POSSIBLE ADAPTIVE GROWTH RESPONSES OF *CHROMOLAENA ODORATA* DURING HEAVY METAL REMEDIATION

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ABSTRACT

The changes in morphological and crude protein content of *Chromolaena odorata* to heavy metal-induced stress were investigated. This was with a view to providing information on the test plant adaptation potential during remediation of soils contaminated with selected metals . Stems of *C. odorata* were planted in soils treated with Cd (as CdCl) and Pb (as PbCl) at different concentrations of 6.67, 33.3 and 100 mg/kg respectively, singly and in combinations (Cd+Pb). After the exposure of *C. odorata* to heavy metals for 3 months, results showed that residual Pb concentration in soil was 4.31 mg/kg in the 6.66mg (Pb)/kg-polluted soil, compared to the control (0.7 mg/kg). Residual concentration of Cd in soil was 0.22 mg/kg in the soil originally polluted with 6.66 mg (Cd)/kg, compared to a residual concentration of 5.94 mg/kg in the 100 mg (Cd)/kg-polluted soil. Residual metal concentration after plant exposure was lower for Cd in Cd-polluted soil than for Pb in Pb-polluted soils suggesting better plant-assisted remediation of Cd than Pb. Total crude protein content of plants exposed to metals increased with increase in metal concentration in soil. The presence of metal in soil reduced plant height from 127 cm in the control to a range of vales from 93 – 117 cm in the plants exposed to metal contamination. However, at lower concentrations of the Cd in soil, plants showed improved root branching (117.00±11.31), compared to the control (62.35±5.63). Results of the study further show the capability of *C. odorata* for metal remediations and also possible morphological adaptation mechanisms of survival in metal-impacted soils.

Keywords: Heavy metals, bioremediation, crude protein, adaptation, *Chromolaena odorata*

INTRODUCTION

Plants are constantly unprotected from several forms of stress conditions in the environment, both abiotic and biotic. These stressors including herbivory, pathogen attack, drought, salinity, and heavy metals, significantly affect plant growth and ultimately disturb the life of plant (Kraner *et al.*, 2010). They thus pose a severe threat to plant life and accounts for suppressed crop efficiency all over the world by at least 50% (Boyer, 1986; Bray *et al.*, 2000).

A major concern in the world today is the all-round effects of the discharge of several harmful substances from industrial operations and other activities of human. For example, melting and smelting activities, electroplating, vehicular gaseous exhausts, industrial burns, energy and fuel formation, to mention a few, contribute daily to accumulation of heavy metals in the environment (Aiyesanmi *et al.,* 2012). Given the phytotoxic effects metals have on both plants and humans, it becomes appropriate for the scientific community to find ecofriendly methods of metal reclamation from the environment. Phytoextraction of heavy metals, using hyperaccumulator plants, is one very important technique. However, it is very important to also have an idea of how the plant of choice adapts to the metal-contaminated soil; the soil consequently prompts abiotic response in plants.

Plants depend on a blend of nutrients in the soil and air, including solar energy, CO_2, and H_2O for photosynthetic processes. Some of these nutrients are heavy metals like Zn and Cu which are essential micronutrients for the plants. Zinc is important in the ability of plants to produce seeds as well as in disease resistance, whereas Cu is involved in metabolic processes of some plants (Fargasova, 2004; Sagardoy *et al.*, 2009; Yruela, 2009). However, there are a couple of other heavy metals which, even in low concentrations are harmful to plants. Examples are Cd and Pb. Also important is the fact that these metals are among the very commonly found heavy metal contaminants in Nigerian soils (Galadima *et al*, 2011). Both metals not only are also very common in agrarian soils, they cause similar morphological effects (Yadav, 2010). These two were also chosen as the test metals in the study.

During the removal of toxic metals from soils, plants, employ a number of mechanisms for reaction to metal-induced stress. One of such methods of plant adaptation to abiotic stresses is the synthesis of stress proteins and presentation of some morphological adaptive features such as reduced plant growth, broader leaves, earlier folial senescence, as well as reduced photosynthetic activity (Okonokhua *et al.*, 2007; Anoliefo *et al.*, 2010; Ikhajiagbe and Anoliefo, 2012; Ogedegbe *et al.*, 2013). Some of these features displayed by metal-stressed plants may not necessarily be adaptive features but phenotypic expressions of metal toxicity. Besides stress proteins, flavonoids have been reportedly used by plants to regulate defense against abiotic stress. It has also been found to be useful as heavy metal chelators (Brown *et al.*, 1998).

