliefo, (2016a &b) 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: 20g of fine soil was placed in a container and 50ml of distilled water added. The suspension was shaken for 30minutes 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.

Nitrogen: One gram of the soil sample was placed into a Kjedahl digestion flask. One table spoon of a catalyst and 20ml concentrated tetraoxosulphate (VI) acid was added and the mixture was shaken to ensure mixing. At completion of digestion, 10ml distilled water was added and the solution was filtered through a Whatman filter paper. Nitrogen was determined calorimetrically at 625nm.

Organic Carbon: One gram 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:

$$\%TOC = \frac{T_B - T_S}{W_S} x0.3xM1.33$$

T_B = Titre value of blank; T_S = titre value of sample, W_S = Weight of sample

Available Phosphorus: One (01)g of soil was shaken for 5 minutes with 10ml of extracting solution containing 0.03N NH_4F and 0.1 N HCl. The solution was filtered through Whatman filter paper and 3ml of the filtrate was transferred into a test tube and 3ml of ammonium molybdate was added. Thereafter, five (05) drops of a mixture of boric acid, sodium sulphite and sodium sulphate were added. The phosphorus content was determined calorimetrically at 645nm.

Cation Exchange Capacity: Five (05)g of soil were placed into sterile conical flask and 20ml of extracting solution (NH_4OAc) was added into the 250ml volumetric flask containing the soil samples. Whatman filter paper was then used to filter the solution. Also 0.1ml of the filtrate was transferred to a test tube and diluted with 10ml 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. Two (02) 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 minutes. The digest was cooled and 5ml of concentrated nitric acid was added and heated for additional 30 minutes. The latter step was repeated and the solution was reduced to about 5ml 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 minutes 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 contents in the digested samples were analyzed for using the Atomic Absorption Spectrophotometer.

Statistics: Statistical analysis was carried out by determining the mean and standard error of three replicates.

RESULTS AND DISCUSSION
Plant height for *V. amygdalina* grown in soil treated with various levels of Cd + Pb and control is presented in Figure 1. Values recorded show that at the end of the experiment, control had the highest value while the 25 mg/kg and 50 mg/kg had significantly (P < 0.05) lower values. At the same time, the 75 mg/kg and 100 mg/kg treated soils had lost all their plants. The values 77.43±1.45, 62.37±2.04, 59.23±3.96, 0.00 and 0.00 cm were recorded for control, the 25, 50, 75 and 100 mg/kg treatments respectively at the end of the experiment.

The effect of Cd + Pb treatment on the number of leaves of *V. amygdalina* is shown in Figure 2. The results show that the control plants recorded higher number of leaves compared to the treatments. At 12 MAT, control plants recorded significantly higher (P < 0.05) number of leaves while the treatments showed adverse effect along the concentration gradient. The values 35.67±7.53, 14.00±4.35, 12.33±0.88, 0.00 and 0.00 represent results for number of leaves for control, the 25, 50, 75 and 100 mg/kg treatments respectively, at the end of the experiment.

