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Phytoremediation Potential of Four Heavy Metals in Soil by *Chromolaena odorata* (L.) King & Robinson at the Phytotoxicity Screening Benchmarks

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Abstract

The remediative capacity of *Chromolaena odorata* with respect to heavy metal induced growth-stress was investigated. This was with a view to providing information on the adaptive mechanisms adopted by the test plant during the phytoremediation of selected heavy metals such as copper, manganese, zinc and cadmium. Stems of *C. odorata* were planted in soils polluted with Mn, Zn, Cd, and Cu at the ecological screening benchmark (ESB) of 50 mg/kg (Mn and Zn), 4 mg/kg (Cd) and 100 mg/kg (Cu) of metal per kilogram of soil, in varying concentrations of 0 ESB (control), 1 ESB, 3 ESB and 5 ESB. After the exposure of *C. odorata* to heavy metals for 3 months, results showed that the plant accumulated more Zn in the roots than in both leaves and stems put together in the present study. This, points to Zn exclusion. Accumulation of metal occurred generally in the intermediate and younger leaves; the older leaves were senesced. The totality of heavy metals (HM) accumulated by plant at each time of observation was always below phytotoxicity benchmark. This suggests HM avoidance, or perhaps one of several reasons for plant survival. At all times, it was observed that the totality of HM accumulated in all the plant parts put together was always below ESB value at each time.

Keywords: *Chromolaena odorata*, heavy metal, remediation, phytotoxicity, phytoscreening

Introduction

Most industrial processes generate toxic substances into the environment; some of these are heavy metals. To ensure environmental safety and likely sustainability, best practices are usually adopted to minimize the release of these substances. However, when such substances get into the environment, the urgent need is to remove them before accumulation becomes significant and uncontrollable. High levels of these elements can become harmful to organisms. Some other metals including cadmium (Cd), and arsenic (As) are not necessarily important components of metabolism, and as such are major threats to both plants and animals. Metals display cytotoxicity and genotoxicity in both animal and plant levels (Ciriaková 2009). Characteristics of heavy metals ranging from ductility, malleability, conductivity, cation stability, and ligand specificity make them sometimes difficult to remove from the environment. Therefore whenever a plant species is identified to have capacities to remediate elevated concentrations of heavy metals in the environment, emphases are usually on how to either maximize its remediation abilities. This is first made possible by understanding its mechanisms for remediation as well as growth and biochemical responses when exposed to metals of differing concentrations.

Of the harmful effects of heavy metals on plants, they still require some of these elements, though in very small quantities (Hall 2002). Any alteration in the amount required

for each metal can result to an inhibition of growth in plants (Hall 2002).

Although plants can cope with heavy metal stress via a change in cell; the growth stages of plants determines their responses to heavy metals. Maksymiec and Baszynski (1996) reported the resistance of beans (dicotyledonous plants) and alfalfa to heavy metals at the early stages of growth. However, the toxic actions of elevated concentrations of heavy metals in older plants are much more noticeable. As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly. Some plant species like *Peperomia pellucida, Acanthospermum hispidum, Eleusine indica,* and *Chromolaena odorata* have however been reported to subsist even in heavy metal-polluted soils (Anoliefo et al. 2006, 2008).

A study on the remediative capacity of *Chromolaena odorata* with respect to heavy metal induced stress was investigated. The aim was to expound the adaptive mechanisms adopted by *C. odorata* during the phytoremediation of selected heavy metals such as copper, manganese, zinc and cadmium. Researchers have found that when heavy metals are present in high concentrations in the soil they disrupt physiological functions and cause morphological deficiencies in plants, yet some plants still thrive with these disruptions (Cataldo and Wildung 1978, Anoliefo et al. 2006, 2008, Ciriaková 2009). On this basis, it was therefore important for this research to identify possible remediating capacity of the plant and possible mechanisms adopted by the plant.

Materials and Methods

Description of the study area

The experiment was conducted in the Screen House of the Department of Plant Biology and Biotechnology botanical garden, University of Benin, Nigeria, between August 2017 and February 2018. The site was predominated by annual weeds (especially grasses) before it was cleared for use.

Preparation of experimental plot

The marked plot beside the botanical garden was cleared to bare ground and all debris removed. The plot was eventually over-laid with polyethylene materials to ensure that there were no eventual percolations of heavy metals through the material into the underlying soil. Paint buckets measuring 25 cm diameter and 36 cm long were purchased and arranged in the plot.

