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Effect of Mixture of Lead and Zinc on the Growth and Development of *Vernonia amygdalina* Del.

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ABSTRACT The effect of the mixture of lead and zinc (1:1) on the growth and development of *Vernonia amygdalina* Del was investigated. The plant was grown from stem cuttings in buckets (each with 5 kg dry soil) and was allowed to stabilize for a month before being treated with the mixture of lead and zinc. The experiment was made up of control and four concentrations (25, 50, 75 and 100 mg/kg) in three replicates. Data were collected monthly for 12 months for plant height, leaf area, number of branches and girth. Results revealed an adverse effect of treatment. Data recorded for number of leaves were 35.67±7.53, 19.67±4.63, 23.00±1.52, 25.33±4.48 and 21.67±5.54 for the control and four treatments (25, 50, 75 and 100 mg/kg) respectively. Girth values obtained at the experiment for the control, 25, 50, 75 and 100 mg/kg treatments were 15.71±0.00, 11.00±0.90, 12.57±0.00, 14.14±0.90 and 12.57±1.81mm respectively. There was significant difference between height and girth of control and the treated plants. Treatment also resulted in a decrease in soil pH, nutrient element composition of soil and soil microbial population. It however caused an increase in soil carbon. The plants took up more lead than zinc.

Keywords: Growth, Treatment, Lead, Zinc, Mixture, *Vernonia amygdalina*

Introduction

Heavy metals are of great environmental concern due to their toxicity and cumulative behaviour (Yusuf *et al*., 2002). Plants contamination with various heavy metals due to human activity has been observed (Hawiczka, 2008; Sas-Nowosielska, 2009). Other sources of heavy metals pollution in plants are landfills, non-ferrous metal smelters, mines, chemicals, pesticides and fertilizers.

Zinc (Zn) is a micronutrient essential for plants but can be highly toxic when present at excessive concentration, causing reduced growth, leaf chlorosis and nutritional disturbances (Kinraide *et al*., 2004). Lead (Pb) is a non-essential and toxic metal (Lai *et al*., 2012). It causes retardation of plant growth (Edegbai and Anoliefo, 2016a) and has significant negative impacts on seedling biomass, root and shoot lengths (Joseph *et al.,* 2002).

The uptake of toxic elements from contaminated soils by plants often exceeds their acceptable norms and leads to phytotoxicity (Niesiobedzka *et al.*, 2005). Each metal ion has a characteristic manner of uptake, transport and accumulation in the plant, but in the presence of other ions in the soil, there are also noted interactions between the ions (Eapen and D`souza, 2005). These interactions can be neutral, synergistic or antagonistic. In the case of a neutral interaction, the occurrence of one element does not affect the uptake of the others from the soil. A synergistic relationship is one in which there is an observed increase of one ion uptake in the presence of another. An antagonistic relationship is one which is about stopping or reducing the uptake of one metal in the presence of another (Mulielinska *et al.*, 2016).

It is important to investigate the combined effect of pollutant mixtures on agricultural plants. The present study focuses on investigating the effect of the mixture of lead and zinc treatment on the growth and development of *Vernonia amygdalina* Del.

Materials and Methods

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 on latitude 6º 30′ 0″N and longitude 6º 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 at a site which had remained undisturbed for over fifteen (15) years from the old Botanic Garden of the Department of Plant Biology and Biotechnology, University of Benin, Edo State. Top soil (0 – 10cm), of known physicochemical property was collected and dried. Thereafter, 5 kg soil each was placed into 15 pieces of 8 litres buckets perforated at the bottom of each bucket.

Preparation of stems: Uniform (30 cm 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 1 h before planting. Three (3) 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 (3) stem cuttings of *V. amygdalina* were sown in each bucket containing 5 kg soil and later thinned down to one (1) after fourteen (14) days of sprouting. The stands were allowed to stabilize for one (1) month before being exposed to treatment with lead and zinc mixture (1:1). There were 4 concentrations (25, 50, 75 and 100 mg/kg) in 3 replicates and control. Lead and zinc were measured and dissolved in distilled water and dispensed.

