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EFFECT OF MIXTURE OF CADMIUM AND ZINC ON THE GROWTH AND DEVELOPMENT OF *Vernonia amygdalina* Del

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Abstract
Vernonia amygdalina Del was grown in soil treated with a mixture of cadmium and zinc in different concentrations (25, 50, 75 and 100mg/kg) to investigate the effect of the treatments on the growth of V. amygdalina. Identical stem cuttings were collected and sown in buckets filled with 5kg dry soil. These were allowed to grow for a month before the soil samples were treated with the mixture of metals. The experiment included control and four concentrations in three replicates. Data were collected monthly for 12 months. Results on plant height, number of leaves, number of branches and girth showed adverse effect of treatment unlike leaf area which was enhanced. There was significant difference between height values recorded for control and the various treatments - the heights for control, the 25 mg /kg, 50mg / kg, 75mg/ kg and the 100 mg/ kg treatments at 12 MAT were 77.43±1.03, 59.23±2.80, 55.83±1.57, 35.13± 0.86 and 31.47±2.08cm respectively. There was decreased soil pH, microbial load and nutrients. There was however an increase in soil carbon. The presence of zinc reduced the uptake of cadmium in the plant. The increased presence of heavy metals affected the cell division process of the plant, lowered the soil pH - causing an increase in bioavailable heavy metals for uptake, decreased the available nutrients for plant growth due to competition and disrupted the soil's natural microbial flora. Though the accumulation of heavy metals in the plant were below tolerable limits, prolonged consumption may result in poisoning.

Key Words: *Mixture, Treatment, Cadmium, Zinc, Vernonia amygdalina*

Introduction

The concentration of heavy metals in soils is affected by both natural and anthropogenic factors. Natural conditions that affect the content of heavy metals in soils are - the parent rock, soil formation processes and grain size distribution of a given soil (Dragovic *et al*., 2008; Skwierawska, 2013). Metals like cadmium, copper and zinc as soil pollutants can originate from geochemical processes evoked by volcanic eruption or the weathering of parent rock (Kabata-Pendias, 2004). The highest quantities of heavy metals enter soils from the metallurgic and mining industries (Vasquez-Murrieta *et al*., 2006), and from transportation routes and emission of fumes (Khan *et al.*, 2011; Qiao *et al.*, 2011). Plants growing on soil contaminated with heavy metals may tend to take up more of these elements, which

are then transferred to subsequent links in the feeding chain (Zalewska, 2012; Nadgorska-Socha *et al.*, 2013).

Cadmium is quite a mobile element in soil water and thus freely taken up by plants. It has been identified as one of the most phytotoxic heavy metals (Ederlin *et al*., 2004; Pilon-Smits, 2005). Zinc is a micronutrient essential for plants but can be highly toxic when present at excessive concentration (Kinraide *et al.*, 2004). Cadmium frequently accompanies zinc minerals in the environment (Ullrich *et al.*, 1999) and due to their chemical similarity, they both can be taken by plants as divalent cations. It is well known that metals in mixture may act independently or interact to produce additive, synergistic, or antagonistic effects (Wilde *et al.*, 2006). Studies conducted to investigate the Cd-Zn interaction on Cd and Zn uptake and accumulation have revealed mostly antagonistic interaction between these two metals (Wu and Zhang, 2002; Aravind and Prasad, 2003; Balen *et al.*, 2011), although synergistic effects were also reported (Nan *et al.*, 2002).

Experiments on the toxicity of mixtures of pollutants may reflect the actual toxicity to ecosystems in a more realistic way than experiments in which toxicants are tested individually (Spurgeon *et al*., 1994). This study investigates the effect of a mixture of cadmium and zinc on a highly consumed Asteraceae plant (*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 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.

Cadmium: The cadmium (Cd) used for this study was obtained from cadmium sulphate ($CdSO_4$).

Zinc: The zinc (Zn) used for this study was obtained from zinc sulphate ($ZnSO_4$).

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 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 cadmium and zinc mixture. There were 4 concentrations (25, 50, 75 and 100mg/kg) in 3 replicates and control. The cadmium and zinc 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 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 Esalvernier caliper. (Girth = πd).

