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Growth and Development of *Vernonia amygdalina* Del in Soils Treated with Lead

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Abstract
The effect of lead on the growth and development of Vernonia amygdalina Del. was investigated. The plant as grown from uniform green cuttings in buckets (each with 5 kg dry soil) and allowed to stabilize for a month before being treated with lead. The experiment was made up of control and four concentrations (25, 50, 75 and 100 mg/kg) in three replicates. Data were collected on a monthly basis for 12 months. The results for plant height, number of leaves, leaf area, number of branches and girth showed adverse effect of lead treatment on growth except for leaf area which apparently was enhanced. The soil treatment with lead, at 12 months after planting (MAT), had 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 and leaf area values of 17.45 ± 4.84, 21.47 ± 8.83, 14.55 ± 2.01, 32.12 ± 9.58 and 24.85 ± 7.07 cm^2 for control, the 25, 50, 75 and 100 mg/kg treatments respectively. Lead caused decrease in pH, nutrient element composition of soil and soil microbial population. It however caused increase in soil carbon. Lead taken up by plant was within tolerable limits. These effects were along the concentration gradient.

Keywords: Treatment, growth, nutrient, gradient, lead, *Vernonia amygdalina*

Introduction
Lead (Pb) is one of the toxic heavy metal pollutants common to all urban systems. The metal harmfully affects neurovascular systems of humans and is also toxic to all other life forms including marine life, and has negative influence on crop yields (1, 2, 3). Lead occurs naturally in poor quality fossil fuels and it continuously pollutes roadside soils and industrial environments all over the world.

For several years now, lead has been the intense focus of environmental health research and this is understandable, considering the perennial effect of lead toxicity (4, 5, 6). It is therefore, imperative to decipher whether vegetables, fruits and food crops are safe for human consumption. There have been several reports on lead contamination in Nigeria (4, 7, 8, 9, 5, 6) and this pollution spans across the water, soil and air environments (4). Lead levels in soil and air vary according to the location and nearness to lead based activities and vehicular density. The sources of food contamination have often been traced to fumes from car exhausts (9). All these sources of pollution invariably contribute to food contamination.

Lead persists in the environment once it is introduced (10) and plants which are able to concentrate lead accumulate it in their roots and foliage (11). Okoronkwo and collegues(6) reported the levels of lead present in the soil from an abandoned waste dump in Umuahia, South-eastern Nigeria with mean concentrations of 111.75 ± 17.78 and 76.63 ± 19.94 mg/kg respectively. It is also not uncommon to find vegetable farms being irrigated with sewage water and vegetables grown in these farms have also been shown to have high concentrations of lead and other heavy metals, with large concentrations in the roots and foliage. Generally, lead contaminations occur in vegetables grown on contaminated soils (12). Lead poisoning is a global reality, and fortunately is not a very common clinical diagnosis yet in Nigeria, except for few occupational exposures (13). It is considered as environmental hazards, as they are toxic for human being and other organisms (14, 15, 16).

Vernonia amygdalina stem is used as chewing stick; the leaf is eaten raw or as vegetable for food, while the decoction of the root serves as medicaments. A number of Asteraceae plants have been found to bioaccumulate heavy metals, for example, *Helianthus annuus* which bioaccumulates Pb (17). There is need to know if the same can be said of this plant and lead. This study investigated the effect of lead on the growth and development of *Vernonia amygdalina* and provides data on the safety of consuming *V. amygdalina* grown in the lead polluted soil.

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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 Vegetation. Latitude 6° 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. As much as possible, 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 (0-10 cm), of known physicochemical property, was collected and dried. Thereafter, 5 kg soil each was placed into 15 pieces of bottom –perforated - 8 - litres buckets.

Lead: The lead (Pb) used in this study was obtained from lead nitrate ($PbNO_3$) – a soluble salt.

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.

Methodology

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 5 kg 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 lead. There were 4 concentrations (25, 50, 75 and 100 mg/kg) in 3 replicates and control. Lead was 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 the 12 month period.

Field Data Collection

Plant Height: For plant height measurements, previously identified plant stands were tagged and growth followed to ensure progressive appraisal and uniformity.

Number of leaves: The total number of leaves for 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 (18)

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

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

Soils were dried at ambient temperature (22-25°C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried and less than 2 mm 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: Twenty grammes (20 g) of fine soil was placed in a container and 50 ml of distilled water added. The suspension was shaken for 30 min and allowed to settle. Electrical conductivity and pH of the solution were then measured using a pH meter **(**Model 215**)** and conductivity meter. The pH meter was first standardized using a buffer solution.

