TOXICITY OF CADMIUM TO *VERNONIA AMYGDALINA* DEL

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ABSTRACT
Vernonia amygdalina Del. was grown in soil treated with different concentrations (25, 50, 75 and 100 mg/kg) of cadmium to investigate the effect of the heavy metal on the growth of V. amygdalina and its bioaccumulation potential which may have implications on the safety of its consumption of the plant. Identical stem cuttings were collected and sown in buckets filled with 5 kg dry soil. These were allowed to grow for a month before the soil samples were treated with cadmium. The experiment included control and four concentrations in three replicates. Data was collected monthly for 12 months. Results of plant height, number of leaves, leaf area, number of branches and girth showed an adverse effect of treatment. There was decreased soil pH, microbial load and nutrients. There was however an increase in soil Carbon. The effect increased along the concentration gradient. Cadmium uptake by plant was within acceptable limits.

Keywords Cadmium, Growth, Heavy Metal, Bioaccumulation

1.0 INTRODUCTION

Cadmium, in its purest form, is a soft silver white metal that is found naturally in the earth's crust. Cadmium rarely exists in its pure form in the environment. It is usually found in combination with other elements. Such compounds include cadmium oxide, cadmium chloride and cadmium sulfide. Human activities such as irrigation of farmland with industrial effluent and/ or sewage water and the use of fertilizer have largely contributed to the cadmium load in the environment. Cadmium is quite a mobile element in soil water and thus freely taken up by plants. Cadmium has many uses in industry and consumer products like batteries, pigments, metal coatings and plastics.

Among heavy metals, cadmium appears to be one of the most dangerous elements to all kinds of organisms (Wojcik and Tukiendorf, 2005). Cadmium has been identified as one of the most phytotoxic heavy metals (Fodor, 2002; Ederli *et al*., 2004; Pilon-Smits, 2005). Although it is considered to be a non-essential element for metabolic processes, it is easily absorbed by plants and even in small amount, it causes toxicity symptoms (Wojcik and Tukiendorf, 2005; Benavides *et al.,* 2005). It is a highly toxic contaminant that affects many plant metabolic processes (Li *et al*., 2008). Cadmium has been shown to inhibit enzymatic activities in plants (Jadia and Fulekar 2009). High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayuso, 2008). The consumption of cadmium has been reported to cause gastro intestinal, haematological, musculoskeletal, renal neurological and reproductive adverse health effects (ATSDR, 1999).

Subramani *et al.* (1997) revealed that the germination and seedling growth of black grain (*Vigna mungo* L. Hepper) showed a gradual decline with the increase in the concentration of Cd treatments. As a result of the immobility of plants they are invariably exposed to potentially toxic compounds at any place they are located through-out their life.

Ye *et al*. (1997) observed that when *Typha latifolia* was treated with cadmium, leaves became chloretic before harvest. According to those authors, leaf and root elongation, shoot and root dry weights were significantly reduced by cadmium and the uptake/ accumulation of cadmium also was observed in seedlings of *Typha latifolia* even in the treatment with 50 µg ml-1 cadmium solution. Although cadmium adversely affects all growth parameters, root growth is affected the most and faster reduction in root biomass than shoot resulting in an increased shoot/root biomass ratio (Jalil *et al*., 1994). According to Zhang *et al*. (2002) addition of cadmium to the nutrient solution significantly decreased shoot and root weight, shoot height, root length and tiller per plant in wheat genotypes but growth inhibition was different in root and shoot and among genotypes. In *Triticum aestivum* seedlings, cadmium treatment led to inhibition of root growth and ion uptake (Abdel-Latif, 2008).

According to Liao *et al*. (2002) cadmium causes cellular damage by denaturing proteins due to the binding of cadmium ions to sulfhydryl residues or by displacing cofactors from a variety of proteins and enzymes. Wu and Zhang (2002) reported that cadmium is one of the most aggressive heavy metals and can be taken up by the roots, translocated readily to above ground tissues and get accumulated in the fruits/seeds and hence become a potential threat to human health as it enters the food chain.