A number of plant species have been studied for capability to reclaim both organic and inorganic compounds from contaminated soils including *Chromolaena odorata* (Anoliefo and Vwioko, 2001; Anoliefo *et al.*, 2003; Agunbiade and Fawale, 2009). The phytoremediative capability of this weed, as reported by the authors, is hinged on the possession of such characteristics as the possession of a large biomass, short life span, high breeding frequency as well as a high tolerance for heavy metal contaminated soil. *C. odorata* is an invasive weed and it is found in most parts of Nigeria.. *C. odorata* has been recognized as having great potential for accumulation of heavy metals, particularly Pb and Cd from metal contamination caused by solid waste disposal (Agunbiade and Fawale, 2009). Hence, it was considered very useful in this project.

In the present study, three parameters were studied in relation to the plant. First is the presentation of certain morphological parameters including plant height, root formation as well as foliar presentations. It is hoped that comparing features in contaminated and control soils will help to provide information on distinctions in morphological responses especially relating to metal specificity. In the place of chromatographic assay of various stress proteins produced, the other option of assessing the amount of crude proteins produced as well as means total chlorophyll content index upon exposure to metal contamination was explored to give a very close idea as to how the plant responds biochemically with protein formation as induced by plant stress conditions. Finally, in order to further clarify its phytoremediative potential, residual concentrations of heavy metals in contaminated soils was also investigated.

MATERIALS AND METHODS

Methodology

Top-layered soil (0 – 10 cm) was collected from a garden beside the Botanic Garden of the Ugbowo Campus of the University of Benin, into heaps. These were sun-dried to constant weight; and then 20 kg each was measured into buckets already prepared for this study, with 5 2 mm-diameter perforations underneath each bucket. The soil in each bucket was moistened with water to water holding capacity, which was earlier determined to be 192.9 ml/kg soil, and was made ready for use. Twenty (24) hours later, the moistened soils in buckets were divided into three (3) groups of 3 sub-groups each; consisting of nine (9) treatments altogether and a control. These were quintuplicated, amounting to fifty (50) buckets. Soils in the first batch were polluted with Cd (as CdCl) at the concentration of 6.66mg of metal per kilogram of soil, 33.3mg/kg and 100.0mg/kg respectively. The second batch was polluted with Pb (as PbCl) in three similar concentrations; whereas the third batch was polluted with both Pb and Cd in equal proportions, but totally into 6.66, 33.3 and 100.0 mg/kg respectively. These separate measured quantities were dissolved in 1 litre of water with which each 20-kg-soil was moistened again and thoroughly stirred. For example, in the 6.66 mg (metal)/kg (soil) treatment, 6.66 x 20 = 133.2 mg of metal was dissolved in 1litre of water and poured into 20 kg soil. The same factor applied for the other concentrations. The control soil did not receive any heavy metal contamination.

Planting of *C. odorata*

Healthy-looking *C. odarata* stem cuttings of equal sizes (2.0 - 2.3cm in thickness) and length (30cm) were planted vertically into the soil with 10 cm of stem cutting buried into the soil. One stem cutting was planted per bucket.

Husbandry and Analyses

Since the aim of the study was to observe performance of *C. odorata* in response of metal contamination, every other weed was removed by hand pulling as soon as they appeared. Since the experimental buckets were exposed to prevailing weather condition (rainy season, March – July, 2015), water requirements of the soil was augmented by wetting the soil in each bucket with 500 ml of tap water (pH 6.5-6.9). The setup was studied for 3 months for plant morphological parameters including plant height and number of primary branches. Stem girth was determined by using a Vernier caliper. Main root length was measured after plant was uprooted, washed and sun-dried. Number of folded leaves, leaves with chlorotic and necrotic lesions, as well as number of senesced leaves per plant. Mean total chlorophyll content index was measured by using a chlorophyll content meter; CCM-200 plus. CCM-200 is a non-destructive chlorophyll content measuring meter. The average meter reading of five leaves per plant was taken as the CCI. Chlorophyll has several distinct optical absorbance characteristics that the CCM-200 plus exploits in order to determine relative chlorophyll concentration. Crude protein content of whole plant was determined by using the methods of Ene-Obong and Carnovale (1992). Residual metal content of soil was also determined following the methods of SSSA (1971) and AOAC (2005)

RESULTS

In order to follow up with soil physicochemical conditions during exposure of plants to metal contamination, soil conductivity (Fig. 1) and pH (Fig. 2) were determined. Values recorded showed that conductivity ranged from 20.7 µs/cm in the 33.3 mg(Cd)/kg-polluted soil to 27.5µs/cm in the 6.66 mg(Cd+Pb)/kg-polluted soil at 1 month after planting (MAP). At 2 MAP, an increased conductivity value of 34.5 µs/cm was obtained in the 6.66 mg (Cd)/kg-polluted soil, whereas a lower conductivity value (21.2 µs/cm) was obtained in 33.3 mg (Cd+Pb)/kg-polluted soil. Soil conductivity was stable in the control (22.2 – 24 µs/c). The pH of the soil was modified by metal presence (Fig. 2) during the 3 months of observation. Soil pH of the control experiment varied between 5.3 – 5.5 in the 3 months, compared to a range between 5.5 - 6.7 in the 6.66 mg(Cd)/kg-polluted soil. Higher pH values in this study were obtained in the 6.66 mg (Pb)/kg-polluted soil; 6.8, 5.5, and 6.8 in the first, second and third months respectively (Figure 2).