After the soil treatment, data was collected on a monthly basis for 12 months after treatment (MAT). 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 followed to ensure progressive appraisal and uniformity.

Number of leaves: The total number of leaves of *V. amygdalina* was taken by visual (manual) 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 (manual) 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: Soils were dried at ambient temperature (22-25 ºC), 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*:* 20 g of fine soil was placed in a container and 50ml 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.

pH of soil: The pH of the soil samples was determined following Jackson (1967). The oven dried soil sample weighing 12.5 g was suspended in 25 ml of distilled water and stirred continuously. The pH was measured using calibrated pH meter.

Nitrogen: 1.0 g of the soil sample was placed into a Kjedahl digestion flask. One table spoon of a catalyst and 20 ml concentrated tetraoxosulphate (VI) acid was added and the mixture was 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 calorimetrically at 625nm.

Organic carbon: 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 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 150 ml. 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 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:

$$\% \text{ TOC} = \frac{\text{Titre value of blank} - \text{Titre value of sample } \mathbf{x}\ 0.3\ \mathbf{x}\ \text{M}1.33}{\text{Weight of sample}}$$

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Available phosphorus: 1.0 g of soil was shaken for 5 minutes with 10 ml of extracting solution containing 0.03N 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 3 ml 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 calorimetrically at 645 nm.

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 10 ml 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 latter step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 5 ml 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 60 ml with distilled water. Lead and zinc 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. Data were subjected to one way analysis of variance (ANOVA) using SPSS-20® software.

Results

Figure 1 shows the mean height values for all *V. amygdalina* treated plants and control plants. There was significant difference ($P < 0.05$) between control and all the treatments. The lowest height was recorded in the 25mg/kg treatment (30.90±0.51 cm) at the end of the experiment at 12MAT.

Figure 2 shows the number of leaves recorded for the Pb + Zn treated and control plants of *V. amygdalina*. The mean values recorded show a consistent increase in control values while other treatments increased and decreased at various times during the experiment. There was no significant difference ($P < 0.05$) between control values and those for the treatments. The pattern of effect was inconsistent with treatment concentration. When control had a mean number of leaves of 35.67±7.53, the 25mg/kg, 50mg/kg, 75mg/kg and the 100mg/kg treatments had 19.67±4.63, 23.00±1.52, 25.33±4.48 and 21.67±5.54 values respectively.

Leaves area values representing the effect of Pb + Zn treatment and control on *V. amygdalina* are shown in Figure 3. Results did not show any consistent pattern along the concentration gradient. There was no significant difference ($P<0.05$) between control and treatments. Leaf area values were 17.45±4.85 for control and 15.07±0.30, 20.80±2.85, 23.84± 4.01 and 14.27±1.52 cm^2, for the 25, 50, 75 and the 100 mg/kg treatments respectively at the end of the experiment.

Figure 4 shows the effect of Pb + Zn treatment on the number of branches of *V. amygdalina*. Control recorded increase throughout the duration of the experiment while other treatments had lower values than control, though inconsistent with pollution concentration. There was no significant difference ($P < 0.05$) between control and all the treatments. The lowest mean number of branches was recorded in the 100mg/kg treatment (3.33±0.88).

Figure 5 shows the effect of Pb + Zn treatment on the girth of *V. amygdalina.* The various treatments recorded lower mean values than the control value at the time of termination of the experiment. Control value was significantly different ($P<0.05$) from the values recorded for the 25, 50, 75 and the 100 mg/kg treatments.15.71±0.00, 11.00±0.90, 12.57±0.00, 14.14±0.90 and 12.57±1.81 mm were recorded for control, the 25, 50, 75 and the 100 mg/kg treatments at the end of the experiment.