Studies on the elucidation of molecular basis of Cd^{2+} uptake into plant cells revealed that for Cd^{2+}, a non – essential metal ion, there would be no specific uptake mechanism. Ca^{2+} channels have long been studied using Cd^{2+} ions and evidences for Cd^{2+} uptake into plant cells via Ca^{2+} channels have come from the studies by Perfus-Barbeoch *et al*. (2002). According to Clemens (2006) there is clear yet so far mostly indirect evidence, that Cd^{2+} is taken up into plant cells by Fe^{2+} , Ca^{2+} and Zn^{2+} transporters/channels of low specificity.

Cadmium tolerance and hyper accumulation potential have been reported as a characterisitic of *Bidens pilosa* which is a widely growing weed from tropical and subtropical zones (Sun *et al.*, 2009). Similarly, *Lonicera japonica* plant exposed to cadmium for 21 days showed accumulation of cadmium in the leaves, stem and roots with increased cadmium accumulation in the medium (Liu *et al.*, 2009).

Vernonia amygdalina is put to diverse use by people of different cultures. Virtually all parts of the plant are consumed and in some cases raw. This experiment is aimed at investigating the toxic effect and bioaccumulation potential of *V. amygdalina* treated with cadmium

2.0 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. 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.

2.1 Collection of Plant Materials and soil Samples

2.1.1 Stem: The stem cuttings of *Vernonia amygdalina* used in the study were obtained from a hedge composed primarily of *V. amygdalina* within the Senior Staff Quarters of the University of Benin, Benin City, Edo state.

2.1.2 Soil: The soil (top soil, 0-10 cm) used for this research was carefully 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 . The soil was dried and thoroughly mixed. Five (05) kg dried soil was weighed, each into 15 perforated buckets.

2.1.3 Heavy Metals: Cadmium (Cd) used in this study was obtained from its soluble salt - cadmium sulphate ($3CdSO_4 + 8H_2O,$). The quantities of cadmium (Cd) corresponding to the various treatment concentrations were calculated by relating the molecular weights of the individual elements to those of the compounds i.e. by simple proportion.

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

2.2 METHODOLOGY

The buckets earlier perforated and properly identified were laid out on the prepared site in a completely randomized setting. Three (03) uniform young stem cuttings (30cm long, similar girth with 3 - 4 buds) of *V. amygdalina* were sown in each bucket and later thinned 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. There were 4 concentrations in 3 replicates. The concentrations were 25 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg and control (0 mg/kg). The various pollutants were measured and dissolved in distilled water and dispensed.

After the soil treatment, data was 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.

2.2.1 FIELD DATA COLLECTION

2.2.2 Plant Height: The plant height for V. *amygdalina* was recorded from a tagged branch.

2.2.3. Number of leaves: The total number of leaves for V. *amygdalina* were counted and recorded..

2.2.4 Leaf area: A leaf from a tagged branch was used and the area was determined using the proportional method of weighing a cut-off traced paper with standard paper of known weight to area ratio (Eze, 1965).

2.2.5 Number of branches: The number of branches was taken by visual counting of branches on the tagged plants at the required interval.

2.2.6 Girth: The diameter of the shoot was obtained using the Esal vernier caliper. (Girth = πd)

2.3 ANALYSES

Soil microbial analysis, physicochemical analysis and analysis for Cadmium in both soil and plant parts were done according to established procedures as follows:

2.3.1 Microbiological Analysis

Ten (10.0) g of each soil samples was mixed with 90.0ml of sterile distilled water in a beaker. Then the samples were serially diluted using tenfold serial dilution and 0.1 ml of the appropriate dilution pour plated onto nutrient agar (NA) and Potato dextrose agar (PDA) respectively for bacteria and fungi isolations. The nutrient agar plates were incubated at 37°C for 24 hours under aseptic condition while Potato dextrose agar plates were incubated at 28^0C for 72 hours.

