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THE EFFECT OF ZINC TREATED SOIL ON THE GROWTH AND DEVELOPMENT OF *VERNONIA AMYGDALINA* DEL. PLANT

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ABSTRACT

The effect of zinc on the growth and development of *Vernonia amygdalina* was investigated in this study. Identical stem cuttings of the plants were collected and sown in buckets filled with 5kg dry soil. The experiment was made up of control and four different concentrations (25, 50, 75 and 100mg/kg) in three replicates. Data were collected monthly for 12 months. Result on plant height, number of leaves, number of branches and girth of stem showed an adverse effect of treatment except for leaf area which was enhanced. For example, when control value at 12 MAT was 35.67 ± 7.54, for number of leaves, the 25 mg / kg, 50 mg / kg, 75 mg / kg and 100 mg / kg recorded 11.33 ± 0.88, 11.67 ± 1.20, 12.33 ± 2.19 and 12.33 ± 2.03 respectively. There was decreased soil pH, nutrients and microbial load. There was however an increase in soil carbon. These effects were along the concentration gradient. Zinc uptake by plant was within tolerable limits.

Keywords: treatment, gradient, zinc, *Vernonia amygdalina*

INTRODUCTION

Zinc (Zn) is a very common substance that occurs naturally in air, water and soil. However, zinc concentrations are rising unnaturally due to its addition through human activities. Most zinc are added through industrial activities such as mining, coal and waste combustion and steel processing. Some soils are heavily polluted with zinc, and these are found in areas where zinc has to be mined or refined or where sewage sludge from industrial areas has been used as fertilizer (Tkalec *et al*., 2014). Zinc plays a fundamental role in numerous cellular functions (Broadley *et al*., 2007; Cherif *et al*., 2011) and is involved in the catalytic function of many enzymes and structural stability of various cell proteins (Vallee and Falchuk, 1993). Plants often have a zinc uptake that their systems cannot handle due to the accumulation of zinc in soils. Only a number of plants have a chance of survival on zinc rich soils. That is why there is not much plant diversity near zinc- disposing factories (Tkalec *et al*., 2014).

A number of Asteraceae plants have been found to bioaccumulate heavy metals, for example, *Helianthus annuus* which bio-accumulates Pb (Boonyapookana *et al*., 2005). The current study investigated the effect of zinc on the growth and development of *Vernonia amygdalina* and provides data on the safety of consumption of *V. amygdalina* grown in zinc polluted soil.

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 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 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.

Table 1: Field Layout

REPLICATES		
$Z^{3.2}$	O	$Z^{1.1}$
O	$Z^{4.3}$	$Z^{2.3}$
$Z^{2.1}$	$Z^{2.2}$	$Z^{3.3}$
$Z^{4.2}$	$Z^{1.2}$	O
$Z^{1.3}$	$Z^{3.1}$	$Z^{4.1}$

KEY
O = Control
Z = Zn
$Z^{1.1}$, $Z^{1.2}$, $Z^{1.3}$ = Replicates of 25 mg / kg
$Z^{2.1}$, $Z^{2.2}$, $Z^{2.3}$ = Replicates of 50 mg / kg
$Z^{3.1}$, $Z^{3.2}$, $Z^{3.3}$ = Replicates of 75 mg / kg
$Z^{4.1}$, $Z^{4.2}$, $Z^{4.3}$ = Replicates of 100 mg / kg

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 5kg 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 zinc. There were 4 concentrations (25, 50, 75 and 100 mg/kg) in 3 replicates and control (0 mg/kg). Zinc 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 12 month period.

Field Data Collection

Field data collections were carried out according methods outlined by Edegbai and Anoliefo (2016a) as follows:

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 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

Analyses were carried out according methods outlined by Edegbai and Anoliefo (2016a). 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: Twenty (20) grammes of fine soil was placed in a container and 50ml of distilled water added. The suspension was shaken for 30mins 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: 1.0g 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: 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:

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

Available Phosphorus: 1.0g 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, 5 drops of mixture of boric acid, sodium sulphite and sodium sulphate were added. The phosphorus content was determined calorimetrically at 645nm.