Nitrogen: One gramme (1.0 g) of the soil sample was placed into Kjedahl digestion flask. One table spoon of a catalyst and 20 ml concentrated tetraoxosulphate acid was added and the mixture was hand shaken to ensure mixing. At completion of digestion, 10 ml distilled water was added and the solution was filtered through a Whatman filter paper. Nitrogen was determined colorimetrically at 625 nm.

Organic Carbon: One gramme (1.0 g) of the soil sample was placed in a 250 ml conical flask. Then 10 ml of $K_2Cr_2O_7$ and 20 ml conc. 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. Ten (10) ml of phosphoric acid and 8 drops of diphenylamine solution were then added. A blank determination was done by using 10ml $K_2Cr_2O_7$ and 20 ml concentrated H_2SO_4 solution and titrated to a green colour with Ferrous Ammonium sulphate solution.

The total organic carbon (TOC) was calculated as:

$$\% \text{ T O C} = \frac{\text{Titre value of blank- titre value of sample} \times 0.3 \times \text{M1.334}}{\text{Weight of sample}}$$

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

Cation Exchange Capacity: Five grammes (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 % stronium 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 (2) g portions of the samples were weighed accurately and 10.0ml of concentrated HNO_3 was added to each. The samples were digested on a hot plate for 15 minutes. The digest was cooled and 5 ml of concentrated nitric acid was added and heated for additional 30 minutes. The later 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 10 ml 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 ashless filter paper and diluted to 60 ml with distilled water. Lead content 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 (19).

Result

Mean plant height recorded for *V. amygdalina* grown in soil treated with various levels of lead and control is presented in Figure 1. The presence of lead appeared to trigger growth up to 10 MAT, beyond which period, control recorded the tallest plants. All the treatments including control had consistent and steady increase in plant height while the experiment lasted. The growth pattern was however inconsistent with pollution concentration. There was significant difference ($P < 0.05$) between control mean and that of the 50mg/kg treatment only. At 12 MAT, the 50 mg/kg lead treatment had the least mean height. At the end of the experiment, control height was 77.43±1.45cm while the 25, 50, 75 and 100 mg/kg treatments had mean values of 72.47±7.33, 57.27±5.71, 68.67±6.13 and 66.47±5.60 cm respectively.

Figure 2 shows the mean number of leaves of *V. amygdalina* recorded for control and lead treated soils. At the end of the experiment, the control plants recorded more leaves than the plants in the lead treated soil. At 12 MAT, control mean number of leaves was significantly higher ($P < 0.05$) than the 100 mg / kg treatment only. At this time (12 MAT), control mean number of leaves was 35.67±7.53 while the 25, 50, 75 and 100 mg/kg treatments had mean values of 31.67±3.28, 29.33±0.66, 29.00± 1.54 and 8.67 ±0.66 respectively.

Mean leaf area results of the effect of lead treatment on *V. amygdalina* are shown in Figure 3. The values did not show any consistency along the concentration gradient. At the end of the experiment, control mean was lower than the lead treatments except the 50 mg/kg lead treated soil. There was no significant difference ($P < 0.05$) between the values recorded for the various treatments and control. Data collected at 12MAT for control and the 25, 50, 75 and 100 mg/kg treatments were 17.45 ± 4.84, 21.47 ± 8.83, 14.55 ± 2.01, 32.12 ± 9.58 and 24.85 ± 7.07 cm^2 respectively.

Figure 4 shows the mean number of branches recorded for control and various soil treatments with lead. At the end of the experiment (12 MAT), control mean value was higher than the values recorded for the lead treatments. There was significant difference ($P < 0.05$) between control and the 25 mg/kg and the 50 mg/kg treatments. There was no consistent pattern with the treatment concentration. At this time, the values 4.67 ± 0.67 (control), 2.67 ± 0.67 (25 mg/kg), 2.67 ± 0.67 (50 mg/kg), 3.00 ± 0.57 (75 mg/kg) and 3.33 ± 0.33 (100 mg/kg) were recorded.