Soil Physicochemical Analyses

Soil analyses was carried out as reported in Edegbai and Anoliefo (2016a). Soils were dried at ambient temperature (22-25ºC), crushed in a porcelain mortar and sieved through a 2mm (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 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. The cadmium and 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

Figure 1 shows the effect of Cd + Zn mixture on the height of *V. amygdalina.* There was a consistent increase in values from 0 MAT to 12 MAT for all the treatments. The rate of increase however decreased with increase in treatment concentration and there was significant difference ($P < 0.05$) in values recorded between control and all the treatments. The values - 77.43±1.03, 59.23±2.80, 55.83±1.57, 35.13± 0.86 and 31.47±2.08 cm represent the heights for control, the 25 mg/kg, 50mg/kg, 75mg/kg and the 100 mg/kg treatments at 12 MAT.

The effects of Cd + Zn treatment on the number of leaves of *V. amygdalina* is shown in Figure 2. Control recorded an increase in number of leaves consistently from the 0 MAT to 12 MAT. All the treatments showed a decrease in number of leaves throughout the duration of the experiment. However there was no pattern consistent with the treatment concentration. There was significant difference ($P < 0.05$) between control number of leaves and that of the other treatments. The values - 35.67±7.54, 10.67±1.76, 9.67±0.88, 10.00±1.53 and 7.33±0.88 number of leaves were recorded at 12 MAT for control, the 25mg/kg, 50mg/kg, 75mg/kg and the 100 mg/kg treatments.