Cation Exchange Capacity: 5g 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. 2g 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. Zinc content in the digested samples was 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 zinc and control is presented in Figure 1. All treatments including Control recorded a consistent and steady increase in height. At the end of the experiment, control plants recorded significantly ($P < 0.05$) higher mean heights than the Zinc treated soils. The pattern of growth was not consistent with the treatment concentration. At 12 MAT, control (0 mg / kg) mean height was 77.43 ± 1.45 cm, 25 mg / kg treatment was 62.80 ± 0.32 cm, 50 mg / kg was 54.03 ± 1.21 cm, 75 mg / kg was 53.80 ± 2.44 cm and 100 mg / kg was 60.73 ± 1.52 cm.

Figure 2 shows the mean number of leaves of *V. amygdalina* recorded for control and zinc treated soils. The result shows that the control plants recorded higher number of leaves than the plants from the zinc treated soil. At 12 MAT, control plants had more leaves than all the other treatments. There was significant difference ($P < 0.05$) between control values and those recorded for the various treatments with zinc. When control (0 mg / kg) value at 12 MAT was 35.67 ± 7.54, the 25 mg / kg, 50 mg / kg, 75 mg / kg and 100 mg / kg recorded 11.33 ± 0.88, 11.67 ± 1.20, 12.33 ± 2.19 and 12.33 ± 2.03 respectively.