Growth as seen in plant height (Figure 1) and number of leaves (Figure 2) revealed a significant adverse effect of treatment compared to control. Higher concentrations of treatment (75 mg/kg and 100 mg/kg) resulted in the death of plants. When *V. amygdalina* was treated with cadmium alone (Edegbai and Anoliefo, 2016a) growth inhibition and death of plants also occurred. In that experiment, control plant height was 77.43±1.45 cm while the 25, 50, 75 and 100 mg/kg had heights of 39.77±2.32cm, 28.77±4.32 cm, 0.00cm and 0.00cm respectively. However, when treated with lead alone, all plant stands survived through the period of the experiment with height values of 77.43±1.45,72.47 ± 7.33, 57.27 ± 5.71, 68.67 ± 6.13 and 66.47 ± 5.60 cm for control and the 25, 50, 75 and 100 mg/kg treatments. (Edegbai and Anoliefo, 2016b).The severity of adverse effect of these treatments compared to the current treatment is in the order Cd >CdPb>Pb. It is apparent that both heavy metals were antagonistic to each other.
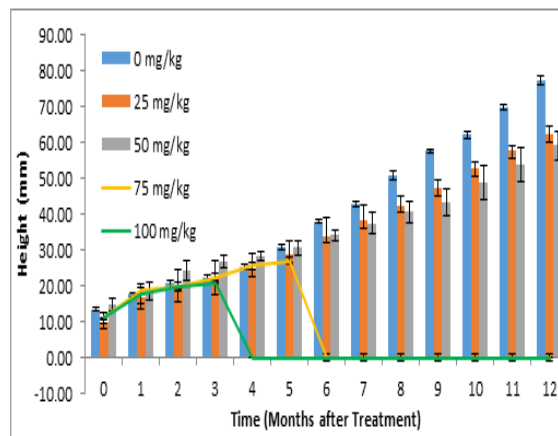

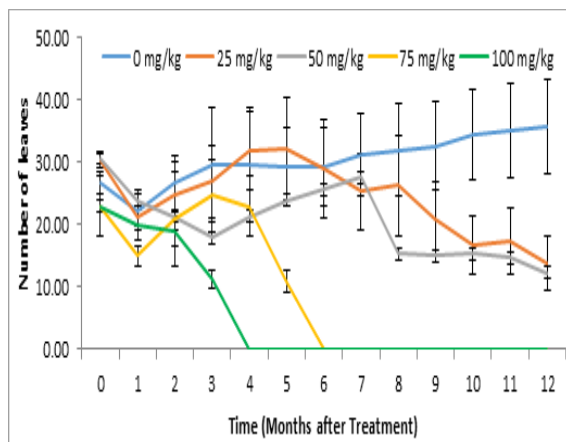**Fig 1**: Effect of Cd + Pb mixture on the height (cm) of *V. amygdalina*

Fig 2: Effect of Cd + Pb mixture on the number of leaves of *V. amygdalina*

Mean leaf area results of *V. amygdalina* for control and cadmium and lead mixtures treated soil are shown in Figure 3. Control values were consistently lower than the values recorded for the 25 mg/kg and 50 mg/kg treatments while the 75 mg/kg and 100 mg/kg treatment had lost all plant stands at 6 MAT and 4 MAT respectively. The values - 17.45 ± 4.84, 37.29 ± 8.55, 23.4 ± 8.46, 0.00 and 0.00 cm^2 are leaf area values taken at the termination of the experiment for control, the 25, 50, 75 and 100 mg/kg treatments respectively.

Figure 4 shows the effect of Cd + Pb mixture on the number of branches of *V. amygdalina* from 0 MAT to 12 MAT. There was a consistent increase in control values until 10 MAT. There was however a decrease in values recorded for the 25 mg/kg treatment at 6 MAT while 75 mg/kg and 100 mg/kg lost all branches at 6 MAT and 4 MAT respectively. There was no significant difference ($P < 0.05$) in the mean value for control (4.67 ± 0.66), the 25 mg/kg (4.00 ± 0.00) and the 50 mg/kg (3.33 ± 1.33) treatments.

Figure 5 shows the effect of Cd + Pb mixture on the girth (mm) of *V. amygdalina.* All the treatments recorded increases in girth from 0 MAT to 12 MAT except the 75mg/kg and 100mg/kg treatments which lost all plants by 6 MAT and 4 MAT respectively. There was no significant difference ($P < 0.05$) between control, the 25 mg /kg and the 50mg/kg, though these were significantly different from the 75mg/kg and 100 mg/kg treatments. The values - 71 ± 0.00, 15.71 ± 1.81, 16.76 ± 1.04, 0.00and 0.00 mm were girth values recorded for control, the 25, 50, 75 and 100 mg/kg treatments respectively at the end of the experiment.