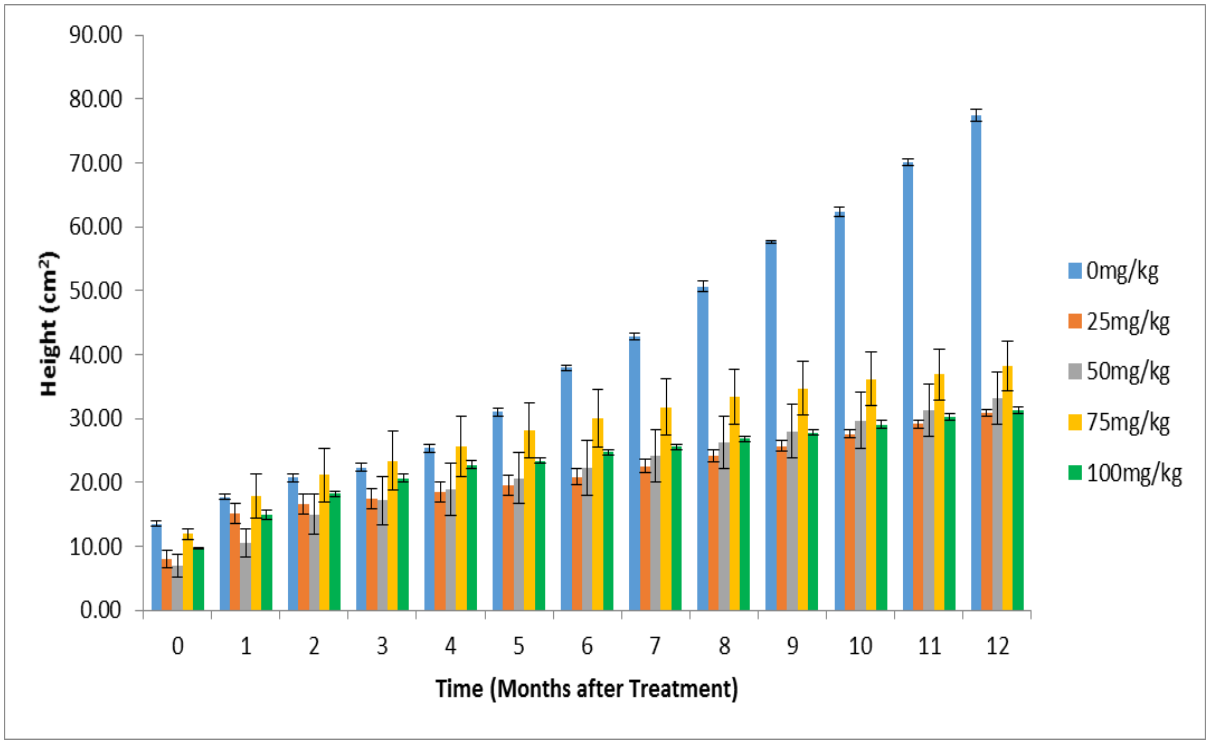

Figure 1: Effect of Pb + Zn mixture on the Height (cm) of *V. amygdalina*

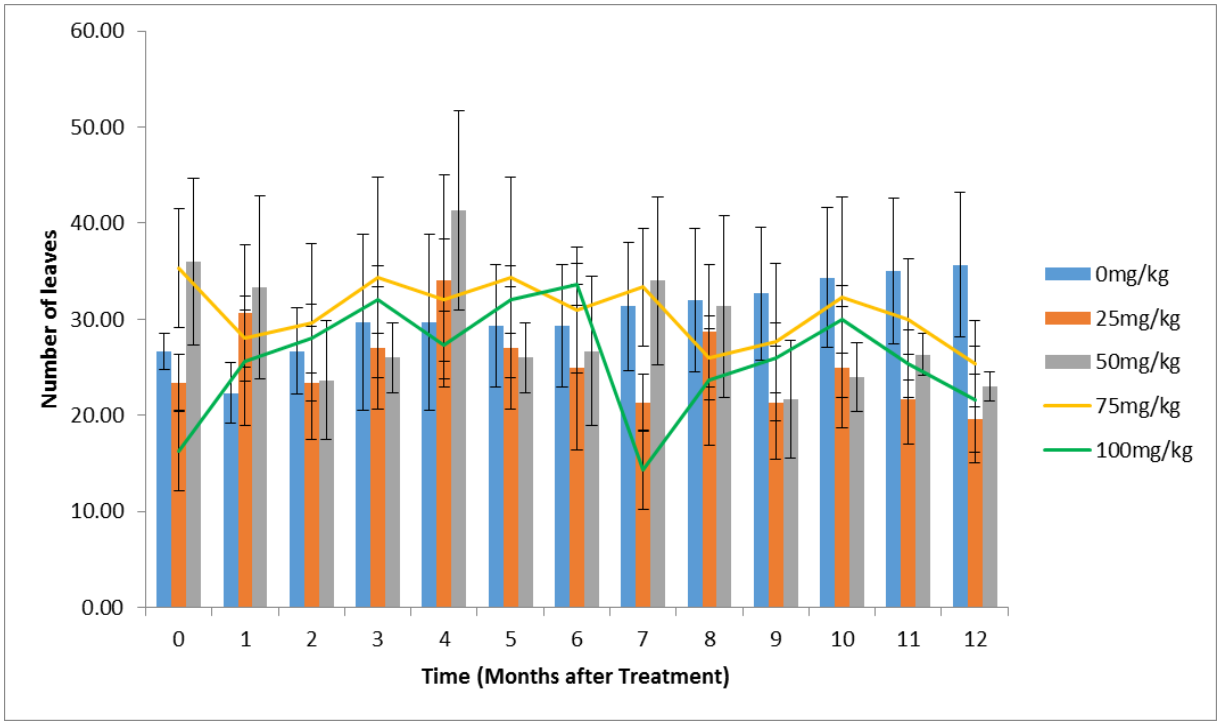

Figure 2: Effect of Pb + Zn mixture on the number of leaves of *V. amygdalina*

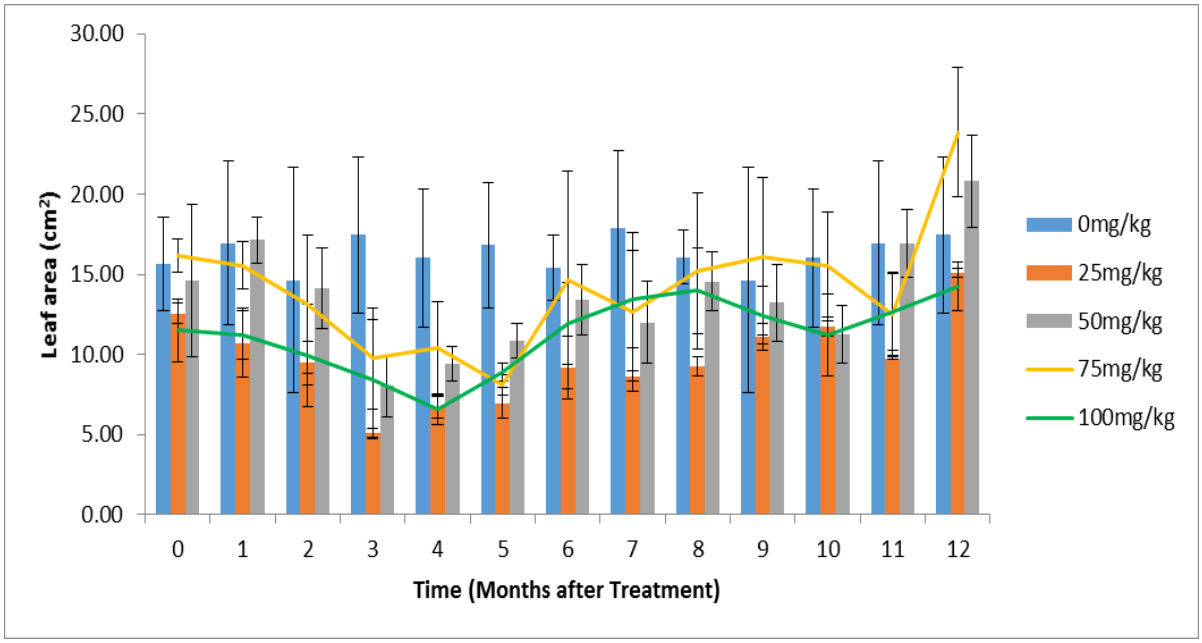

Figure 3: Effect of Pb + Zn mixture on the Leaf area (cm^2) of *V. amygdalina*

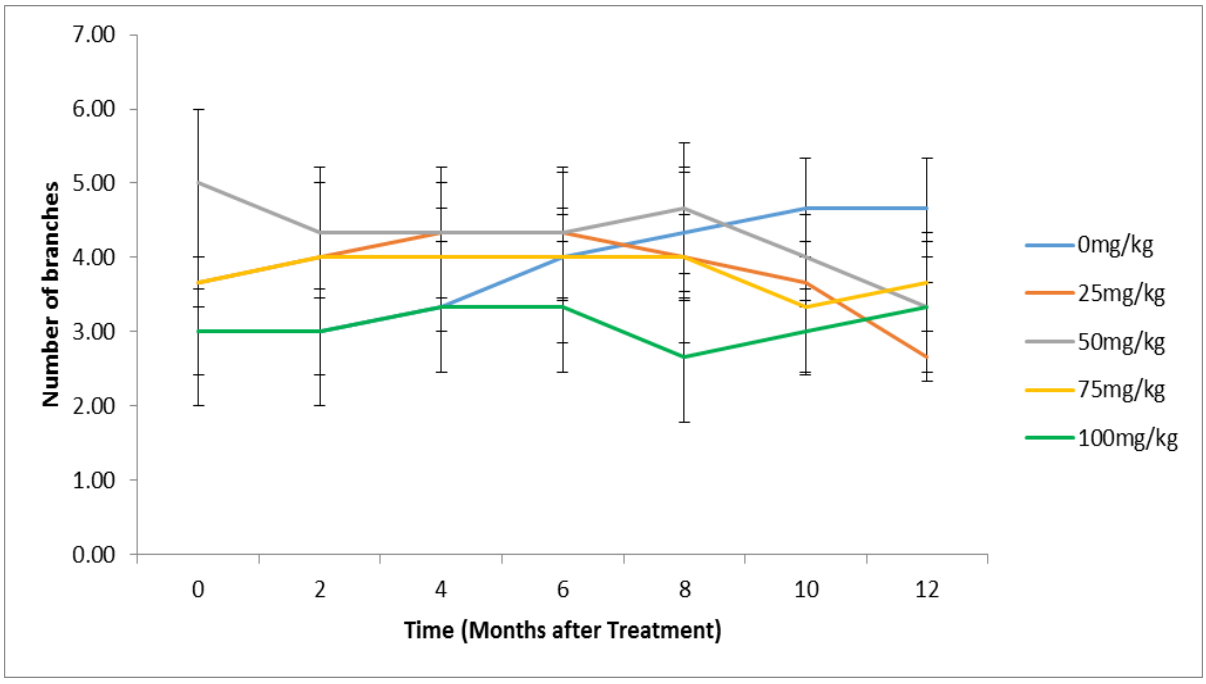

Figure 4: Effect of Pb + Zn mixture on the number of branches of *V. amygdalina*

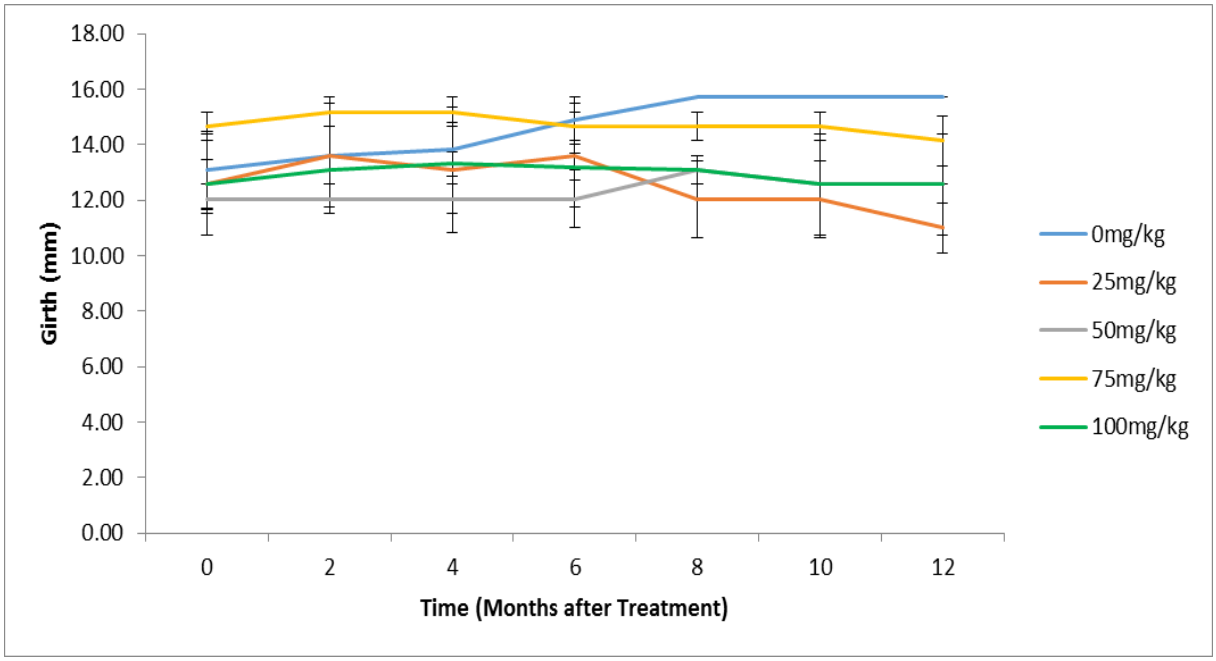

Figure 5: Effect of Pb + Zn mixture on the girth (mm) of *V. amygdalina*