Soil collection and pretreatment

Soil used in this study was collected from ten (10) randomly selected spots in the botanical garden. The choice of the spot was such that no experiment had been conducted in this plot; particularly those related to the use of any form of soil enhancers like fertilizers or contaminants like heavy metals. A sample of this pooled soil was sent to the laboratory for physicochemical analysis prior to use in the experiment. The results of the analysis have been presented in Table 1. The soil was eventually brought from the botanic garden and sun-dried to constant weight, after which 20 kg of the constant sun-dried soil were measured.

Metal acquisition

Metals used for this research were manganese, cadmium, copper and zinc. These metals were required in their chloride forms, e.g., manganese chloride ($MnCl_2$), cadmium chloride ($CdCl_2$), copper chloride ($CuCl_2$), and zinc chloride ($ZnCl_2$). Distilled water (3.38 litres) was used to dissolve the metals measured for each of the 20 kg soil. This measurement of water was obtained initially as the water-holding capacity of the soil. The experiment was carried out in such a way that the buckets were contaminated at 3 concentrations on the basis of their ecological screening benchmarks (1 ESB, 3 ESB and 5 ESB). The ESB of manganese, cadmium, copper, and zinc were 50, 4, 100, and 50 mg/kg, respectively (Efroymson et al. 1997). These were in three replicates for each concentration; this amounted to 12 replicates

for each concentration with a control, amounting to 48 buckets in total. These buckets were allowed attenuation for 3 weeks, after which the treatment plants were introduced. Water holding capacity of the soil used in the experiment was observed to be 190.3 ml/kg of soil. This meant that for the 20 kg of soil required for the experiment, 190 multiplied by 20 kg of soil was roughly 3.8 litres of water per experimental bucket. This quantity of water was used to dissolve metals measured by aid of a sensitive balance. The 3.8 litres of metal-dissolved water was utilized in moistening the soils in each designated experimental bucket.

Treatment preparations

The experiment was done such that the buckets contained each of the four selected metals at three concentrations on the basis of their ecological screening benchmarks as earlier stated. Each of the contaminated buckets amounted to three replicates and these were provided for four separate experimental groups, amounting to a total of twelve replications. The control experiment was the garden soil with no form of heavy metal treatment added.

Analysis of physicochemical parameters

Physical and chemical properties of soil were determined prior to contamination with heavy metals following standard procedures. Total organic carbon (TOC) was determined according to the methods outlined by Nelson and Sommers (1982). For the determination of soil pH and soil conductivity, 20 g of air-dried soil were sieved and 20 ml of distilled water was added to it and allowed to stand for 30 minutes. The mixture was stirred occasionally with a glass rod. Soil pH was determined by using a pH meter (Model PHS-3C), and the soil conductivity read through a conductivity meter (Model DDS-307). For determination of exchangeable bases, 5 g air-dried soil was weighed into a 5 g plastic bottle. Another 100 ml of neutral 1 M ammonium acetate was added, and the mixture was mechanically shaken for 30 minutes and thereafter filtered into a 100 ml volumetric flask through Whatman filter paper No 42. This was made up with the acetate to the mark. Na (589-nm wavelength) and K (766.5 nm wavelength) were determined via flame photometry, and then Ca and Mg were determined by atomic absorption spectrophotometry (AAS Model Perkin Elmer). Heavy metal contents in samples of soil as well as those of plant tissues were determined by atomic absorption spectrophotometry (Model Perkin Elmer) following the methods of AOAC (2005) and El-Sharabasy and Ibrahim (2010). Accordingly, Cd, Cu, and Zn were determined at wavelengths of 228.8, 224.8, and 213.9 nm, respectively and at detection limits of 0.007, 0.027 and 0.008 µg/kg. For heavy metal assessment of the plant and soil samples, each sample was air dried for 72 hrs and then oven dried at 105 °C for 2 hrs. It was ground to fine powder, and 1 g of it was measured. 10 ml of freshly prepared mixture of HNO_3/HCl (3:1) was measured and added to the 1 g sample in a boiling tube and heated slowly for about 1 hr. The clear digest was diluted with about 20 ml distilled water and filtered into a 100 ml standard flask using Whatman filter paper 110 nm. The filtrate was made up to the mark and analyzed in the AAS. In order to check for reliability of results, calibration of the machine was done by preparing standard solutions at 0, 0.5, 1, 1.5, 2, 2.5, 5, 10, and 15 ppm of the elements to be determined. These were used to obtain calibration curves to help ascertain the accuracy of the results. A blank (digested reagents without the sample) determination of the elements was also carried out to check for any interference. The different elements were then determined by extracting 2 ml of the digested sample into the FAAS when the calibration of the AAS machine was completed. The results were checked against the detection limit of the machine. Residual heavy metal constituents of plant tissues (root, stem and leaves of exposed plants) were determined after 6 months.