2.3.2 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 < 2 mm samples were stored in polythene bags for subsequent analysis. The < 2mm fraction was used for the determination of selected soil physicochemical properties and the heavy metal fractions.

2.3.3 Sample Preparation 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. Metal content in the digested samples were analyzed for Cd using the Atomic Absorption Spectrophotometer.

3.0 RESULTS

Plant height for *Vernonia amygdalina* grown in soil treated with various levels of cadmium and control is presented in Figure 1. Control recorded a consistent and steady increase in height while the Cadmium treated soil showed mean height values increasing at a much slower rate. The 100mg/kg cadmium treated soil lost all plants by the 4th MAT, while the 75mg/kg cadmium treated soil lost all plants by the 5th MAT. At the end of the experiment, control plants recorded significantly higher ($P < 0.05$) mean heights than the plants in the cadmium treated soil. At the time of termination of the experiment, control plant height was 77.43±1.45 cm while the 25 mg /kg, 50mg / kg, 75mg/ kg and the 100 mg/ kg had heights of 39.77±2.32cm, 28.77±4.32 cm, 0.00±0.00 cm and 0.00±0.00 cm respectively. It was observed however that the 25mg/ kg treatment in the early months, recorded the highest height values up to 5 MAT

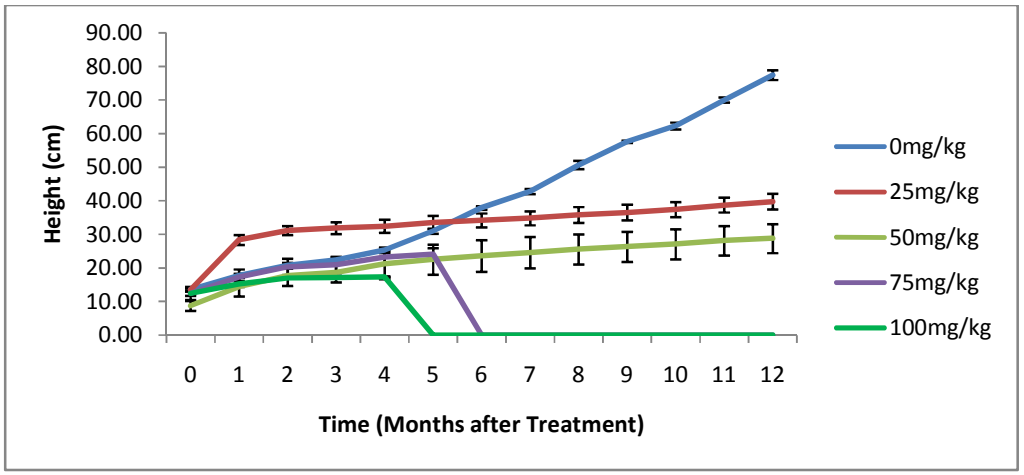

Figure 1: Effect of Cd treatment on the height (cm) of *V. amygdalina*

Figure 2 shows the mean number of leaves recorded for *V. amygdalina* grown in control and cadmium treated soil. The result showed that the control plants recorded higher number of leaves than the plants in the cadmium treated soil. Between 0 MAT and 4 MAT, the 25mg/kg cadmium treated soil recorded more leaves than all the treatments including control. Beyond this period however, there was a sharp drop in its number of leaves. At 12 MAT, control plants recorded significantly higher number of leaves. There was adverse effect along the concentration gradient. Thus, when control had 35.67±7.53 number of leaves at 12 MAT, the 25 mg /kg, 50mg / kg, 75mg/ kg and the 100 mg/ kg treatments had 13.33±4.48, 12.67±2.90, 0.00 ±0.00 and 0.00 ±0.00 number of leaves respectively. There was significant difference ($P < 0.05$) between the control and the various cadmium treated soils.