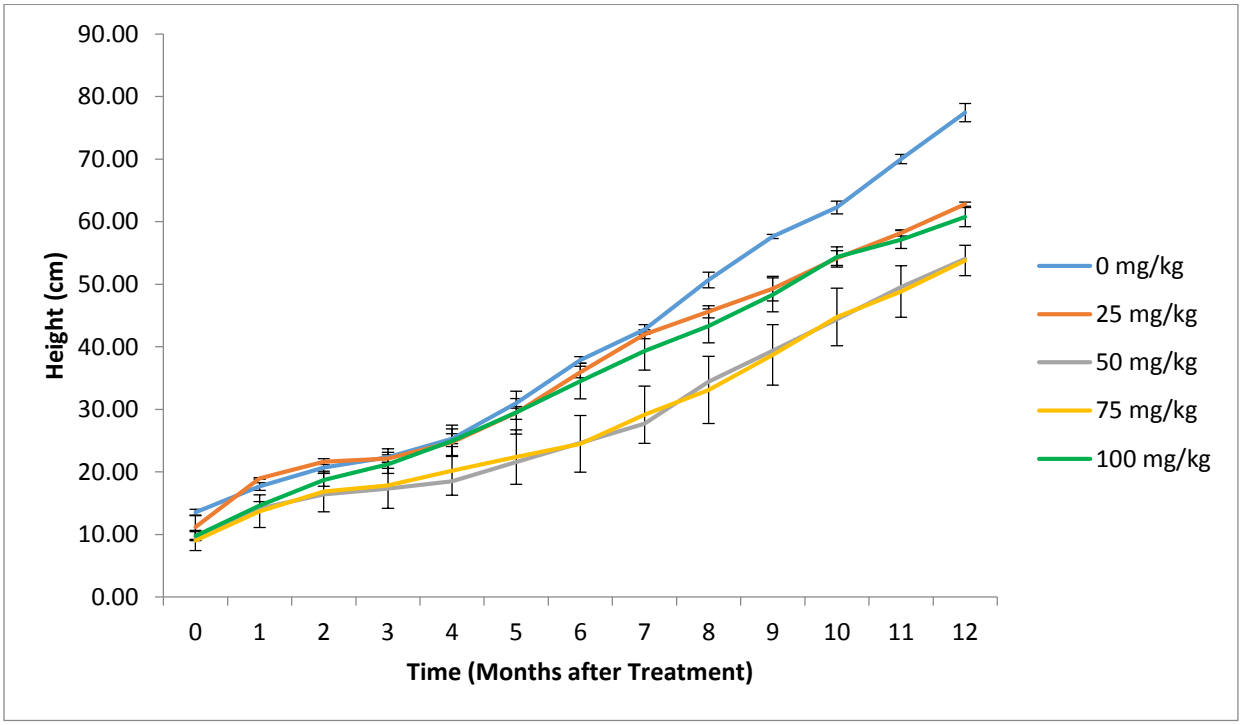

Fig. 1: Effect of Zn treatment on the height of *V. amygdalina*

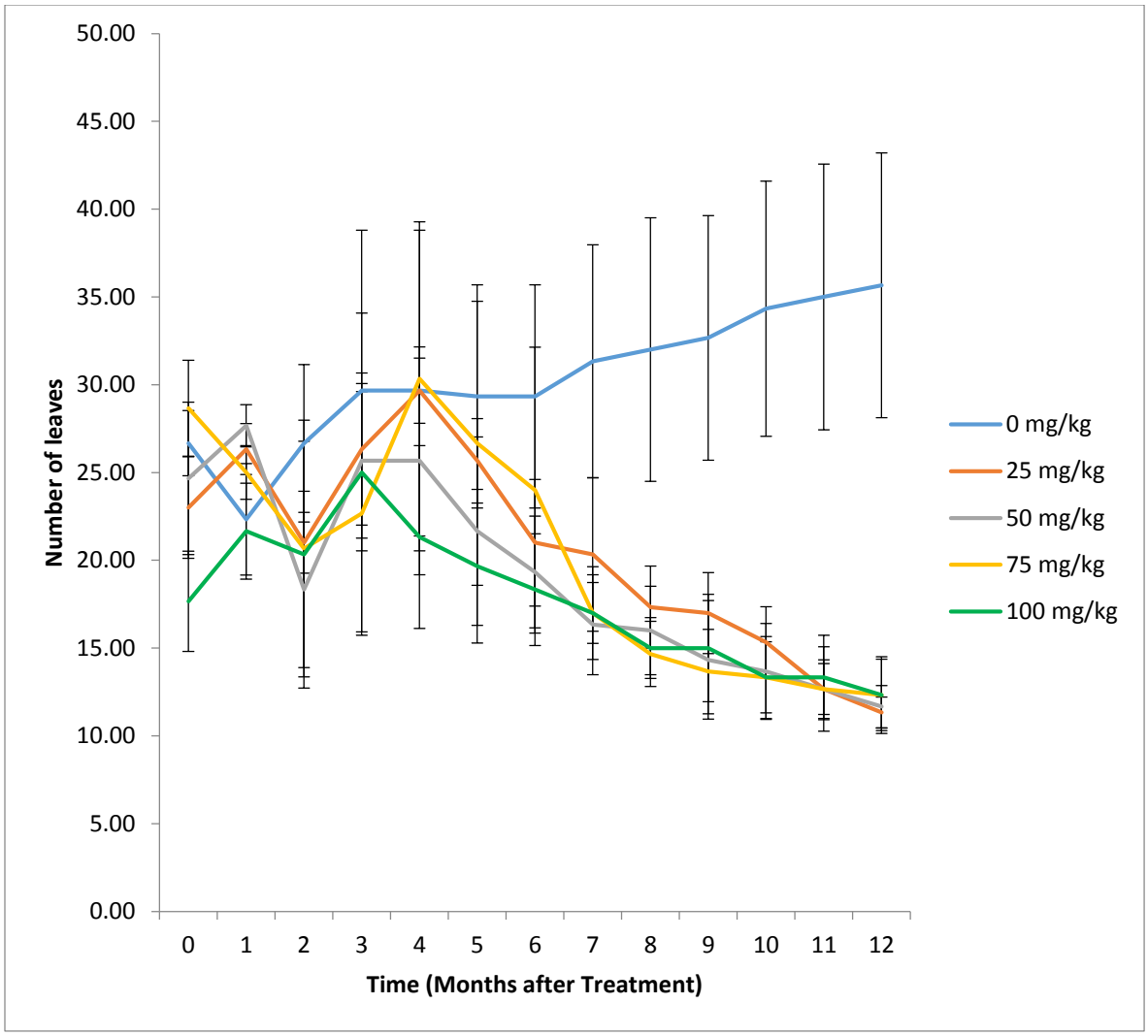

Fig. 2: Effect of Zn treatment on the number of leaves of *V. amygdalina*

Prodgers and Inskeep (1981) had observed that excessive amounts of toxic elements usually caused reduction in plant growth. Levitt (1980) suggested that heavy metals in the plant environment operate as stress factors and they cause physiological changes and in the process they can reduce vigour, or in the extremes totally inhibit plant growth. It has been reported that yield of *Brassica* sp. was adversely affected by high Zn and Cr contents in soils (Ebbs and Kochian, 1997). Edegbai and Anoliefo (2016c) observed that when treated with Cd + Pb + Zn, growth in height and the number of leaves of *V. amygdalina* were significantly lower compared to control. The outcome of this study is in conformity with the findings in this study.