Residual Pb concentration in the soil was 4.31 mg/kg in the 6.66mg (Pb)/kg-polluted soil, compared to the control (0.7 mg/kg; Fig. 3). Comparatively, residual Pb concentration in soil was concentration-dependent. Residual metal concentrations in 6.66 mg(Pb) and 33.3 mg(Pb)-polluted soil were not significantly different from each other (p>0.05), indicating that significant Pb remediation attributed to plant presence was better at lower metal concentrations in soil. Concentration of Cd in Cd-polluted soil sown with *C. odorata* was 0.22 and 0.27 mg/kg in both 6.66mg (Cd)/kg and 33.3 mg (Cd)/kg - polluted soil respectively (Fig. 4), compared to a residual concentration of 5.94 mg/kg in the 100 mg (Cd)/kg-polluted soil. Again, insignificant differences existing in metal concentration at lower metal levels indicated better plant-related remediations of Cd when levels in the soil were lower. Comparing the results presented in both Fig. 3 and Fig. 4, residual metal concentration after plant exposure was lower for Cd in Cd-polluted soil than for Pb in Pb-polluted soils. Perhaps, plant-assisted remediation of heavy metals was better in Cd contaminations.

In the soil polluted with a mix of both heavy metals (Cd and Pb) (Fig. 5), residual heavy metal concentration after removal of *C. odorata* plants was lower for Cd than for Pb. Soil concentrations of Pb ranged from 5.55 – 11.40 mg/kg, compared to 0.7 – 6.1 mg/kg of Cd. Again, plant-assisted remediation of Cd was better compared to that of Pb.

Whole plant crude protein content of *C. odorata* was assayed at 3 months (Fig. 6). Crude protein content of plants in the control was 12.8%, compared to 10.44% in the 100 mg(Cd)/kg-polluted soil. Crude protein of plants sown in 33.3 mg (Cd)- and 100 mg(Cd)-polluted soils were 16.06% and 18.95% respectively. Protein content of Pb-contaminated treatments however ranged from 4.74 – 16.90%, while those in the Cd/Pb-polluted soils ranged from 9.20 - 12.05% dry wt. The amount of protein present in each treatment increased with increase in concentration of heavy metals in the soil.

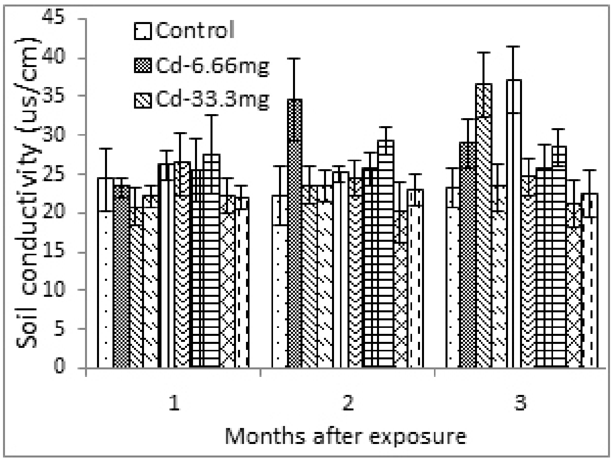

Figure 1: Soil conductivity after plant was exposed to the metal-polluted soil for 3 months

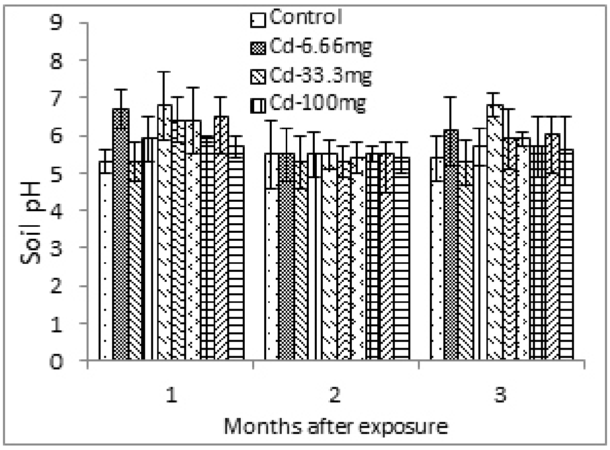

Figure 2: Soil pH after *Chromolaena odorata* plant was exposed to the metal-polluted soil for 3 months