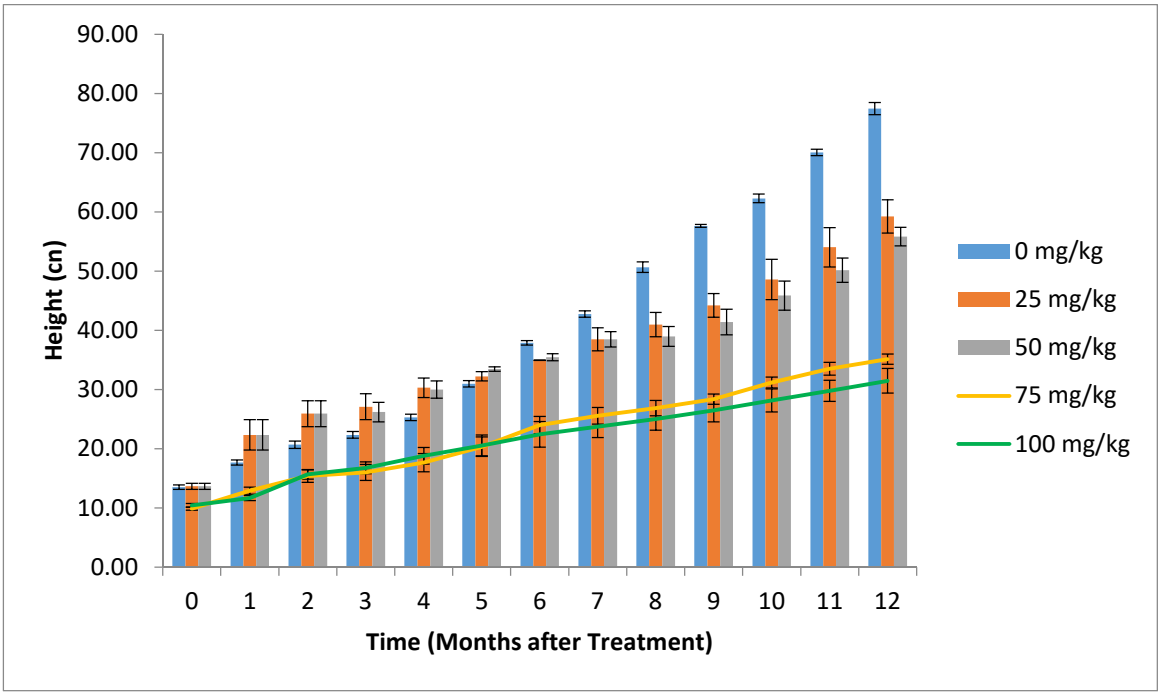

Fig. 1: Effect of Cd + Zn mixture on the height (cm) of *V. amygdalina*

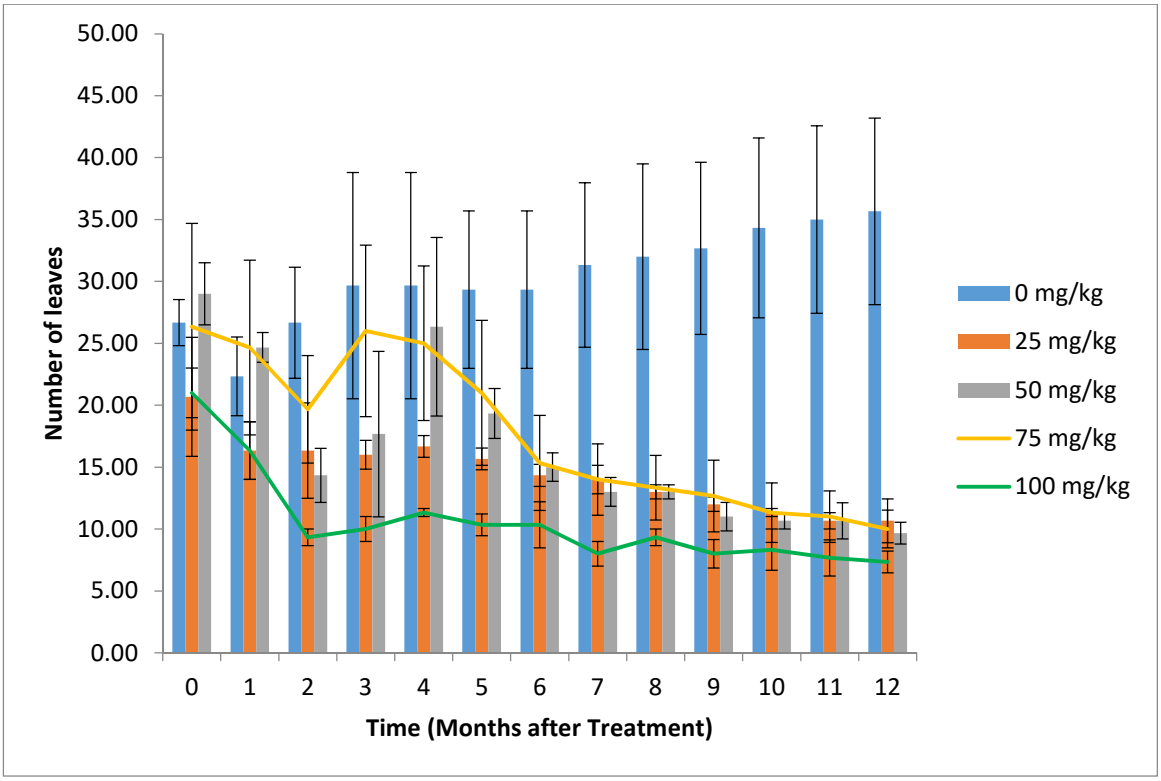

Fig. 2: Effect of Cd + Zn mixture on the number of leaves of *V. amygdalina*

Figure 3 shows the effect of Cd + Zn treatment and control on the leaf area of *V. amygdalina.* Results recorded here show that treatment with the heavy metals caused an increase in leaf area of the plant though without a definite pattern of effect based along the concentration gradient. Control mean leaf area was consistently smaller than those recorded for all the treatments. When control was 17.45±4.85 cm^2, the 25mg/kg treatment was 24.23±6.58cm^2, the 50mg/kg was 37.94±6.91cm^2, 75mg/kg was 29.50±5.91cm^2 and the 100 mg/kg treated plants was 27.35±7.32cm^2

Figure 4 shows the means values for the effect of Cd + Zn treatment on the number of branches recorded for *V. amygdalina.* Mean values recorded for control and the various treatment concentrations from 0 MAT to 12 MAT show that only control did not lose any branches throughout the experiment. All the other treatments recorded loss of branches at various times during the experiment. The effect was inconsistent with treatment concentration. 4.67±0.67, 2.00±0.00, 3.33±0.33, 4.00±1.00 and 3.00 ±0.58 were the means of number of branches at 12 MAT for control, the 25mg /kg, 50mg/kg, 75mg/kg and 100mg/kg treatments respectively.