Mean leaf area results of *V. amygdalina* for control and zinc treated soil are shown in Figure 3. Control values were consistently lower than values recorded for the various soil treatments with zinc. Values did not show any pattern consistent with treatment concentration. Thus at 12 MAT, control (0 mg / kg) value was 17.45 ± 4.85 cm^2 while the 25 mg / kg, 50 mg / kg, 75 mg / kg and 100 mg / kg treatments were 26.70 ± 6.14, 20.68 ± 1.79, 23.04 ± 3.26 and 20.32 ± 0.00 cm^2respectively. There was no significant difference ($P < 0.05$) between control and the other treatments.

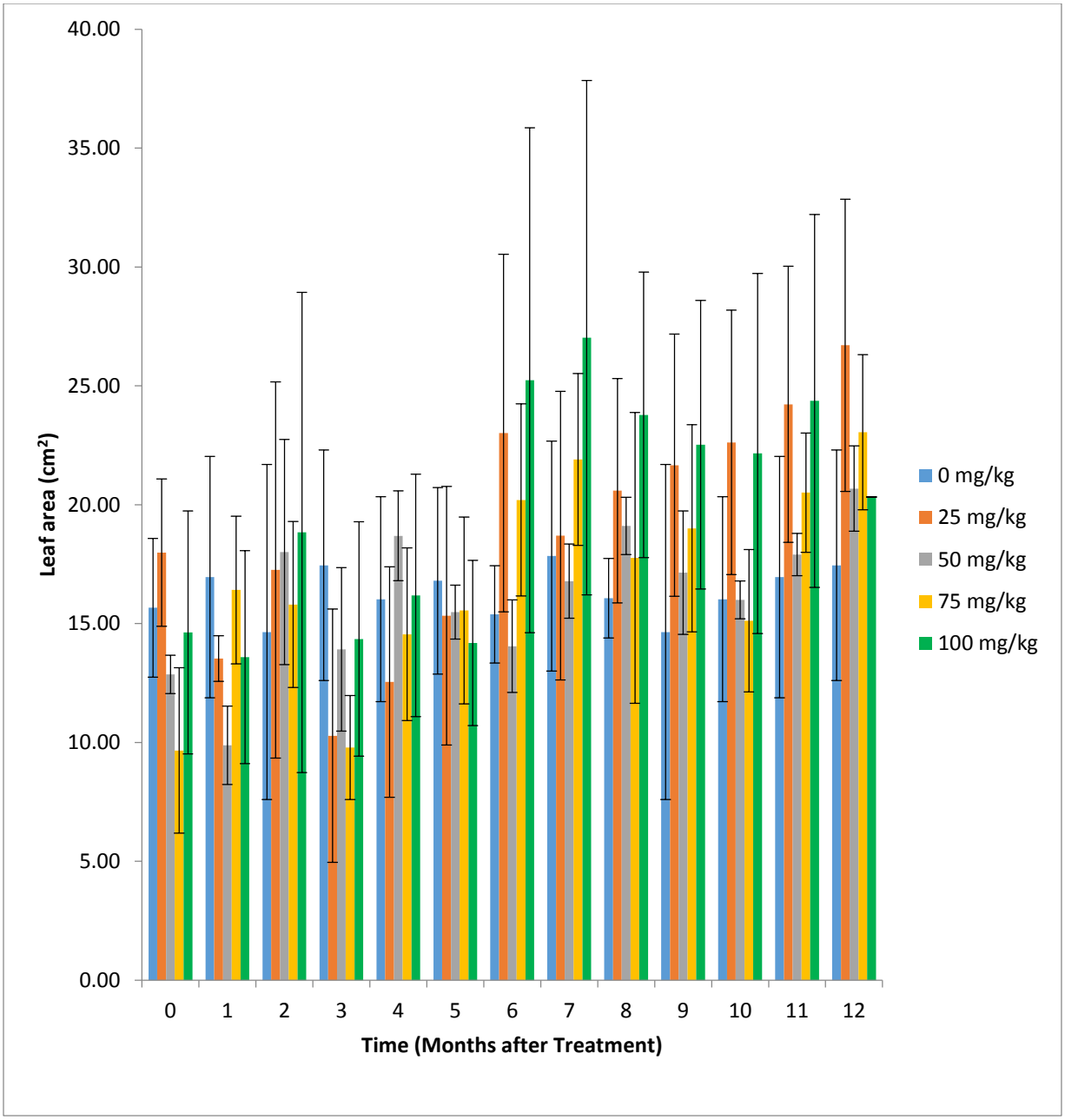

Fig. 3: Effect of Zn treatment on the leaf area (cm^2) of *V. amygdalina*

Edegbai and Anoliefo (2016a) recorded similar results on leaf area when they treated *V. amygdalina* with lead. This is however different from the observation of Anoliefo and Osubor (1998) who observed that in sunflower, lead reduced leaf area, dry mass and plant height.

Figure 4 shows the mean number of branches of *V. amygdalina* recorded for control and various soil treatments with zinc. Control values were higher than the treatments at 12 MAT. The growth pattern was inconsistent with the concentration gradient. The mean values recorded for control (0 mg / kg) and various treatments at 12 MAT were 4.67 ± 0.67, 2.67 ± 0.67, 3.67 ± 0.66, 4.00 ± 1.15 and 3.33 ± 0.88 respectively.

Figure 5 shows the average values recorded for girth of stem of *V. amygdalina* for control and various zinc treated soil. Control girth increased until 8 MAT and remained the same till the end of the experiment while the 25 mg / kg, 50 mg / kg and 75 mg / kg treatments recorded lower values at 12 MAT. At the end of the experiment 15.71 ± 0.00, 11.78 ± 3.93, 11.52 ± 2.62, 13.62 ± 2.09 and 14.66 ± 2.09 mm were recorded for control (0 mg / kg) and the 25 mg / kg, 50 mg / kg, 75 mg / kg and 100 mg / kg respectively.