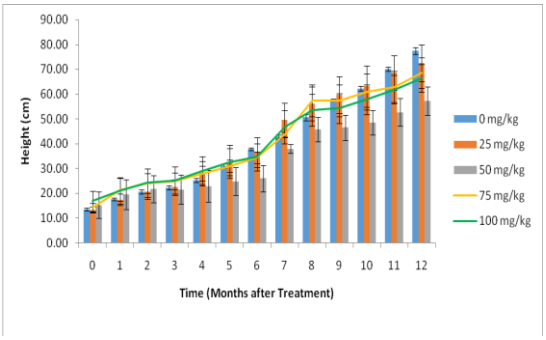

Figure 1: Effect of Pb treatment on the height (cm) of *V. amygdalina*

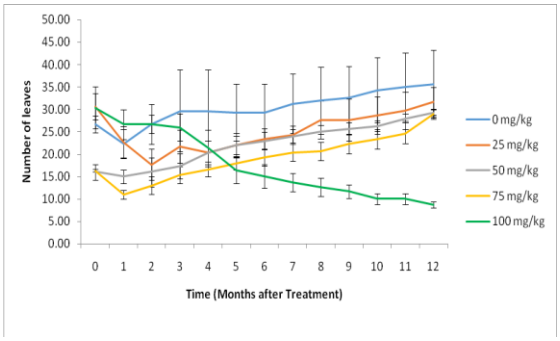

Figure 2: Effect of Pb treatment on the number of leaves of *V. amygdalina*

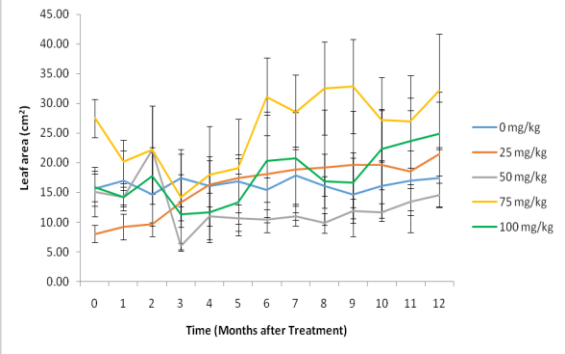

Figure 3: Effect of Pb treatment on the leaf area (cm^2) of *V. amygdalina*

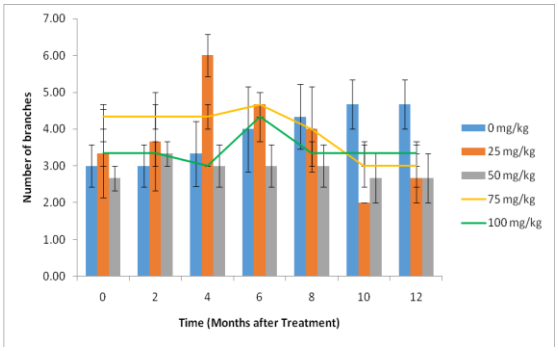

Figure 4: Effect of Pb treatment on the number of branches of *V. amygdalina*

Figure 5 shows the mean values recorded for girth of stem of *V. amygdalina* for control and various lead treated soil. Control girth as well as the girth of all the treatments increased throughout the duration of the experiment, except the 100 mg/ kg treatment which reduced at 8 MAT. There was no significant difference ($P < 0.05$) between the values recorded for control and the various lead treated soils. Control mean girth was 15.71 ± 0.00 mm while the 25, 50, 75 and 100 mg/kg treatments were 15.19 ± 3.43, 14.14 ± 2.39, 17.8 ± 0.52 and 14.66 ± 1.04 mm respectively.

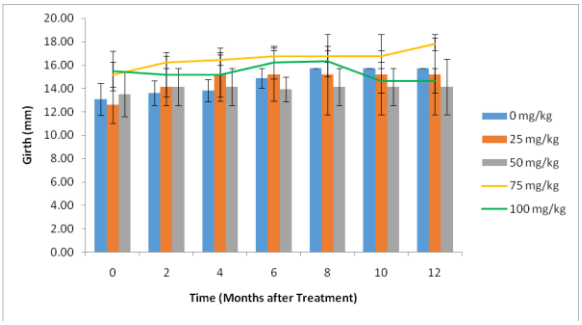

Figure 5: Effect of Pb treatment on the girth of *V. amygdalina*

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 lead and the effect was along the concentration gradient. Similarly, lead contamination depressed the nutrient elements (N, Ph, Ca and Mg) composition of the soil. Conversely however, the carbon content of the soil increased as the lead treatment concentration increased.