The effect of Cd + Zn treatment and control on the girth of *V. amygdalina* is shown in Figure 5. Mean values recorded for girth increased for all the treatments throughout the experiment. The least girth was recorded for the 100mg/kg treatment. At 12 MAT girth mean values of 15.71±0.00, 15.19±1.39, 17.91±0.94, 14.14±0.91 and 12.57±1.81mm were recorded for control, the 25mg/kg, 50mg/kg, 75mg/kg and the 100mg/kg treatments respectively.

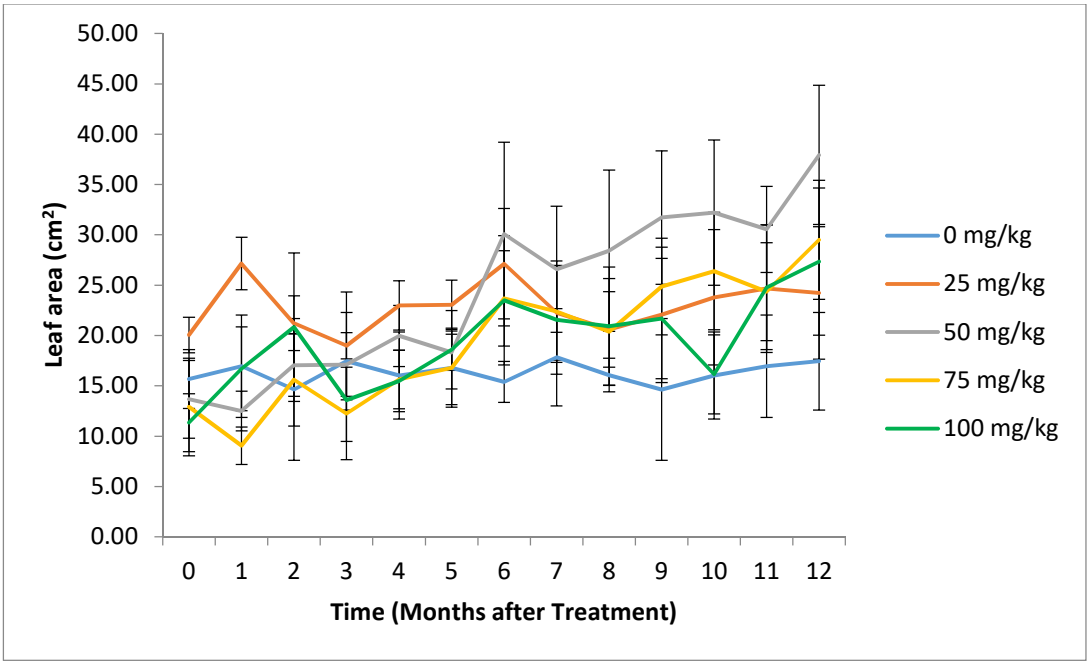

Fig. 3: Effect of Cd + Zn mixture on the leaf area (cm^2) of *V. amygdalina*

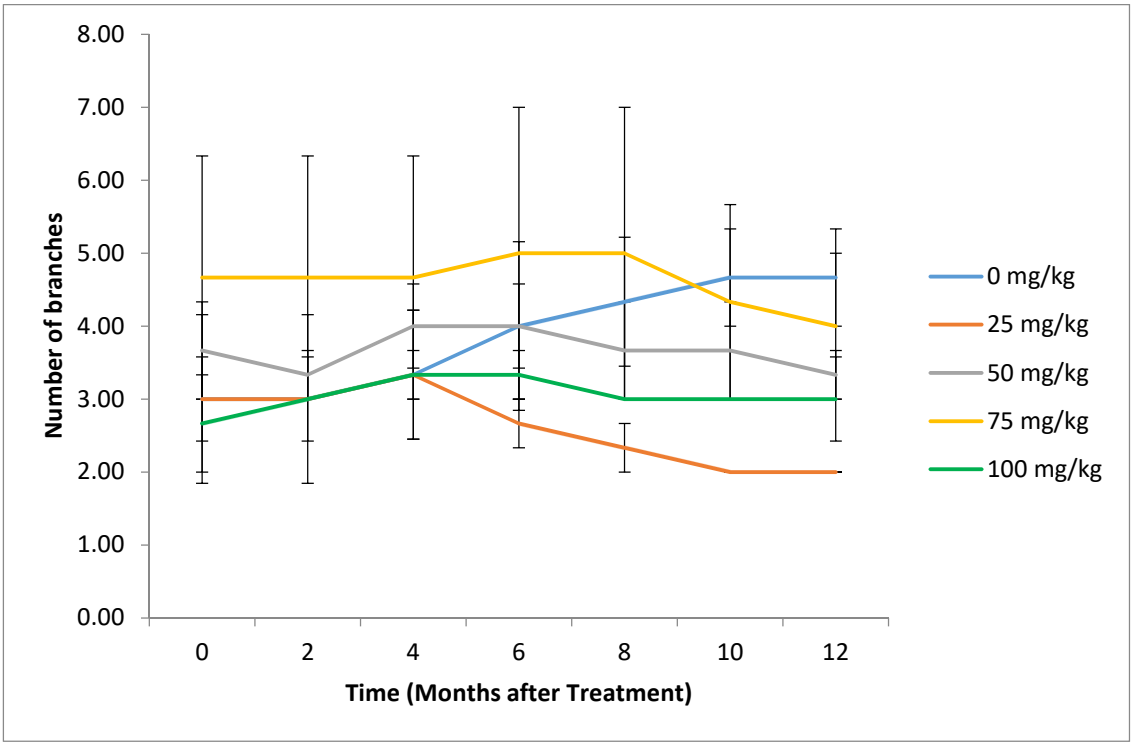

Fig. 4: Effect of Cd + Zn mixture on the number of branches of *V. amygdalina*

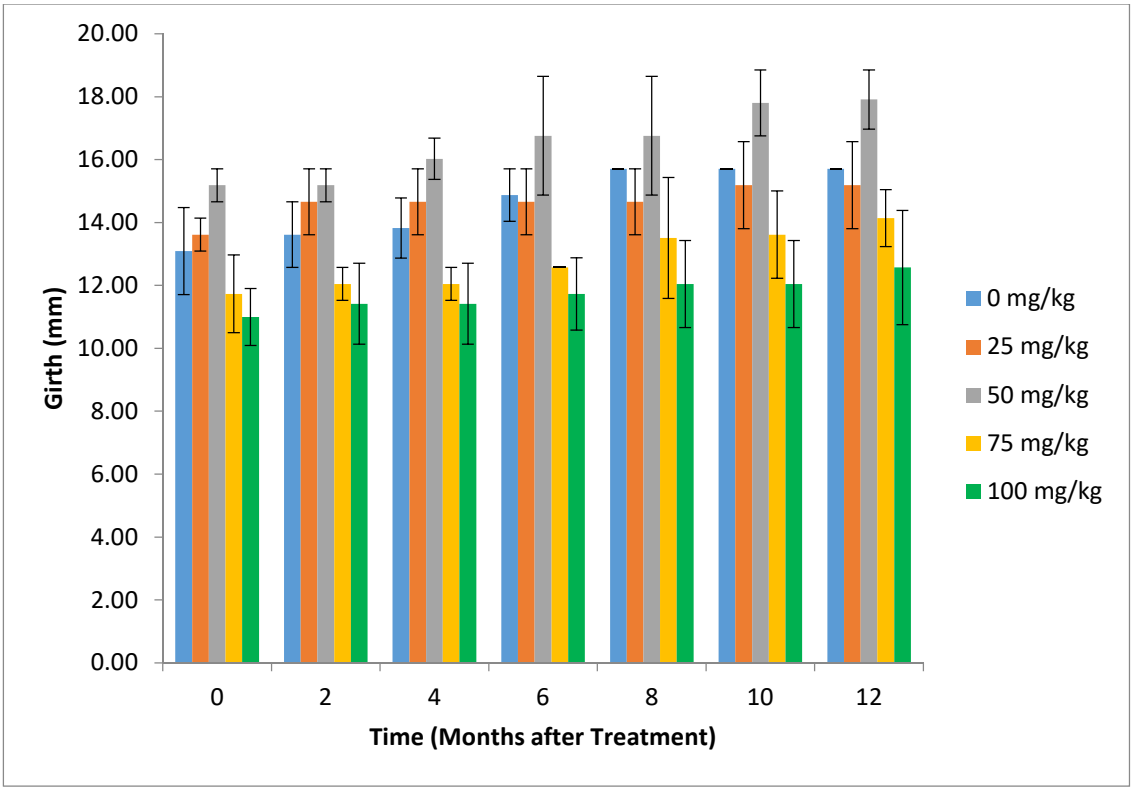

Fig. 5: Effect of Cd + Zn mixture on the girth of *V. amygdalina*

The mixture of Cd and Zn treatment resulted in a decrease in soil pH values along the concentration gradient (Table 1). Similarly, the quantities of nitrogen, phosphorus, calcium and magnesium also decreased as the treatment concentration increased. Soil available carbon however increased along this line.

The amounts of the heavy metals found in *V. amygdalina* plants (Table 2) at 12 MAT shows that there was increased uptake of the metals as the concentration of the treatment increased. The order of uptake by quantity was Zn > Cd. Microbial population (Table 3) was adversely affect by the presence of the heavy metals in the soil.

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