In order to determine the concentration of unavailable HM in the study, concentration of loosely bound metals herein otherwise referred to as available HM was determined using the methods of Minkina et al. (2018). The difference between total metal content earlier measured and the content of loosely bound metal was referred to in this study as unavailable metal.

Statistical Analyses

The statistical tools used were ANOVA, T-test, and principal cluster analyses where necessary. The software used was the SPSS-23®.

Results and Discussion

Results showed a significant reduction in heavy metal (HM) contents. HM availability status from only ionic forms of HM to both ionic and organic forms of the respective HMs identified both in plant parts as well as in residual soil concentrations. There was twice as much HM in the ionic form in the leaves of the test plant, irrespective of the HM, as there was HM in the unavailable (organic) form. Mn concentrations in plant leaves were 8.48–9.12 mg/kg, whereas the organic forms of the HM were 2.73–3.21 mg/kg (Table 2). Of the HMs accumulated in plant leaves, Mn was the most accumulated, followed by Cu; Cd was the least. Similarly, accumulation of organic forms of the respective HM was lower than the respective ionic forms. The roots accumulated more HM than in leaves or stems, particularly in Zn-exposed plants (10.18–12.76 mg/kg).

Table 3 shows HM distribution in plants leaves at 6 months after exposure to experimental conditions. There was evidence of organic forms of HM in the soil at the lower plant partitions. Dead leaf tissues were reported for Mn, Cd, Zn, and Cu-affected plant leaves. Significant HM accumulations were reported in both intermediate and young plant leaves, with Cd accumulations been reported as the least (0.07–0.09 mg/kg). Apart from Zn accumulations, there were more ionic forms of Mn, Cd, and Cu in plant leaves than the organic forms.

The residual HM composition of soil after 6 months has been presented in Table 4. Cd at 1ESB and 3ESB levels were removed from soil (conc. < 0.001 mg/kg) by the test plant, thus indicating > 99% remediation efficiencies. Generally, results showed that the remediation efficiency of average total HM decreased gradually as the initial HM concentrations in soil increased. Remediation efficiency of Mn in Mn+1ESB- and Mn+3ESB-polluted soils was 95.4% and 67.5% respectively. Similarly, Cd remediation efficiency in Cd+5ESB-polluted soil was 72.4%, compared to > 99.0% in Mn+1ESB-polluted soil (Table 4).

Table 5 shows metal accumulation indices for heavy metals in older *Chromolaena odorata* plants. Given that SR ratio > 1, a plant is labeled a hyperaccumulator, else a HM excluder (Rotkittikhun et al. 2006). Results showed a shoot to root ratio of > 1.00 for Mn, Cd, and Cu accumulations, indicating that these HMs were hyperaccumulated (Harrison and Chirgawi 1989, Rotkittikhun et al. 2006). However, Zn may have been excluded at all the metal concentrations in soil, presenting an SR ratio of < 1.00.

In the present study, phytoconcentration efficiency (%) was used to indicate the capacity for HM deposition unto plant shoot (Ikhajiagbe et al. 2018). The metals had better phytoconcentration efficiencies at lower soil HM concentrations. Sequestration coefficients show capacity for HM sequestration. The results showed that the plants in Cd-polluted soils showed higher capacity for HM sequestration. It was reported in the study that *C. odorata* significantly accumulated HM in both ionic and non-ionic forms of the metals in different plant parts; the availability of HM in organic forms actually implied that the plant has the capacity for HM sequestration. As reported by Cataldo and Wildung (1978), heavy metals which enter the plant via the roots can either be stored or actively transported to the shoots. A good example is the active transport of Cd in the roots of oat via the tonoplast as a free ion through a Cd/H^+ antiport (Dierberg et al. 1987).