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

Concentration (mg / kg)	pH	Carbon (%)	Nitrogen (%)	Phosphorus(%)	Ca (ppm)	Mg (ppm)
0	8.1	0.82	0.29	3.71	1.26	0.82
25	6.9	0.97	0.21	3.2	1.03	0.72
50	6.6	1.12	0.18	2.9	0.94	0.64
75	6.3	1.19	0.17	2.51	0.83	0.56
100	6.1	1.23	0.14	2.33	0.78	0.41

Table 2 shows the concentration of lead accumulated by the plant at the end of the experiment. The amount of lead accumulated by the plant increased with increase in the concentration of lead treatment. Lead was not detected in the control plants.

Table 2: Lead accumulated by *V. amygdalina* in the treated soil at 12 MAT

Concentration (mg / kg)	Pb (ppm)
0	ND
25	0.175
50	0.289
75	0.534
100	0.871

ND- Not Detected

Discussion

In this study, growth [as estimated by height (Figure 1), number of leaves (Figure 2) and leaf area (Figure 3)] may have apparently been stimulated by Pb treatment for a period of the study. For example, plant height up to 10 MAT. Beyond this time, the adverse effect of the metal became manifest. The effect was inconsistent with pollution concentration. All the plants survived the entire experiment. Anoliefo and Umweni (20) observed that growth of *Launae taraxcifolia* plants in lead treated soils compared favourably with control.

There was an enhancement in the leaf area for the treated plants (Figure 3) as compared to the control at the end of the experiment except for the 50 mg/kg treatment which recorded a lower value than the control. This outcome is at variance with the observation of (21) that in sunflower, lead reduced leaf area, dry mass and plant height. Also, (22) reported significant decrease in leaf area of *Cucumeropsis manni* Naudin in cadmium treated soil. In addition, some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species (23, 24). The results of this study are at variance with their findings. No significant difference was observed for girth of stem for control and the various treatments.

The treatment with heavy metals resulted in increased acidity. There was increased acidity along the concentration gradient. Soil pH is a very important factor that controls the mobility and availability of metals and soil nutrients. (25). (26) and (27) reported that the solubility of heavy metals was significantly related to their total concentration, together with soil pH. Bioavailability of metals and their potential uptake is determined by the fraction of free metals present in the soil solution in relation to the total content of metals in the solid phase (28, 29). The chemical properties of metals in soil and their retention in the solid phase of soil is affected by pH, quantity of the metal, cation-exchange capacity, content of organic matter and mineralogy of soil. Changes in chemical properties of soils affect concentration of free metals and result in changes in their availability for plants (30). With increasing pH, content of organic matter and clay, the solubility of most metals decreases due to their increased adsorption. The bioavailability of zinc, lead and copper from soil decreases with increasing pH. The decreased availability of metals is affected by higher adsorption and precipitation in alkaline and neutral environments (31, 32).

Also, the carbon constituent of the soil increased as the concentration of the treatment increased. This could be due to slowing down of the mineralization rate of soil organic C and increase in the amount of hardly biodegradable organic C. (33). They also observed that increasing soil heavy metals pollution resulted in increase in the particulate organic matter and its proportion in total soil organic carbon.

The results of the other analyses show that % N, % P, % Ca, % Mg, % K and % Na constituents of the soil decreased with increased concentration of the treatment. 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

(34). Walker and others (35) observed that Pb decreased the uptake of phosphorus in *Zea mays*. Kibria (36) also reported that Pb application significantly decreased potassium concentration in straw and roots of rice shoot and root of radish and leaf, stem and root of Indian spinach. It was reported that above its critical level, an increase in Pb^{2+} decreased K concentration in shoot of cowpea (37).

The present concentrations of lead (the highest was 0.871 mg/kg) in the plant are lower compared to the recommended tolerable levels proposed by the joint FAO/WHO Expert Committee on Food Additives for leaves, stem and root of different vegetables, which is 5.0 mg kg-1 (38, 39).

Conclusion

The findings of this study raise causes for concern on the safety of consumption of *V. amygdalina* (the bitter leaf) without recourse to its source. Though the quantity of Pb bioaccumulated in the plant may be within tolerable limits, with the level of consumption of the plant, one should be weary of the implications of its consumption over a long period. Morphologically, control and treatment plants did not present marked differences.

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