Concentration	pH	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Ca (ppm)	Mg (ppm)
0	8.1	0.82	0.29	3.71	1.26	0.82
25	6.0	1.07	0.20	3.09	0.98	0.66
50	5.7	1.13	0.17	2.71	0.92	0.62
75	5.5	1.20	0.14	2.53	0.79	0.57
100	5.2	1.28	0.10	2.33	0.71	0.48

Table 2: Heavy metal accumulation in plant at the end of the experiment

Concentration	Cd (ppm)	Zn (ppm)
0	ND	0.014
25	0.038	0.067
50	0.056	0.095
75	0.093	0.175
100	0.146	0.255

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	9.2×10^4	1.8×10^4
50	8.0×10^4	1.6×10^4
75	7.6×10^4	1.5×10^4
100	6.7×10^4	1.3×10^4

KEY: Cfu/g: Colony forming unit per gram

Discussion

Results on plant height and number of leaves revealed an adverse effect of treatment as control plants recorded higher values. Compared to when cadmium alone was used as shown by studies done by Edegbai and Anoliefo (2016b), it can be deduced that there was a less toxic effect when the elements in the current study were mixed compared to when cd alone was used. Cadmium was highly toxic when it was used alone on the same plant resulting in death of the plants in higher concentration after some months.

Results on leaf area showed that there was an increase in the leaf area with the treatment. The use of cadmium alone had an adverse effect on treated plant as control plants recorded higher leaf area Edegbai and Anoliefo (2016b). It can be deduced that the presence of zinc in the mixture suppressed the toxicity of cadmium. It has been found that zinc can lessen physiological damage caused by cadmium (Wu and Zhang, 2002).

Results on number of branches and girth of stem revealed an adverse effect of treatment. This is in line with the findings

of Kleckerova *et al.* (2011) who stated that the application of Cd and Zn led to an inhibition in growth parameters (root and shoot biomass) compared to control untreated plants and as discovered in this study, the inhibition was highest in the 100 mg/kg treated plants. This inhibition is a result of the mutual influence of both metals. However, the toxic effect of the mixture is less than that observed when cadmium alone was used. This result is at variance with the observation of Alia *et al.* (2015) who stated that the toxicity of cadmium and zinc mixture was higher than that observed in the individual metal applications but less than their additive sums.

Result on soil pH revealed an increased acidity with increasing concentration of mixture. Mobility and availability of heavy metals depend on factors such as soil pH, content of organic matter and type of metal (Wyszkowska *et al.*, 2013). Increase in acidity results in increase in the heavy metals available in solution in the soil and consequently to plants (Edegbai and Anoliefo, 2016a).

The carbon constituent of the soil increased with increasing concentration of treatments. The study done 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 organic C.