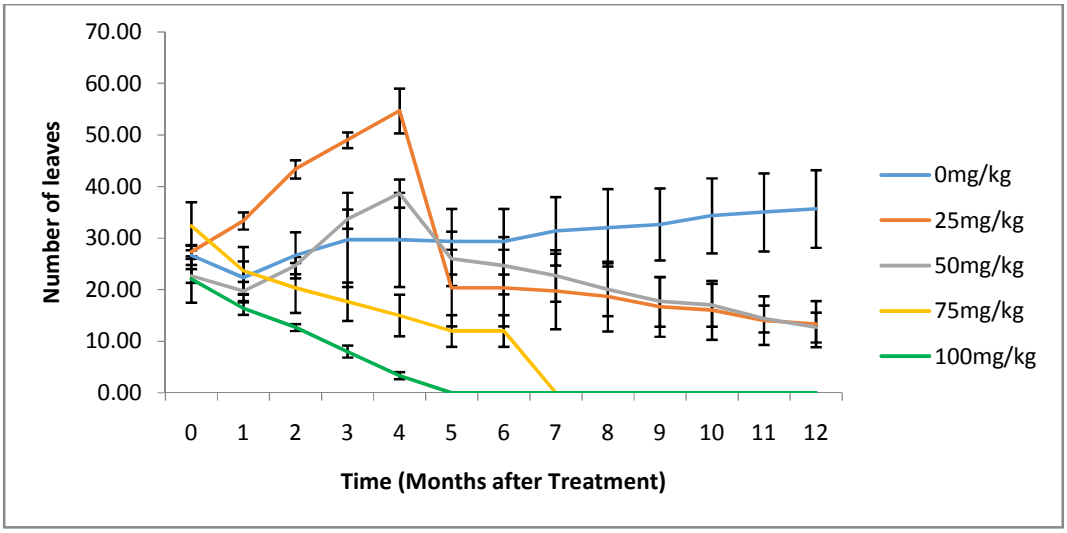

Figure 2: Effect of Cd treatment on the number of leaves of *V. amygdalina*

Mean leaf area values for *V. amygdalina* raised in control and cadmium treated soil are shown in Figure 3. Control values were consistently higher than the values recorded for the various soil treatments with cadmium, with decreasing values (increasing effect) along the concentration gradient. At 12 MAT, control leaf area was 17.45±4.84 cm^2 while the 25 mg /kg, 50mg / kg, 75mg/ kg and the 100 mg/ kg treatments had values of 6.17 ±1.04 cm^2, 4.74 ±0.77 cm^2, 0.00 ±0.00 cm^2,0.00 ±0.00 cm^2 respectively showing a significant difference ($P < 0.05$) between control and the treatments.