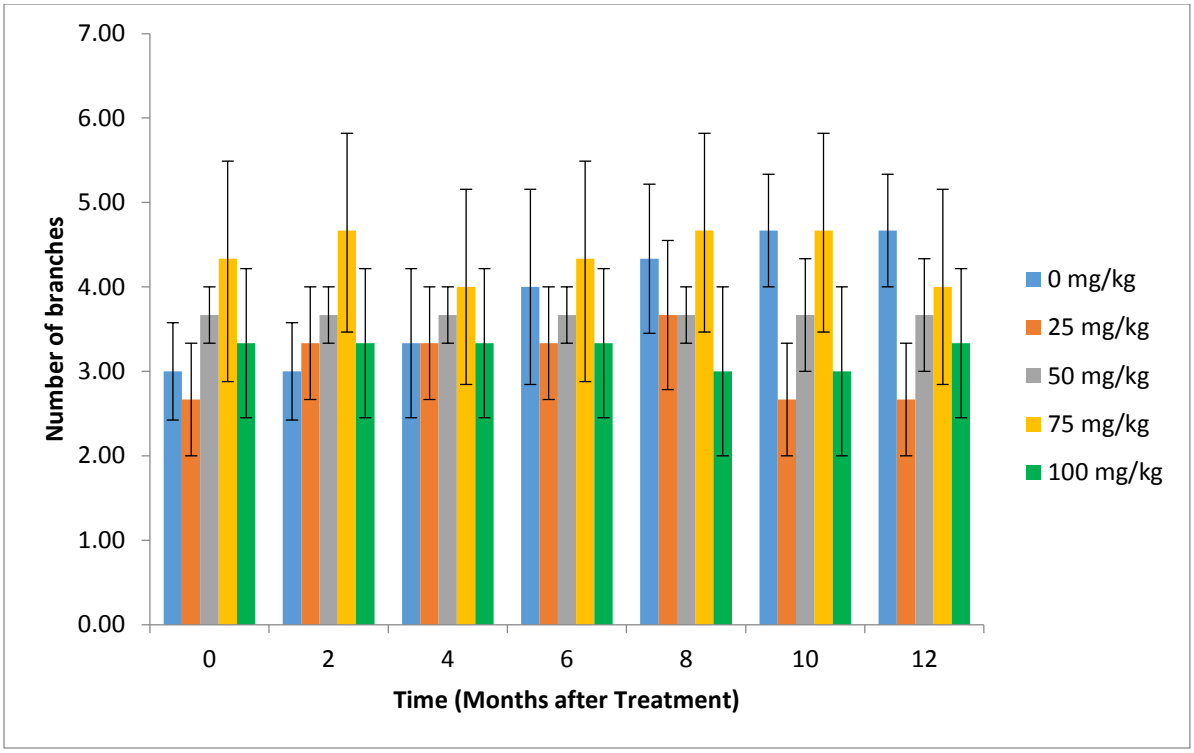

Fig.. 4: Effect of Zn treatment on the number of branches of *V. amygdalina*

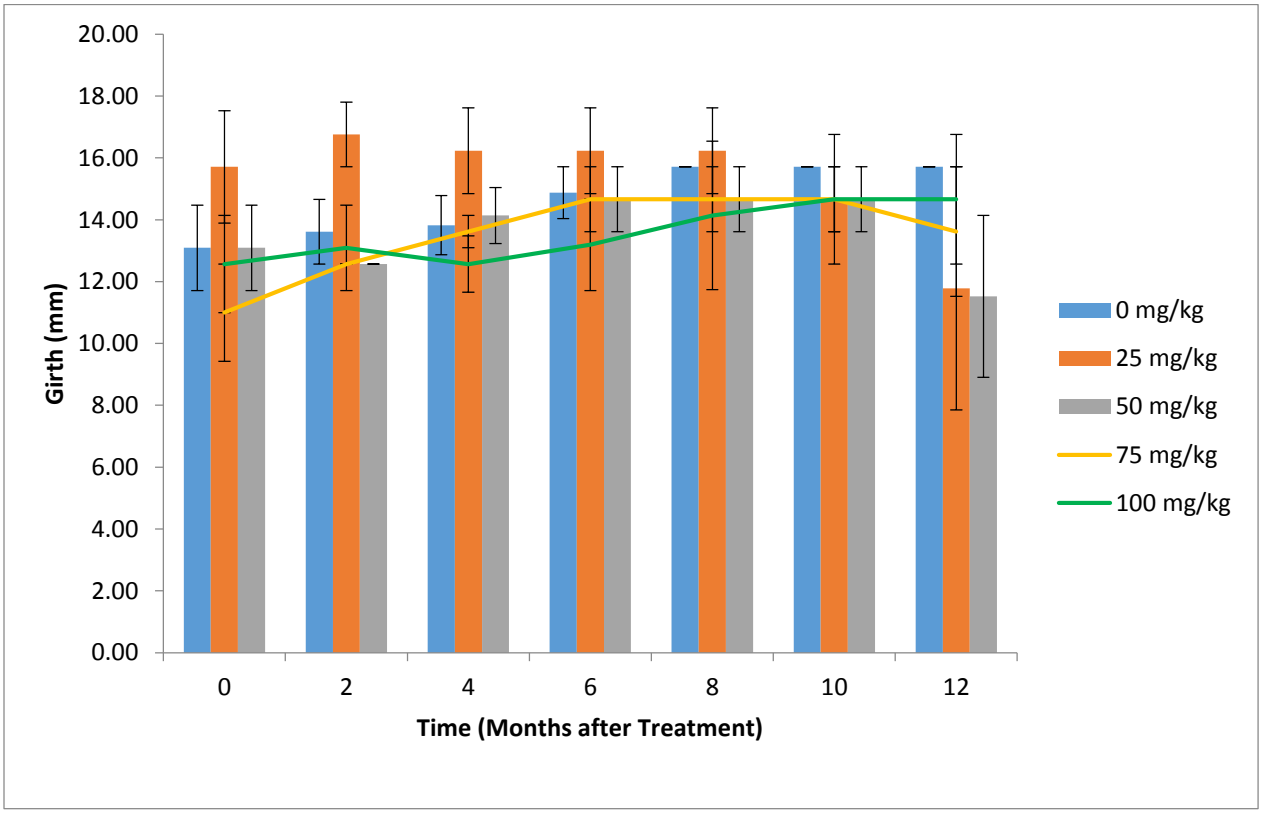

Fig. 5: Effect of Zn treatment on the girth (mm) of *V. amygdalina*

Panda (2007) had stated that metal toxicity reduces vigour and growth of plants, causes death and in extreme cases interferes with photosynthesis, respiration, water relation, reproduction and causes changes in certain organelles, disruption of membrane structure and functions of different plant species. In this study, growth as observed in number of branches and the girth of the plants was adversely affected by zn treatment.

Table 2 shows the physicochemical properties of soil samples, zinc accumulated by *V. amygdalina*, and bacterial and fungal counts of soil samples at the end of the experiment. There was a decrease in soil pH and nutrient composition with increasing treatment. The carbon content of the soil however increased with increasing concentration of treatment.

Table 2: Physicochemical properties of soil samples, zinc accumulated by *V. amygdalina,* and bacterial and fungal counts of soil samples of post *V. amygdalina* cultivated soil at the end of the experiment (12 MAT)

Treatment (mg/kg)	pH	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Bacterial (cfu/g)	Fungal (cfu/g)
0	8.1	0.82	0.29	3.71	1.26	0.82	0.014	1.37×10^5	6.7×10^4
25	7.3	0.88	0.23	3.54	1.09	0.76	0.158	1.27×10^5	4.4×10^4
50	7.1	0.97	0.2	3.38	1.05	0.73	0.245	1.09×10^5	3.9×10^4
75	6.8	1.09	0.17	3.31	1.01	0.68	0.309	9.6×10^4	3.8×10^4
100	6.5	1.15	0.16	3.11	0.96	0.64	0.584	8.8×10^4	3.4×10^4

Soil pH is an essential factor that determines the movement and availability of heavy metals and soil nutrients. The decrease in pH values along the concentration gradient is in line with the findings of Edegbai and Anoliefo (2016a and 2016b) when they treated *V. amygdalina* with lead and cadmium respectively. Adeniyi *et al.* (2005) reported that the solubility of heavy metals was significantly related to their concentration together with the soil pH.