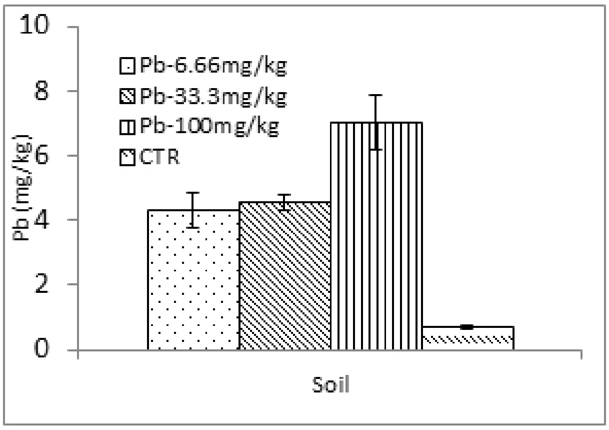

Figure 3: Concentration of Pb in the Pb -spiked soil three months after exposure to *Chromolaena odorata*

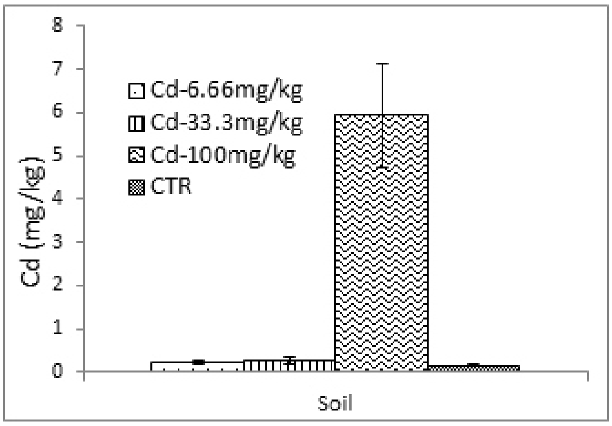

Figure 4: Concentration of Cd in the Cd -spiked soil three months after exposure to *Chromolaena odorata*.

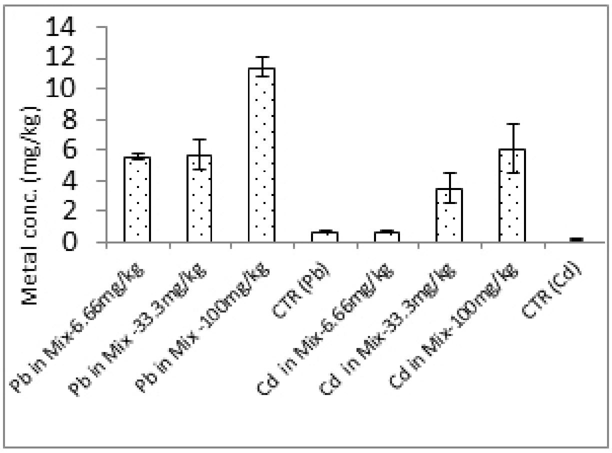

Figure 5: Concentration of Pb and Cd in the Pb-Cd-spiked

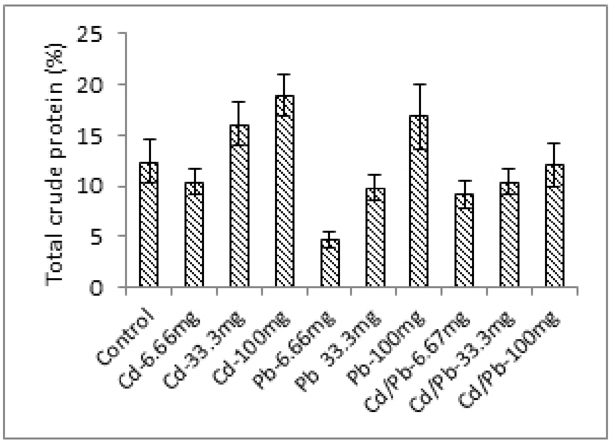

Figure 6: Total crude protein content of the plant after exposure to the metal-polluted soil for 3 months.

At 3 months following exposure of plants to heavy metals, the maximum height attained by metal-exposed plants was 127.00 cm in the control (Table 2). Plants sown in 33 mg(Pb)/kg-polluted soils had the least plant height of 93.00 cm. Branch height ranged from 86.00 -115.00 cm, with the control having the maximum value and the least obtained in the plant sown in 33.3 mg (Pb+Cd)/kg-polluted soil. However, there was lesser number of root branches per plant in the control (62.35) compared to those in Pb-polluted soils (42.67 – 46.27). It was also observed that plants in lower concentrations of the Cd-polluted soil had better root branching (117.00±11.31) compared to the control. Similarly, better root branching was observed in the mixed-metal-polluted soils.