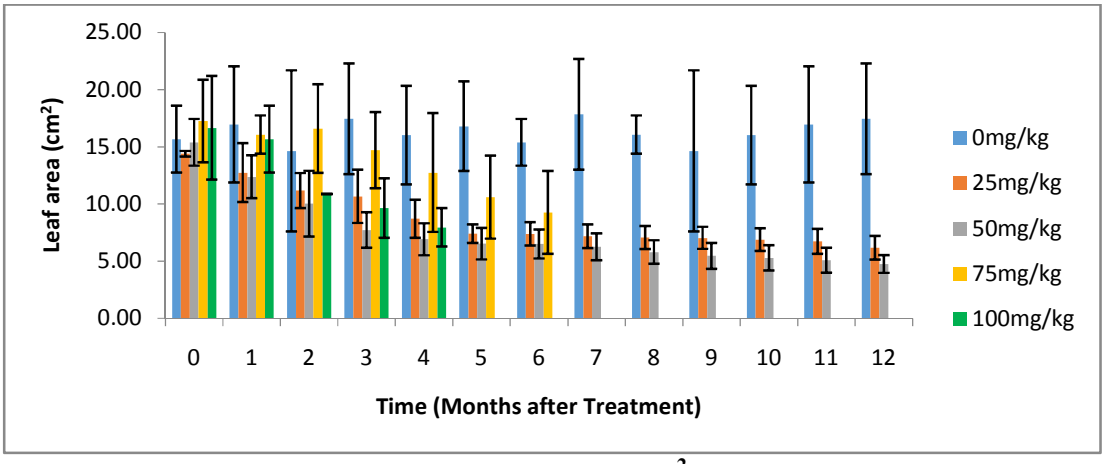

Figure 3: Effect of Cd treatment on the leaf area (cm^2) of *V. amygdalina*

Figure 4 shows the mean number of branches recorded for control and various soil treatments with cadmium. At 4 MAT, the 75mg/ kg had the highest number of branches. Beyond this time, control values were higher than the values for all the cadmium treated plants. However there was no significant difference ($P < 0.05$) between control, the 25mg/kg and the 50mg/ kg cadmium treated soils at 12 MAT when control and the 25 mg /kg, 50mg / kg, 75mg/ kg and the 100 mg/ kg treatments had values of 4.67±0.66, 4.33±1.33, 2.67±0.33, 0.00±0.00 and 0.00±0.00 respectively.

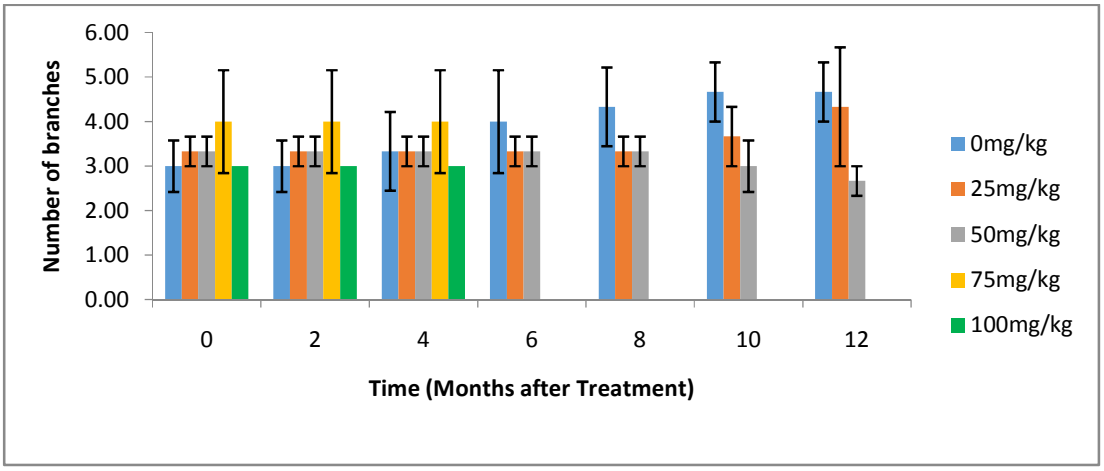

Figure 4: Effect of Cd treatment on the number of branches of *V. amygdalina*

Figure 5 shows the mean values recorded for girth of stem of *V. amygdalina* for control and various cadmium treated soils. Control girth increased until 8 MAT and remained the same till the end of the experiment while cadmium treatments 25mg/kg and 50mg/kg recorded shrunken values beyond 8 MAT. The 75mg/kg and the 100mg/kg treatments had lost all plants by 6 MAT. At 12 MAT, control and the 25 mg /kg, 50mg / kg, 75mg/ kg and the 100 mg/ kg treatments had values of 15.71± 0.00, 15.71± 0.00, 13.09 ±1.39, 0.00 ±0.00, and 0.00 ±0.00 cm respectively. Control girth was significantly different ($P < 0.05$) from the girth of the 50mg / kg and the 100 mg/ kg treatments.