Results on leaf area (Figure 3) revealed that treatment favoured the leaf area as control recorded lower values. In an earlier report, Cadmium alone resulted in decreased value for leaf area in treated plants while lead alone did not record any significant difference between control and treated plants (Edegbai and Anoliefo, 2016a &b). The mixture revealed less adverse effect as compared to the individual metals with the 25 mg/kg and the 50 mg/kg (Figure 3) treatments. Treatment had no significant adverse effect on number of branches (Figure 4) and girth (Figure 5) of stem at lower concentrations.
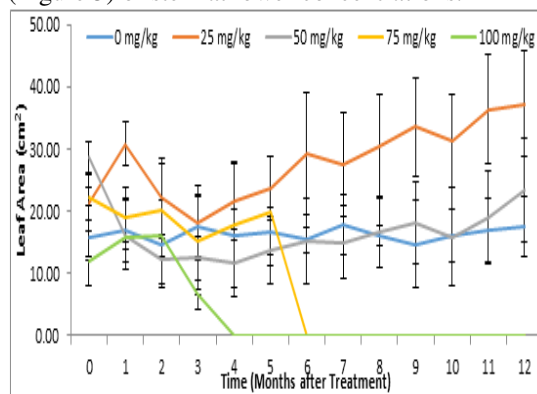

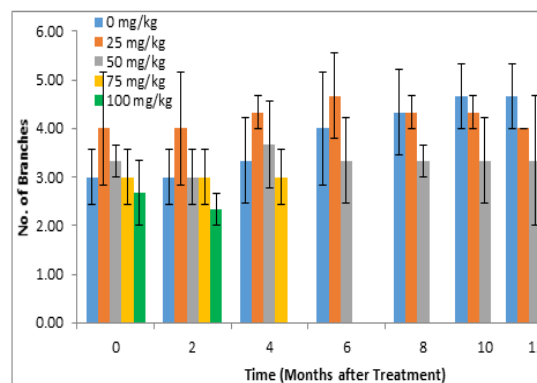**Fig 3**: Effect of Cd + Pb mixture on the leaf area (cm^2) of *V. amygdalina*

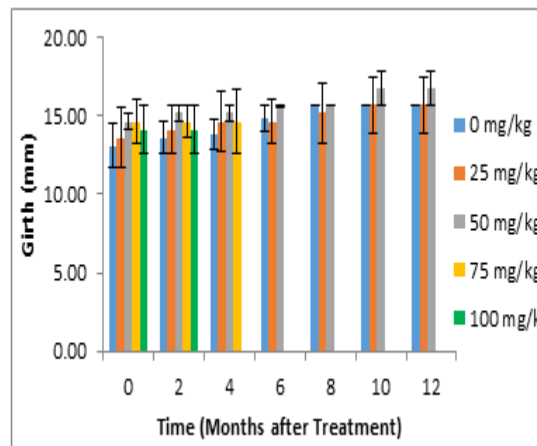**Fig 4**: Effect of Cd + Pb mixture on the number of branches of *V. amygdalina*

Fig 5: Effect of Cd + Pb mixture on the girth (mm) of *V. amygdalina*

Table 1 shows the results of pH and chemical analyses for soil at the end of the experiment. The pH was depressed by the presence of cadmium and lead and the effect was along the concentration gradient. The treatment also depressed the nutrient elements (N, Ph, Ca and Mg) composition of the soil. Conversely however, the carbon content of the soil increased as the cadmium and lead treatment concentration increased.

Result on soil pH (Table 1) revealed an increase in acidity along the concentration gradient. The mobility and availability of heavy metals and soil nutrients is greatly determined by soil pH. Increased acidity results in increase in the heavy metals available in solution in the soil and consequently to the plants. Depending on the reaction of the plant species to the presence of the heavy metals, they could exhibit effects commensurate with the degree of exposure. Sauve *et al.* (1997), Nolan *et al.* (2003) and Su *et al.* (2007) reported that the solubility of heavy metals was significantly related to their total concentration,

together with soil pH. The increased acidity along the concentration gradient is similar to results obtained by Edegbai and Anoliefo (2016a &b) when they treated *V. amygdalina* with cadmium and lead separately.