Table 2: Plant Parameters after 3 Months Following Exposure to Metal Contamination

Treatments	Plant height (cm)	No. of primary branches	Stem girth (cm)	Main root length (cm)	Number of root branches
Control	127±0.54	6.32±0.44	0.80±0.09	33.67±4.07	62.35±5.63
Cd-6.66mg/kg	108±0.97	5.42±0.51	0.70±0.01	25.07±2.75	117.00±11.31
Cd-33.3mg/kg	110±0.98	3.62±0.43	0.70±0.00	27±8.18	63.67±2.04
Cd-100mg/kg	107±1.51	10.35±0.49	0.80±0.01	42±12.22	55.33±5.51
Pb-6.66mg/kg	105±0.54	12.32±0.50	0.75±0.01	47±16.30	46.17±3.55
Pb-33.3mg/kg	94±1.10	2.22±0.51	0.50±0.04	33±5.95	43.00±6.55
Pb-100mg/kg	93±0.47	2.42±0.48	0.60±0.02	36.5±11.63	42.67±2.36
Cd/Pb-6.67mg/kg	117±0.60	5.52±0.49	0.70.02	25.3±3.79	75.33±3.20
Cd/Pb-33.3mg/kg	103±0.50	2.34±0.53	0.60±0.001	31.73±6.44	72.00±10.79
Cd/Pb-100mg/kg	107±0.51	3.01±0.48	0.50±0.04	33±5.96	90.00±13.76

Results are mean±standard error for 5 determinations

As shown on Table 3 the control plants had an average of 3.23 folded leaves, compared to 4.34 – 14.33 in the metal-exposed plants. There were a significantly high number of leaves with chlorotic lesions (8.32±1.10) in the plant sown in 100 mg(Pb)/kg-polluted soil than in the other treatments. Similarly, in this same soil treatment, a significantly higher number of senesced leaves per plant were obtained (15.24±3.52). Leaf chlorophyll content index ranged from 13.47 – 18.17 CCI in the plants exposed to metal contamination, compared to 22.60 CCI in the control (Table 3).

Table 3: Morphological Parameters of Plant Leaves after 3 Months Following Exposure to Heavy Metals

Treatments	No of folded leaves/ plant	Number of leaves with chlorotic lesions	Number of leaves with necrotic lesions	No. of senesced leaves	Mean total Chlorophyll content index
Control	3.23±0.22	2.53±0.59	1.00±0.01	8.12±1.16	22.60±3.27
Cd-6.66mg	4.34±1.94	3.98±1.00	2.42±0.50	2.91±1.00	15.00±2.73
Cd-33.3mg	6.93±1.85	5.63±0.83	5.12±0.19	7.01±0.50	18.17±1.59
Cd-100mg	11.54±2.40	5.23±1.01	6.53±0.07	4.24±0.48	14.73±2.22
Pb-6.66mg	14.33±2.21	4.22±0.10	4.22±0.47	7.93±0.54	13.47±1.13
Pb-33.3mg	7.11±1.42	4.32±0.67	4.28±0.69	4.24±0.47	16.40±2.19
Pb-100mg	8.32±1.27	8.32±1.10	5.04±0.11	15.24±3.52	16.50±0.96
Cd/Pb-6.67mg	8.55±1.20	4.43±0.81	3.63±0.52	4.13±0.57	16.30±1.72
Cd/Pb-33.3mg	11.32±1.71	3.03±1.22	4.20±0.15	6.43±0.43	16.73±2.66
Cd/Pb-100mg	12.43±2.20	4.46±0.85	5.19±0.01	9.29±3.33	16.00±1.64

DISCUSSION

The study was carried out in order to determine the morphological changes that occur in *Chromolaena odorata* as an adaptation response to heavy metal composition of soil. Changes in metal concentration in the soil are a factor of the inherent soil physicochemical condition. For example, soil pH has been reported to be an important factor in the remediation of organic and inorganic soil contaminants (Vidali, 2001; Ikhajiagbe *et al.*, 2012, 2013). As reported by Nasir *et al.* (2015), increased soil electrical conductivity is an indication of the increased presence of cations in the soil. Whereas, increased conductivity can be damaging, low conductivity signifies that available soil nutrients are limiting, and this impedes microbial activity. The damaging effects of this phenomenon include reduced water absorption by plants resulting in foliar necrosis and stunted growth. Given that increased soil conductivity is indicative of water-related stresses in plants including poor water conduction, foliar necrosis and stunted growth (Nasir *et al.*, 2015), these characteristics were not exhibited by the test plants. Increased soil conductivity may have inhibited plant activity and supported plant damage due to heavy metal presence (Paradelo *et al*, 2015). However, no direct correlation could be made between conductivity and heavy metal concentration. The results of the concentration of heavy metal present in the soil after 3 months decreased significantly.