Table 1 shows the effect of the heavy metals on the physicochemical properties of the soil at 12 MAT. There was a decrease in soil pH and essential nutrient level as concentration of metal treatment increased. The carbon content however increased with increased concentration of treatment.
Table 2 shows the heavy metal uptake of *V. amygdalina* at the end of the experiment. The rate of uptake of both metals follow the pattern Pb>Zn. The rate of uptake also increased with increasing concentration of the metal.
Table 3 shows adverse effect of treatment with Pb and Zn mixture on the bacteria and fungi count of the soil samples at the end of the experiment. Effect was along the concentration gradient.
Comparative effect (Table 4) shows that there was significant difference in height between control plants and treated plants. Girth of stem also recorded significant difference between the treatments and control.

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

Concentration (mg/kg)	pH	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Ca (ppm)	Zn (ppm)
0	8.1	0.82	0.29	3.71	1.26	0.82
25	7.1	1.05	0.21	2.91	0.93	0.64
50	6.7	1.11	0.19	2.64	0.87	0.57
75	6.4	1.2	0.15	2.3	0.83	0.44
100	6.1	1.31	0.11	2.13	0.77	0.38

Table 2: Heavy metals (Pb &Zn) accumulated in *V. amygdalina* at the end of the experiment (12 MAT)

Concentration (mg/ kg)	Pb (ppm)	Zn (ppm)
0	ND	0.014
25	0.103	0.063
50	0.131	0.088
75	0.198	0.136
100	0.384	0.243

ND- Not detected

Table 3: Bacterial and fungal counts of soil samples at the end of the experiment (12 MAT)

Concentration (mg/kg)	Bacterial (cfu/g)	Fungal (cfu/g)
0	1.37×10^5	6.7×10^4
25	5.4×10^4	1.2×10^4
50	4.7×10^4	1.1×10^4
75	3.4×10^4	9.0×10^3
100	2.8×10^4	8.5×10^3

Key: cfu/g: colony forming unit per gram

Table 4: Comparative effect of various concentration of Pb+Zn treatment on the growth of *V. amygdalina* 12MAT