The results on other analyses show that %N, %P, %Ca, %Mg, %K and %Na constituents of the soil decreased with increasing concentration of treatments. This is in line with the observations of study done by Alia *et al.* (2015) who stated that combined doses of Cd and Zn led to a reduction in Na, K, Ca, Fe, Mg and Mn in *Spinacia oleracea* and another study done by Wu and Zhang (2002), it was discovered that Cd and Zn jointly reduce the uptake of other essential elements like Mn, Fe, K, Mg and Ca in plants. Plants cultivated in soil contaminated with heavy metals are subject to modification of the content of macronutrients (Ciecko *et al*., 2004).

There was a decrease in microbial count with increasing treatment concentration. Soil pollution with heavy metals in different quantities and forms causes changes in the counts of microorganisms and activity of microbial enzymes which is a true reflection of the actual microbiological condition of the soil (Wyszkowska *et al.*, 2009). In cases when they do not lower counts of microorganisms, they still reduce their diversity (Wakelin *et al.*, 2010).

Plant analysis showed higher zinc uptake than cadmium in the plant. It has been found that Zn can suppress Cd uptake in plants (Aravind and Prasad, 2005). Other researchers also found that Zn supply can inhibit Cd adsorption and thereby cause a low Cd concentration in plants (Nan *et al.*, 2002). The reduced uptake of Cd as a result of the addition of zinc might result from competitive transport and absorption interaction between the two ions.

Conclusion

The toxicity of cadmium is suppressed by the presence of zinc in mixture. The effect of mixture of metals is toxic in high concentrations and still remains a cause for concern as it resulted in adverse effects in the plant. The accumulation of metals by plants also means its successive transfer to the food chain when such plants are consumed by man.

References

Alia, N., Sadar, K., Said, M., Salma, K., Sadia, A., Sadaf, S., Toqeer, A. and Miklas, S. (2015). Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. *International Journal of Environmental Research and Public Health,* 12: 7400 – 7416.

Aravind, P. and Prasad, M.N.V. (2003). Zinc alleviates cadmium induced toxicity in *Ceratophyllum demersum*, a fresh water macrophyte. *Plant Physiology and Biochemistry,* 41: 391 – 397.

Aravind, P. and Prasad, M.N.V. (2005). Modulation of cadmium-induced oxidative stress in *Ceratophyllum demersum* by zinc involves ascorbate-glutathione cycle and glutathione metabolism. *Plant Physiology and Biochemistry* 43: 107-116.

Balen, B., Tkalec, M., Sikic, S., Tolic, S. and Cvjetko, P. (2011). Biochemical responses of *Lemna minor* experimentally exposed to cadmium and zinc. *Ecotoxicology,* 20: 815 – 826.

Ciecko, Z., Kalembasa, S., Wyszkowski, M., Rolka, E. (2004). The effect of elevated cadmium content in soil on the uptake of nitrogen by plants. *Plant, Soil and Environment,* 50 (7): 283 – 294.

Dragovic, D., Mihalovic, N., Gajic, B. (2008). Heavy metals in soils: distribution, relationship with soil characteristics and radio nuclides and multivariate assessment of contamination sources. *Chemosphere,* 72**:** 491 – 495.

Edegbai, B.O. and Anoliefo, G.O. (2016a). Growth and development of *Vernonia amygdalina* Del in soils treated with lead. *NISEB Journal,* 16(1): 20 – 26.

Edegbai, B.O. and Anoliefo G.O. (2016b). Toxicity of cadmium to *Vernonia amygdalina* Del. *European International Journal of Science and Technology,* 5(4): 110 – 120.

Ederlin, L., Reale, L., Ferrauti, F. and Pasqualin, S. (2004). Responses induced by high concentration of cadmium in *Phragmites australis* roots. *Physiologia Plantarum,* 121: 66 – 74.

Eze, J.M.O. (1965). Studies on growth regulation, salt uptake and translocation PhD Thesis, University of Durham, United Kingdom. pp 31 – 33.

Kabata-Pendias, A. (2004). Soil-plant transfer of trace elements- an environmental issue. *Geoderma* 122: 143.

Khan, M.N., Wasim, A.A., Sarwar, A., Rasheed, M.F. (2011). Assessment of heavy metal toxicants in the roadside soil along the N-5, National highway, Pakistan. *Environmental Monitoring and Assessment,* 182: 587 – 595.