Reduction in metal concentration in soil may be plant-based; though there are several other mechanisms of heavy metal reduction that are not necessarily tied to plants, for example infiltration and leaching as well as microbial methylation of the heavy metals, as well as microorganism-based metal sequestration or chelation. Plants are able to phytoaccumulate the metals in plant parts (Cunningham and Berti, 1993).

Plants also contain organic acids in their roots, and these expressively boost the bioavailability of soil-bound heavy metals (Kanazawa *et al.*, 1995; Pellet *et al.*, 1995; Larsen *et al.*, 1998). These organic acids also have been found to chelate rhizospheric metals, which are largely highly phytotoxic, to form a significantly less toxic complex. Lasat (2000) earlier reported the importance of phytochelatins in the detoxification of accumulated Cd in plants.

In the present study, enhanced reduction of Cd over Pb in soil was recorded. This may be as a result of other possible forms of remediation made active by the plant with respect to Cd-reclamation. Once inside the plant, Cd stimulates the activity of NADPH oxidases, resulting in extracellular superoxide, H_2O_2 accumulation and lipid peroxidation and oxidative burst (Brahim *et al.*, 2010). The Cd eventually stimulates the assembly of phytochelatins through increased activity of phytochelatin synthase; and the consequence is the immmobilization Cd by sequestering the phytochelatin-Cd conjugant in vacuoles decreasing free Cd concentration.

In spite of reductions in metal concentration in soil, inhibitory effects of the metals in plant morphology was recorded.

The phytotoxicity of heavy metals is commonly revealed in significant growth reductions and foliar anomalies. There was general reduced or stunted growth of *C. odorata* in the metal-contaminated soil. The inhibitory effects of Cd on plant morphology, including fresh and dry mass accumulation, height, root parameters, leaf number and size have been reported (Moya *et al.*, 1993; Vassilev and Yordanov, 1997; Foy *et al.*, 2005). Vassilev and Yordanov (1997) reported leaf red-brownish discolouration, as well as leaf epinasty and leaf chlorosis on exposure to Cd. Research shows that plants in Cd-polluted soils display observable signs of physiological and morphological damage, including chlorosis, reduced growth, necrosis and subsequent death (Sanita di Toppi and Gabbrielli 1999; Wojcik and Tukiendorf 2004; Mohanpuria *et al.*, 2007; Guo *et al.*, 2008). On the other hand, an increased level of Pb in the soil can cause anomalous morphology in plants. Paivoke (1983) reported irregular radial thickening in pea roots exposed to Pb. However, proliferation effects on the repair process of vascular plants are also prompted by Pb phytotoxicity (Kaji *et al.* 1995).

According to Yadal and Jyoti (2014), plant roots are damaged first due to slow accumulation of the heavy metal in the roots, affecting the overall plant growth. However, in this experiment main root length and number of roots did not show any significant inhibitory effect of metal contamination. It is therefore suggested that both Cd and Pb did not have any effect on the below-ground parameters.

Stressed plants have been previously reported to accumulate certain proteins in response to the stress. In the present study, specific stress proteins were not assayed, but an idea of the total nitrogen present in the plant in response to the stress was determined, using total crude protein content of the plant. Crude protein content does not precisely specify total stress protein; it only measures the nitrogen content (on the bases of all protein containing amino acids, and thus, nitrogen) present in plants. It however gives insight on the total amount of protein present. Total crude protein content of the plants increased with increased concentration of heavy metals. This indicates the increased protein synthesis in the plant in response to increasing stress, probably to combat stress imposed by the heavy metal. Report showed that metal-treated plants show increased amount of hormones and antioxidants (Emamverdian *et al.,* 2015). Upon exposure to abiotic stress, plants develop free radicals, which can disrupt normal functioning of the metabolism and cellular mechanisms of the plant and in attempts to remediate this, stress proteins are synthesized to trigger regulation. Heavy metals accumulation of plants led to improved synthesis of crude protein and other components in plants. These include proteins, abscorbic acid, phytochelatins, abscisic acid, and some antoxidants. One major hormone that is synthesized is Proline. It is an essential hormone that helps plants in extending stem length, adaptation, recovery and signaling when dealing with stress in plants (Emamverdian *et al*., 2015) and is accumulated in plants under Cd, Pb, Zn and Al stress, as cd impose decrease in water potential.