The carbon constituent (Table 1) increased along the concentration gradient. Similarly, individual cadmium and lead treatment resulted in an increase in carbon constituent. Zhang and Wang (2007) revealed that high amount of heavy metals in polluted soil could slow down the mineralization rate of soil organic C and increase the amount of hardly biodegradable C.

The results on other analyses show that %N, %P, %Ca and %Mg (Table 1) decreased with an increase in the concentration of treatment. Wu *et al.* (2007) discovered that the presence of Cd and Pb reduced the plant uptake rate of essential elements like Mn, Fe, K, Mg and Ca. Plants cultivated in soil contaminated with heavy metals are subject to modification of the chemical composition of not only the content of heavy metals but also macronutrients (Ciecko *et al.*, 2004).

Table 1: Physicochemical properties of Cd + Pb post *V. amygdalina* cultivated soil at the end of the experiment (12 MAT)

Concentration mg/kg	pH	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Ca (ppm)	Mg (ppm)	K (ppm)	Na (ppm)
0	8.1	0.82	0.29	3.71	1.26	0.82	0.21	0.11
25	5.9	1.12	0.18	2.80	0.88	0.70	0.11	0.05
50	5.6	1.17	0.13	2.52	0.81	0.63	0.08	0.03
75	5.4	1.26	0.11	2.27	0.73	0.46	0.06	0.02
100	5.1	1.34	0.09	2.01	0.65	0.34	0.05	0.01

Table 2 shows the concentration of cadmium and lead accumulated by the plant at the end of the experiment. The amount of both metals accumulated by the plant increased with increase in the concentration of their treatment. The metals were not detected in the control plants. The highest uptake of 0.138ppm and 0.387 ppm were recorded for 100 mg/kg treatment with Cd and Pb. When treated with cd alone (Edegbai and Anoliefo, 2016a) and pb alone (Edegbai and Anoliefo, 2016b) uptake values of 0.283ppm and 0.871 ppm were recorded for the 100 mg/kg treatment. It presents an antagonistic relationship between the metals. Presence of one hindered the uptake of the other. Similar results were obtained by Alia *et al* (2015). Results of microbial analyses (Table 3) showed reduction in the quantity of bacteria and fungi in the soil. The effect increased as the concentration of treatment increased. Similar results were obtained with individual treatments of cadmium and lead. Wyskowska *et al.* (2008) revealed that heavy metals resulted in a decrease in microbial biomass and a reduction in their activity in the soil. In cases when they do not lower counts of microorganisms, they still reduce their diversity (Xie *et al.*, 2009; Wakelin *et al.*, 2010).

Table 2: Composition of heavy metals in *V. amygdalina* cultivated soil at the end of the experiment (12 MAT)

Concentration mg/kg	Cd (ppm)	Pb (ppm)
0	ND	ND
25	0.045	0.097
50	0.053	0.177
75	0.087	0.209
100	0.138	0.387

ND= Not Detected

Table 3 Results of microbial analyses

Concentration mg/kg	Bacterial (cfu/g)	Fungal (cfu/g)
0	1.37×10^5	6.7×10^4
25	7.3×10^4	2.7×10^4
50	6.3×10^4	2.6×10^4
75	5.5×10^4	2.4×10^4
100	4.8×10^4	2.0×10^4

Conclusion: In this study, the impact of mixture of the Cd and Pb on the growth and development of *V. amygdalina* was investigated. The interaction between the heavy metals, though antagonistic, resulted in inhibition of growth of *V. amygdalina.* Uptake of the metals was reduced as a result of this competitive interaction.

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