The final control with respect to availability of metals to plants is the selective absorption from the soil solution by the root. However, metals might be bound to certain exchange sites on the root and not really taken up (Efroymson et al. 1997). They may go into the root passively in inorganic or organic complexes alongside the flow of water or by metabolically regulated membrane transport systems meant to absorb nutrients which the metal pollutant mimics. The present study further posits that the test plant displayed capabilities for HM remediation via one of 3 mechanisms; phytoextraction (HM concentrated in leaves and stem), phytostabilization (HM accumulated in organic forms) and rhizoexclusion (HM concentrated more in the roots than the shoots). Phytoextraction is the most suitable approach to remediate heavy metals from the soil without altering the soil's structure and productiveness. It is best suited for the remediation of highly polluted areas since the plant absorbs and concentrates harmful metals (Rulkens et al. 1998). In order for plants to absorb toxic metals, they must be mobile in the soil solution. One of the ways plants achieve this is via the secretion of phytosidophores into the rhizosphere in order to bind and solubilize metals that are bound to the soil (Robinson 1986). The metals are first bound by the cell wall of the root, after which uptake across plasma membrane is mediated by high affinity binding sites located intracellularly (Hirsch et al. 1998). *Chromolaena odorata* accumulated more Zn in the roots than in both leaves and stems put together in the present study. In this study, *C. odorata* significantly accumulated HM in both ionic and non-ionic forms of the metals in different plant parts with the availability of HM in organic forms actually implying that the plant has the capacity for HM sequestration. The present study further posits that the test plant displayed capabilities for HM remediation via one of the 3 mechanisms; phytoextraction (HM concentrated in leaves and stem), phytostabilization (HM accumulated in organic forms) and rhizoexclusion (HM concentrated more in the roots than the shoots). Accumulation of metals occurred generally in the intermediate and younger leaves; the older leaves were senesced.

Table 1: Physical and chemical properties of soil before contamination. These are background mean concentrations (n = 5) (mean ± S.E.M)

Category	Parameters	Mean value (n = 5)
Physical and chemical parameters	pH	5.97 ± 0.67
	Electric conductivity (µs/cm)	301.21 ± 23.01
	Total organic carbon (%)	0.49 ± 0.09
	Total nitrogen (%)	4.18 ± 1.06
	Exchangeable acidity (meq/100 g)	0.22 ± 0.08
	Na (meq/100 g)	10.90 ± 2.11
	K (meq/100 g)	1.48 ± 0.62
	Ca (meq/100 g)	14.32 ± 3.10
	Mg (meq/100 g)	12.01 ± 3.22
	NO_2^- (mg/kg)	164.34 ± 23.03
	NO_3^- (mg/kg)	286.16 ± 18.16
Soil texture	Clay (%)	5.43 ± 0.88
	Silt (%)	7.36 ± 1.74
	Sand (%)	84.81 ± 12.12
Heavy metals	Fe (mg/kg)	1011.92 ± 73.38
	Cd (mg/kg)	< 0.001
	Mn (mg/kg)	17.03 ± 3.22
	Cu (mg/kg)	3.93 ± 0.01
	Zn (mg/kg)	30.12 ± 3.06

Table 2: Heavy metal accumulation (mg/kg) in plant parts at 3 months after sowing

	Leaves		Stem		Root	
	Av. HM	Org-HM	Av. HM	Org-HM	Av. HM	Org-HM
Conc. of Mn (mg/kg)						
Mn+1ESB	9.12 ± 0.28	3.21 ± 1.45	6.11 ± 3.45	3.95 ± 2.67	11.78 ± 5.60	3.97 ± 2.21
Mn+3ESB	8.89 ± 0.84	3.02 ± 1.48	7.25 ± 4.50	2.94 ± 1.78	12.76 ± 7.88	4.78 ± 2.98
Mn+5ESB	8.48 ± 0.90	2.73 ± 1.03	6.35 ± 3.36	2.55 ± 1.43	10.18 ± 6.40	2.86 ± 2.77
Conc. of Cd (mg/kg)						
Cd+1ESB	0.35 ± 0.25	0.18 ± 0.01	0.42 ± 0.05	0.32 ± 0.08	0.67 ± 0.01	0.43 ± 0.01
Cd+3ESB	0.49 ± 0.01	0.24 ± 0.02	0.73 ± 0.09	0.61 ± 0.11	0.75 ± 0.45	0.53 ± 0.06
Cd+5ESB	0.47 ± 0.05	0.30 ± 0.5	0.75 ± 0.11	0.49 ± 0.15	0.68 ± 0.61	0.47 ± 0.80
Conc. of Cu (mg/kg)						
Cu+1ESB	3.86 ± 0.79	1.64 ± 0.50	4.62 ± 2.21	2.93 ± 1.79	5.23 ± 4.60	1.97 ± 1.00
Cu+3ESB	4.05 ± 0.08	1.84 ± 0.90	2.88 ± 1.56	1.55 ± 0.85	4.42 ± 2.40	1.25 ± 1.99
Cu+5ESB	3.96 ± 0.94	1.74 ± 0.61	3.76 ± 1.99	2.09 ± 1.11	4.64 ± 2.32	1.43 ± 0.95
Conc. of Zn (mg/kg)						
Zn+1ESB	2.27 ± 0.10	0.61 ± 0.98	3.12 ± 2.15	1.27 ± 0.99	6.54 ± 1.46	2.47 ± 1.34
Zn+3ESB	2.06 ± 0.86	0.43 ± 0.88	2.84 ± 1.45	1.02 ± 0.97	9.64 ± 5.40	5.23 ± 2.31
Zn+5ESB	2.91 ± 0.46	0.62 ± 0.71	3.01 ± 2.64	1.16 ± 0.85	7.32 ± 2.73	3.49 ± 1.87
Significance	0.02	0.02	0.04	0.04	0.02	<0.01
LSD (0.05)	0.94	0.98	2.21	1.24	3.2	1.33