The carbon constituent increased as the concentration of treatment increased. This is also in line with the findings of Edegbai and Anoliefo (2016a and 2016b). Study by 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. Heavy metals were largely enriched in particulate organic matter, which could impact further the mineralization of soil organic matter.

Results of other analysis showed that %N, %P, %Ca and %Mg constituents of the soil decreased with increasing concentration of treatment. Plants cultivated in soil contaminated with heavy metals are subject to modification of the chemical composition of heavy metal content and macronutrients (Ciecko *et al.*, 2004). Wyszkowska *et al.* (2013) reported that high zinc treatment decreased the N, Mg, K and Mn concentrations in all plant parts. 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.

The data for zinc accumulated by *V. amygdalina* at the end of the experiment showed that zinc uptake increased along the concentration gradient. The present concentrations of zinc uptake by the plant (0.584mg/kg) is lower compared to the recommended tolerable level proposed by joint FAO/WHO Expert Committee on Food Additives for leaves, stem and root of different vegetables which is 60.0 mg kg-1 for Zn (Farooq *et al*., 2008).

Data for the microbial count of the soil at the end of the experiment showed that microbial count decreased with increasing concentration of treatment. Wyszkowska *et al.* (2008) stated that heavy metals decrease biomass of microorganisms and reduce their activity in soil. Moreso, in cases when they do not lower counts of microorganisms, they still reduce their diversity (Xie *et al*., 2009; Wakelin *et al.*, 2010).

CONCLUSION

Aspects of growth were significantly impacted in this study. Essential metals like zinc can become harmful to plants when present in high concentrations in the soil. Although, the uptake of Zinc by the plant was within tolerable limit, it still calls for concern as gradual accumulation of this metal by humans can occur when such plants are consumed over time.

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