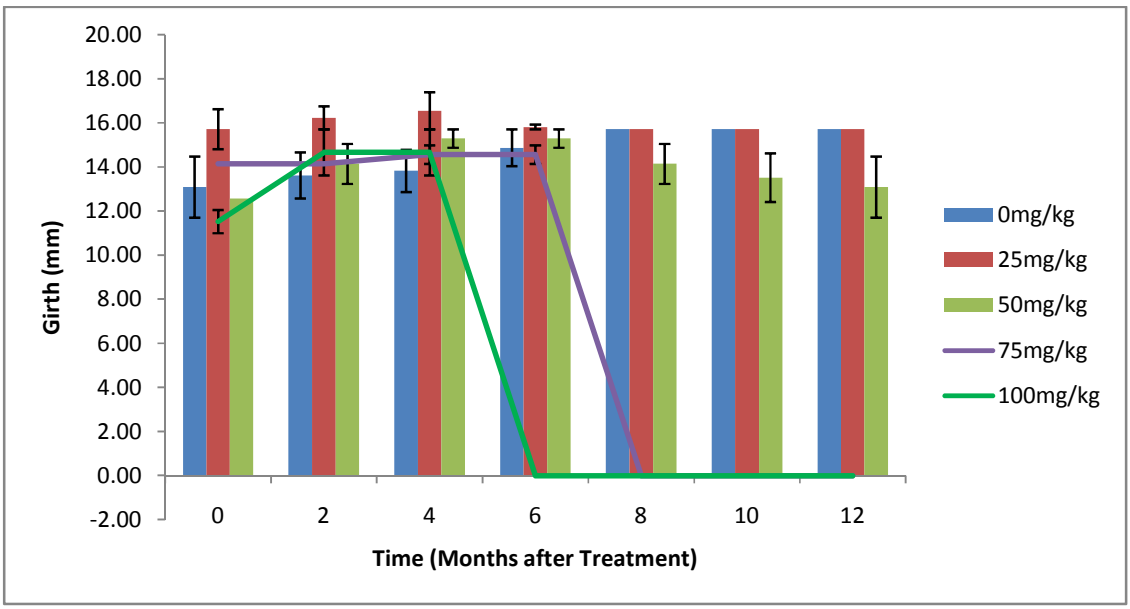

Figure 5: Effect of Cd treatmen on the girth of *V. amygdalina*

Table 1shows the physico-chemical properties of *V. amygdalina* cultivated soil at the end of the experiment (12MAT). There was a decrease in the pH, %N, %P, Ca and Mg content of the soil as the concentration of the treatment increased. Conversely however, there was an increase in the % carbon content of the soil.

Table 1: Physicochemical properties of post *V. amygdalina* cultivated 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	7.8	0.93	0.27	3.48	1.08	0.74
50	7.5	1.08	0.23	3.39	1.03	0.71
75	6.6	1.1	0.19	3.17	0.89	0.65
100	6.4	1.17	0.15	3.08	0.84	0.62

The plant analysis for cadmium is presented in table 2. There was an increase in the amount of cadmium present in the plant as the concentration of the treatment increased. There was no detectable amount of cadmium in the control plants.

Table 2 : Plant analysis for cadmium at the end of the experiment

Concentration (mg / kg)	Cd (ppm)
0	ND
25	0.068
50	0.094
75	0.185
100	0.283

ND- NOT DETECTED

Table 3 shows the bacterial and fungal counts of the soil at the end of the experiment. There was a decrease in the total bacterial and fungal population of the soil along the concentration gradient with the control soils having the highest number of microbial population.