Kinraide, T.B., Perler, J.F. and Parker, D.R. (2004). Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium and low pH. *Plant and Soil,* 259: 201 – 208.

Kleckerova, A., Sobrova, P., Krystofova, O., Sochor, J., Zitka, O., Babula, P., Adam, V., Docekalova, H. and Kizek, R. (2011). Cadmium (ii) and zinc (ii) ions effects on maize plants revealed by spectroscopy and electrochemistry. *International*

Journal of Electrochemical Science, 6: 6011 – 6031.

Nadgorska-Socha, A., Kafel, A., Kandziora-Ciupa, M., Gospodarek, J., Zawisza-Raszka, A. (2013). Accumulation of heavy metals and antioxidant responses in *Vicia faba* plants grown on monometallic contaminated soil. *Environmental Science and Pollution Research,* 20**:** 1124 – 1134.

Nan, Z., Li, J., Zhang, J., Cheng, G. (2002). Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. *Science of the Total Environment,* 285: 187 – 195.

Pilon-Smits, E. (2005). Phytoremediation. *Annual Review of Plant Biology,* 56: 15 – 39.

Qiao, M., Cia, C.H., Huang, Y., Liu, Y., Lin, A. and Zheng, Y. (2011). Characterization of soil heavy metal contamination and potential health risk in metropolitan region of northern China. *Environmental Monitoring and Assessment,* 172: 353 – 365.

Skwierawska, M. (2013). Effect of sulphur forms on concentrations of cadmium and nickel soluble in 1mol HCl dm^{-3} in arable soils. *Journal of Elementology.* 18(2): 277 – 285.

Spurgeon D.J., Hopkin S.P., Jones D.T. (1994): Effects of cadmium, copper, lead, and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environmental Pollution,* 84: 123–130

Ullrich, S.M., Ramsey, M.H. and Helios-Rybeka, E. (1999). Total and exchangeable concentrations of heavy metals in soils near Bytom, an area of Pb/Zn mining and smelting in upper Silesca, Poland. *Applied Geochemistry,* 14: 187 – 196.

Vasquez-Murrieta, M.S., Miguelles-Garduno, I., Franco-Hernandez, O., Govaerts, B. and Dendooven, L. (2006). C and N mineralization and microbial biomass in heavy-metal contaminated soil. *European Journal of Soil Biology,* 42: 89 – 98.

Wakelin, S.A., Chu, G., Lardner, R., Liang, Y. and McLaughlin, M. (2010). A single application of Cu to field soil has long term effects on bacterial community structure, diversity and soil processes. *Pedobiologia,* 53: 149 – 158.

Wilde, K.L., Stauber, J.L., Markich, S.J., Franklin, N.M. and Brown, P.L. (2006). The effect of pH on the uptake and toxicity of copper and zinc in a typical freshwater alga (*Chlorella* sp.). *Archives Environmental Contamination and Toxicology,* 51: 174 – 185.

Wu, F. and Zhang, G. (2002). Alleviation of cadmium toxicity by application of zinc and ascorbic acid in barley. *Journal of Plant Nutrition*, 25: 2745 – 2761.

Wu, F.B. and Zhang, G.P. (2002). Genotypic differences in effect of Cd on growth and mineral concentration in barley seedling. *Bulletin of Environmental Contamination and Toxicology,* 69: 219 – 227.

Wyszkowska, J., Borowik, A., Kucharski, M., Kucharski, J. (2013).Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. *Journal of Elementology,* 15: 769 – 796.

Wyszkowska, J., Kucharski, M., Kucharski, J., Borowik, A. (2009). Activity of dehydrogenases, catalase and urease in copper polluted soil. *Journal of Elementology,* 14(3): 605 – 617.

Zalewska, M. (2012). Response of perennial ryegrass (*Lolium perenne* L.) to soil contamination with zinc. *Journal of Elementology,* 17(2): 329 – 343.

Zhang, M.K. and Wang, L.P. (2007). Impact of heavy metals pollution on soil organic matter accumulation. *Journal of Applied Ecology,* 18(7): 1479 – 1483.