Concentrations are presented as Mean ± S.E.M; Av. HM means ionic form of HM; Org-HM organic form of HM

Table 3: Heavy metal distribution in leaves of older plants after exposure to treatment at 6 months after sowing (partitioning taken into consideration)

Conc. of contaminant in soil	Av. HM	Org-HM	Av. HM	Org-HM	Av. HM	Org-HM	Av. HM	Org-HM
	Lower partition		Intermediate leaves (n = 3)		Young leaves (n = 3)		Total	
	(mg/kg)		(mg/kg)		(mg/kg)		(mg/kg)	
Mn+1ESB	ND	ND	1.736 ± 0.01	0.512± 0.001	1.482 ± 0.10	0.461 ± 0.224	3.218 ± 0.24	0.971 ± 0.88
Mn+3ESB	ND	ND	1.932 ± 0.20	0.706 ± 0.22	1.322 ± 0.02	0.299 ± 0.48	3.252 ± 0.48	1.005 ± 0.41
Mn+5ESB	ND	ND	2.154 ± 0.34	0.928 ± 0.26	2.236 ± 0.34	1.215 ± 0.56	4.390 ± 0.56	2.143 ± 0.33
Cd+1ESB	ND	ND	0.072 ± 0.01	0.028 ± 0.01	0.040 ± 0.66	0.017 ± 0.82	0.112 ± 0.88	0.045 ± 0.42
Cd+3ESB	ND	ND	0.084 ± 0.01	0.041 ± 0.001	0.066 ± 0.72	0.043 ± 0.32	0.151 ± 0.32	0.083 ± 0.11
Cd+5ESB	ND	ND	0.092 ± 0.10	0.048 ± 0.10	0.059 ± 0.84	0.036 ± 0.22	0.151 ± 0.22	0.084 ± 0.24
Cu+1ESB	ND	ND	1.482 ± 0.77	0.749 ± 0.22	1.123 ± 1.21	0.661 ± 0.55	2.605 ± 0.55	1.411 ± 0.11
Cu+3ESB	ND	ND	2.016 ± 0.58	1.283 ± 0.33	1.130 ± 1.00	0.668 ± 0.42	3.146 ± 0.42	1.952 ± 0.34
Cu+5ESB	ND	ND	1.439 ± 0.34	0.706 ± 0.88	1.236 ± 1.06	0.774 ± 0.32	2.675 ± 0.32	1.481 ± 0.35
Zn+1ESB	ND	ND	1.386 ± 0.39	1.238 ± 0.74	0.954 ± 0.82	0.836 ± 0.66	2.343 ± 0.66	2.074 ± 0.42
Zn+3ESB	ND	ND	1.278 ± 0.21	1.164 ± 0.48	1.234 ± 0.01	1.112 ± 0.01	2.508 ± 0.01	2.276 ± 0.43
Zn+5ESB	ND	ND	1.013 ± 0.11	0.865 ± 0.33	0.873 ± 0.10	0.755 ± 0.81	1.886 ± 0.80	1.623 ± 0.88
Significance	NA	NA	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
LSD (0.05)	NA	NA	1.20	0.45	0.9	0.5	1.84	2.1