CONCLUSION

Heavy metal-impacted plants exhibit a number of morphological changes including stunted growth, leaf distortions, and reduced photosynthetic activities probably due to chlorotic and necrotic lesions on leaf surfaces. These were a common feature exhibited by *Chromolaena odorata* in this study. Minimal differences in morphology between metal-impacted and control plants is a clear indication that the test plant might be a tolerant plant and possibly a phytoaccumulator of heavy metals, and thus, can be used for phytoremediation of metal-contaminated soils.

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REFERENCES

Agunbiade, F.O. and Fawale, A.T. 2009. Use of Siam weed biomarker in assessing heavy metal contaminations in traffic and solid waste polluted areas. *International Journal of Environmental Science and Technology* 6 (2): 267–276,

Aiyesanmi, A. F., Okoronkwo, A. E., and Sunday, O. M., 2012. Lead accumulation in siam weed (*Chromolaena odorata*) node weed (*Syndrella nodiflora*): potential phytoremediators. *Archives of Applied Science Research* 4(1): 360-371

Anoliefo, G.O., and Vwioko, D.E. 2001. Tolerance of *Chromolaena odorata* (L) K & R. grown in soil-contaminated with spent lubricating oil. *Journal of Tropical Biosciences,* 1: 20-24.

Anoliefo GO, Vwioko DE, Mpamah P. 2003. Regeneration of *Chromolaena odorata* (L.) K. & R. in crude oil polluted soil: A possible phytoremediating agent. *Benin Science Digest* 1: 9–16

Anoliefo, G.O., Ikhajiagbe, B., Berena A.T., and R.E. Okoro. 2010. Bioremediation of crude oil-polluted soil by using *Vernonia amagdylina* and manure. *International Research Journal of Biotechnology,* 1(4): 37- 43.

AOAC 2005. *Methods of Analysis*. Association of Official Analytical Chemists, Washington D. C. 326p.

Boyer, J. S. 1986. Plant productivity and the environment. *Science* 218: 443-448

Brahim, S., Joke, D., Ann, C., Jean-Paul, N.., Marjo, T., Arja, T., Sirpa, K., Frank, V. B., Karen, S., and Jaco, V. 2010. Leaf proteome responses of *arabidopsis thaliana* exposed to mild cadmium stress. *Journal of Plant*

Physiology 167 (4): 247-254.

Bray, E. A., Bailey-Serres, J., and Wewtinlnyk, E., 2000. Responses to abiotic stress In: Gruissem, W., Buchaama, B., Jones, R., (eds) *Biochemistry and Molecular Biology of Plants*. American Society of Plants Physiologists, Rockville, MD. pp 1158-1249

Brown, J. E., Khorder. H., Hider, R. C. and Rile-Evans C. A. 1998. Structural dependence of Flvonoids interactions with Cu^{2+} ions: implications for their antioxidant properties. *Journal of Biochemistry* 330: 173-178

Cunningham, S. D. and Berti, W. R. 1993. Remediation of contaminated soils with green plants: An overview. *In Vitro Cellular & Developmental Biology Plant.* 29P (4): 207-212.

Emamverdian, A., Ding, Y., Mokhberdoran, F. and Xie, Y. 2015. Heavy metal stress and some mechanism of plant defense response. *Scienti*fic *World Journal.* [Available online; ncbi.nml.nih.gov/pubmed/25688377] Accessed 25/08/2015

Ene-Obong, H.N. and Carnovale, E. 1992. A comparison of the proximate, mineral and amino acid composition of some known and lesser known legumes in Nigeria. *Food Chemistry* 43: 169-175

Fargasova, A. 2004. Toxicity comparison of some possible toxic metals (Cd, Cu, Pb, Se, Zn) on young seedlings of *Sinapis alba* L. *Plant, Soil and Environment* 50 (1): 33 – 38.

Foy, C.D., Chaney, R.L., and White, M. 2005. The physiology of metal toxicity in plants. *Ann. Rev. Plant Physiol. J.* 29: 511-566.

Galadima, A., Garba, Z. N., Leke, L., Almustapha, M. N. and Adam, I. K. 2011. Domestic water pollution among local communities in Nigeria - causes and consequences. *European Journal of Scientific Research* 52(4): 592-603.

Guo, J., Dai, X., Xu, W., and Ma, M. 2008. Over expressing GSHI and AsPCSI simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana. Chemosphere* 72:1020–1026

Ikhajiagbe, B. and Anoliefo, G.O. 2012. Comparative phytotoxicity study of a waste engine oil-polluted soil exposed to different intervals of natural attenuation. *Asian Journal of Experimental Biological Sciences* 3(2)*:* 335-343.