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	1.03×10^5	6.3×10^4
50	8.5×10^4	$6.1`\times10^4$
75	7.9×10^4	5.8×10^4
100	6.5×10^4	5.6×10^4

KEY: Cfu/g: Colony forming unit per gram

4.0 DISCUSSION

In the current study, the treatment with Cd produced adverse effect on the height (Figure 1) of *V. amygdalina* at the end of the experiment (12 MAT). However, between 0 MAT and 5 MAT the 25 mg / kg concentration stimulated the growth of *V. amygdalina.* Beyond this time, the values recorded increasingly were less than control values. At 6 MAT, the 50 mg / kg and 100 mg / kg treatments lost all plant. The effect of cadmium treatment on the number of leaves (Figure 2) leaf area (Figure 3), number of branches (Figure 4) and the girth (Figure 5) of *V amygdalina* showed similar growth response as all showed enhancement at the early stages of growth before manifesting adverse effects of the metal.

Growth in any living organism is the outcome of cell division. This cell division is primarily mitosis. Heavy metals adversely affect the cell division of plants (Brachet and Mirsky, 1981; Duan and Wang 1995) and the effects are different and concentration dependent. Duan and Wang 1995 observed that cadmium caused an extension cell division under a low concentration of 0.01ppm while cell division was shortened but the cell cycle was extended by increasing the dose. In this study there was stimulation of growth measured in height, number of leaves, leaf area, number of branches and girth at the early stages of the experiment with the 25mg/kg treatment concentration. However, at the end of the experiment this trend was reversed. Anoliefo and Osubor (1998) reported decrease of 50% in the vine length of *Cucumeropsis manni* plants grown in cadmium treated soils. They also observed significant decrease in leaf area which is corroborated by this study.

Some plants died in the course of the experiment. Linger *et al*. (2005) and 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.

The girth results in this study are similar to observations reported by Aydinalp and Marinova (2009). The increase in girth may be due to heavy metals deposit in the stem.

The various treatments 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. Increase in acidity results in increase in the heavy metals available in solution in the soil and consequently to the plants. In this study, data for growth revealed that there was adverse effect of treatment along the concentration gradient. Thus, there is a correlation between pH and the growth data as pH values recorded caused an increase in the Cd available for uptake by the plants at the different levels. Adeniyi *et al*., (2005) reported that the solubility of heavy metals was significantly related to their total concentration, together with soil pH.

The carbon constituent of the soil increased as the concentration of the treatments increased. Zhang and Wang, (2007) studied the accumulation and mineralization of soil organic matter under impact of heavy metals pollution. Their results showed 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. With increasing soil heavy metals pollution, the particulate organic matter and its proportion in total soil organic C increased, while the microbial biomass C and its proportion in total organic C decreased. Heavy metals were largely enriched in particulate organic matter, which could impact the further mineralization of soil organic matter. In a word, soil heavy metals pollution could change the mineralization rate of soil organic matter, and affect its accumulation and distribution.

The results of the other analyses show that % N, % P, % Ca, % Mg, % K and % Na constituents of the soil were decreased by increased concentration of the treatment as well as increased heavy metal constituent of the treatments. Interactions between Cd and essential elements lead to changes in plant nutrient content and

physiological disorders as well as retardation of growth and yield (Sandalio *et al.*,2001; Mazen, 2004). 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).

The results for plant analyses showed a direct relationship with the treatment concentration. There is a further direct relationship between the amount of Cd in the plant and the adverse effect on growth manifested in the data collected. According to Nasu *et al*. (1984) the degree of the Cd effect depends on the concentration absorbed. Of all the treatments, there was significant difference between the data for control and all the levels of treatment with cadmium. The present concentrations of Cd 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 0.3 mg kg-1 for Cadmium (Codex Alimentarius Commission, 1984; Farooq *et al.*, 2008).

Conclusion

The aim of this study was to investigate the toxicity of cadmium to *Vernonia amygdalina* L and consequently to man. Available data from this experiment show an adverse effect of treatment on the experimental plant. The bitter leaf plant is put to diverse use. All the parts of the plants are consumed in one way or the other. Cadmium bioaccumulated in this study was within safety limits. It remains a cause for concern though considering what long term accumulation could portend.

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