Av. HM represents available ionic form of metal concentration where as those with the Org- prefix represents the unavailable metals; Concentrations are presented as mean ± SEM of 3 replicates; LSD = least significant difference at p < 0.05; NA = not applicable; ND = not determined

Table 4: Residual metal concentrations of soil at 6 months after sowing of test plant in metal-contaminated soils

	Soil metal concentration (mg/kg)		Total average (mg/kg)	Plant-assisted remediation efficiency (%)
	Av. HM (n = 3)	Org-HM (n = 3)		
Mn+1ESB	0.312 ± 0.015	1.982 ± 0.02	2.294 ± 0.50	95.4 ± 0.11
Mn+3ESB	18.922 ± 0.88	13.621 ± 6.27	32.543 ± 4.39	67.5 ± 6.40
Mn+5ESB	42.054 ± 0.92	26.827 ± 2.36	68.881 ± 2.85	72.4 ± 3.30
Cd+1ESB	<0.001 ± 0.01	<0.001 ± 0.00	<0.001 ± 0.00	> 99.0 ± 5.4
Cd+3ESB	<0.001 ± 0.01	1.954 ± 0.08	1.954 ± 0.08	83.7 ± 4.88
Cd+5ESB	1.8259 ± 0.04	3.756 ± 1.08	5.5819 ± 1.00	72.4 ± 4.50
Cu+1ESB	18.360 ± 7.71	8.534 ± 0.50	26.894 ± 6.16	73.1 ± 4.02
Cu+3ESB	67.772 ± 0.62	42.424 ± 4.50	110.196 ± 0.33	63.3 ± 15.84
Cu+5ESB	94.36 ± 0.68	58.439 ± 1.44	152.799 ± 4.84	69.4 ± 12.48
Zn+1ESB	6.453 ± 5.46	4.967 ± 0.94	11.420 ± 2.45	77.2 ± 3.84
Zn+3ESB	29.646 ± 0.61	20.564 ± 0.96	50.210 ± 0.36	66.5 ± 12.13
Zn+5ESB	46.435 ± 1.68	30.234 ± 0.40	76.669 ± 9.50	69.3 ± 11.12
Significance	P < 0.01	P < 0.01	P < 0.01	P < 0.01
LSD (0.05)	4.92	4.72	17.19	14.06

Concentrations presented as Mean ± S.E.M; Av.HM means ionic form of HM; Org- HM = organic form of HM; Remediation efficiency is determined as a factor of average total HM; LSD = Least significant difference at p < 0.05.

Table 5: Metal accumulation indices for heavy metals in older *Chromolaena odorata* plants

Concentration	Shoot to Root ratio	Phytoconcentration efficiency (%)	Sequestration coefficient (%)
Mn+1ESB	1.42	76.28	26.03
Mn+3ESB	1.25	26.43	25.36
Mn+5ESB	1.54	13.26	24.35
Cd+1ESB	1.15	4.74	33.96
Cd+3ESB	1.61	2.23	32.88
Cd+5ESB	1.74	1.26	38.96
Cu+1ESB	1.81	20.25	29.82
Cu+3ESB	1.82	5.33	31.24
Cu+5ESB	1.90	3.52	30.53
Zn+1ESB	0.81	32.56	21.18
Zn+3ESB	0.42	14.15	17.27
Zn+5ESB	0.71	7.40	17.56

Conclusion

From this study, *C. odorata* significantly accumulated HM in both ionic and non-ionic forms of the metal in different plant parts with the availability of HM in organic forms actually implying that the plant has the capacity for HM sequestration. The present study further posits that the test plant displayed capabilities for HM remediation via one of 3 mechanisms; phytoextraction (HM concentrated in leaves and stem), phytostabilization (HM accumulated in organic forms) and rhizoexclusion (HM concentrated more in the roots than the shoots). Accumulation of metal occurred generally in the intermediate and younger leaves; the older leaves were senesced. The totality of HM accumulated by plant at each time of observation was always below phytotoxicity benchmark (Efroymson et al. 1997). This suggests HM avoidance, or perhaps one of several reasons for plant survival. At all times, it was observed that the totality of HM accumulated in all plant parts put together was always below ESB value at each time. Perhaps, this may be one of several reasons why the plant did not show heavy signs of growth suppression. However, the mechanism by which this occurred, or the mechanism by which the plant did not accumulate enough metals above phytotoxicity benchmarks is unknown thus, the need for further study is suggested.

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