Concentration (mg/kg)	Height	Number of leaves	Leaf area	Number of branches	Girth
0	77.43^a	35.67^a	17.45^a	4.67^a	15.71^a
25	30.90^b	19.67^a	15.07^a	2.67^b	11.00^b
50	33.23^b	23.00^a	20.80^a	3.33^{ab}	12.57^{bc}
75	38.23^b	25.33^a	23.84^a	3.67^{ab}	14.14^{ac}
100	31.30^b	21.67^a	14.27^a	3.33^{ab}	12.57^{bc}

Discussion

Result obtained on plant height (Figure 1) revealed an adverse effect of treatment. Edegbai and Anoliefo (2016a) obtained similar result when *V. amygdalina* was subjected to individual treatment with lead and zinc. The effect of the mixture of metals on the plant is however more severe than that obtained with the individual treatment with the metals. This is in line with the result obtained by Alia *et al*. (2015), who discovered that the combine toxicity of Pb and Zn was more than toxicity due to single dose of each element but less than their additive sums. There was no significant difference in number of leaves (Figure 2), leaf area (Figure 3) and number of branches (Figure 4) recorded for control plants and treated plants. There was however an adverse effect of treatment on the girth of the plant (Figure 5). Lead has been implicated in causing depressed growth of *Zea mays*, (Hussain *et al*., 2013)) and *Avena sativa* (Moustakas *et a*l., (1994). It has been reported that yield of *Brassica* sp. was adversely affected by high Zn and Cr contents in soils (Ebbs and Kochian, 1997).

The various treatment with heavy metal resulted in increased acidity. There was a consistent drop in soil pH along the concentration gradient. Result on soil pH revealed an increased acidity along the concentration gradient. Adeniyi *et al.* (2005) stated that soil pH is a very important factor that controls the mobility and availability of metals and soil nutrients. It was also reported by Sauve *et al.* (1997) that the solubility of heavy metals was significantly related to their total concentration together with soil pH. With increasing pH, content of organic matter and clay, the solubility of most metals decreases due to their increased adsorption.

The carbon content of the soil increased as the concentration of treatment increased. Zhang and Wang (2007) revealed that high amount of heavy metals in the soil could slow down the mineralization rate of soil organic C and increase the amount of hardly biodegradable C.

Result of other analyses showed that %N, %P, %Ca and %Mg decreased along the concentration gradient. This is in line with the findings of Alia *et al.* (2015) who discovered that Pb and Zn jointly reduced the uptake of other essential elements like Mn, Fe, K, Mg and Ca in *S. oleraceae*. Plants cultivated in soil contaminated with heavy metals are subject to modification of both the chemical composition of its heavy metal content and macronutrients (Ciecko *et al*., 2004). 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.

Result on microbial count revealed a decrease along the concentration gradient. Heavy metals decrease the biomass of microorganisms and reduce their activity in the soil (Wyszkowska *et al*., 2008). In cases when they do not lower the counts of microorganisms, they still reduce their diversity (Wakelin *et al*., 2010).

The ranking order for the accumulation of heavy metal in *V amygdalina* in the present study was Pb>Zn. The present concentration of Pb and Zn 0.384, 0.243mg/kg are lower compared to the recommended tolerable levels

proposed by joint FAO/WHO Expert Committee on Food Additives for leaves, stem and root of different vegetables, which are 5.0 and 60.0 mg kg-1 for Pb and Zn respectively (Farooq *et al*., 2008). Pb uptake in the present study was higher than that observed by Edegbai and Anoliefo (2016a) when they subjected *V. amygdalina* to lead treatment. This result shows an antagonistic relationship between both metals. Mulielinska *et al.* (2016) however observed an antagonistic decrease in Pb uptake and accumulation with an increased concentration of Zn. Terelak *et al.* (2000), also stated that lead, due to its relatively limited solubility of minerals, is less mobile than zinc in the environment.

Conclusion

The outcome of this study reveals antagonism between the metals in their interactions. The uptake of the metals, though limited may not overlooked on account of accumulation over time. There were no marked morphological differences between plants exposed to the various treatments and control.

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