Ikhajiagbe B, Anoliefo GO, Oshomoh E O, Uwaila A. Ogedegbe and Nosakhare Airhienbuwa, 2012. Changes in polyaromatic hydrocarbon content of a waste engine oil polluted soil exposed to pH adjustments. *Annual Review and Research in Biology* 2(3): 66-82

Kaji, T., Suzuki, M., Yamamoto, C., Mishima, A., Sakamoto, M., and Kozuka, H. 1995. Severe damage of cultured vascular endothelial cell monolayer after simultaneous exposure to cadmium and lead. *Arch. Environ. Contam. Toxicol.* 28:168–172

Kanazawa, K., Higuchi, K., Nishizawa, N.K., Fushiya, S., Chino, M., and Mori, S. 1994. Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretion under Fe-deficient conditions, in Gramineae. *J. Exp. Bot.* 45:1903-1906.

Kraner, R. M., Minibayeva, F. V., Beckett, P. R., and Seal, E. C. 2010. What is stress? Concepts, defintions and application in seed science.*New Phytologist* 188: 655-662

Larsen, P.B., Degenhardt, J., Tai, C-Y., Stenzler, L.M., Howell, S.H., and Kochian, L.V. 1998. Aluminum-resistant Arabidopsis mutants that exhibit altered patterns of aluminum accumulation and organic acid release from roots. *Plant Physiol.* 117: 19-27.

Lasat, M.M., Pence, N.S., Garvin, D.F., Ebbs, S.D., and Kochian, L.V. 2000. Molecular physiology of zinc transport in the Zn hyperaccumulator Thlaspi caerulescens. *J. Exp. Botany* 51: 71-79.

Mohanpuria, P., Rana, N.K., Yadav, S.K. 2007. Cadmium induced oxidative stress influence on glutathione metabolic genes of Camella sinensis (L.). O Kuntze. *Environ. Toxicol.* 22:368–374

Moya, J., Ros, R., and Picazo, I. 1993. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in Rice plants. *J. Photo. Res.* 36:

75-80.

Nasir, R., Khan, M., Masab, M., Rehman, H.U., Rauf, N.U., Shahab, S.,Ameer, N., Sajed, M., Ullah, M.,Fafeeq, M., and Shaheen, Z. 2015. Accumulation of Heavy Metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water Collected from Tanda Dam Kohat. *J. Pharm. Sci. & Res.* 7(3): 89-97.

Ogedegbe, A. U., Ikhajiagbe, B., and Anoliefo, G.O. 2013. Growth response of *Alternanthera brasiliana* (L.) Kuntze in a waste engine oil-polluted soil. *Journal of Emerging Trends in Engineering and Applied Sciences* 4(2): 322-327

Okonokhua, B.O; Ikhajiagbe, B. and Anoliefo, G.O. 2007. The effects of spent engine oil on soil properties and growth of maize (*Zea mays* L.). *Journal of Applied Science and Environmental Management* 11(3): 147 – 152.

Paivoke, H. 1983. The short term effect of zinc on growth anatomy and acid phosphate activity of pea seedlings. *Ann. Bot.* 20:307–309

Paradelo, R., Moldes, A. B., Rodriguez, M. and Barra, M. T. 2008. Relationship between heavy metals and phytotoxicity in composts. *Ciec. Teccnol. Aliment* 6(2): 143-151.

Pellet, M.D., Grunes, D.L. and Kochian, L.V. 1995. Organic acid exudation as an aluminum tolerance mechanism in maize (*Zea mays* L.). *Planta* 196: 788-795.

Sagardoy, R, Morales, F., Lopez-Millan, A.-F., Abadia, A. and Abadia, J. 2009. Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics. *Plant Biology* 11(3): 339 – 350.

Sanita di Toppi, L. and Gabbrielli, R. 1999. Response to cadmium in higher plants. *Environ. Exp. Bot.* 41:105–130

SSSA 1971. *Instrumental Methods for Analysis of Soil and Plant Tissue.* Soil Science Society of America, Corporated, Wisconsin, U.S.A. Pp27-32.

Vassilev, A. and Yordanov, I. 1997. Reductive analysis of factors limiting growth of cadmium treated plants. *Bulg. J. Plant Physiol.* 23(3-4): 114-133.

Vidali, M. 2001. Bioremediation: An overview. *Pure Applied Chemistry.* 73: 1163-1172.

Wojcik, M. and Tukiendorf, A. 2004. Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana. Plant Growth Regul.* 44:71–80

Yadav, S. K. 2010. Heavy metal toxicity on plants: an overview on the note of gluthathione and phytochelatins in heavy metal the tolerance of plants. *South African Journal of Botany* 76:167-179

Yadal, S. and Jyoti, S. 2014. Phytoremediation of cadmium toxicity by Brassica. Spp: a review (2014). *International Journal of Biology and Biological science level* 3(5): 047-052.

Yruela, I. 2009. Copper in plants: acquisition, transport and interactions. *Functional Plant Biology* 36(5): 409 – 430.