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Differential Morphological Growth Responses of *Chromolaena odorata* under Heavy Metal Influence

Gloria Omoregie[1,3*] and Beckley Ikhajiagbe[2,3,4]

[1]*Federal University of Petroleum Resources Effurun, Department of Environmental Management and Toxicology, Nigeria.*
[2]*University of Benin, Department of Plant Biology and Biotechnology, Nigeria*
[3]*University of Benin, Environmental Biotechnology and Sustainability Research Group, Department of Plant Biology and Biotechnology, Nigeria*
[4]*University of Benin, Applied Environmental Biosciences and Public Health Research Group, Department of Microbiology, Nigeria*

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Abstract

The differential growth responses and morphological changes exhibited by *Chromolaena odorata* in heavy metal-polluted soil were investigated. This was with a view to providing information on the test plant growth adaptation potential during heavy-metal exposure. Fresh- stem cuttings of *C. odorata* were propagated in Manganese, Cadmium, Copper, Lead and Zinc-polluted soils. Heavy-metal (HMs) concentration in soil was based on the respective ecological screening value/benchmark for each metal. The ESV values for the HMs were 50, 4, 100, 50 and 50 mg/kg respectively. Heavy-metal concentrations for the study were 1, 3 and 5 times their respective ESV. The control experiment consisted of plants grown in metal-free soil. The plants were observed for eight months. There was a compensatory growth response of the test plant under heavy-metal exposure. Although growth suppression in some plant parameters occurred as a result of heavy-metal exposure, there were enhanced growth responses with regards to some other parameters. Although, plant height was reduced from 132.2 cm in the control plants to 88.21 – 111.4 cm in the heavy metal-exposed plants, there was > 25% increase in the number of leaves of heavy metal-exposed plants compared with the control plants. The plant showed compensatory growth responses necessitated by heavy metals. Generally, significant foliar chlorosis and necrosis, leaf curling and folding, leaf loss/senescence, refoliation capacity and unique patterns of display of foliar scorching were reported. Despite general growth suppression, the capacity of the plant to subsist at heavy metal concentrations five times higher than benchmark values was noted; an indication that the test plant might be tolerant to heavy metals, and can be used for phytoremediation studies.

Keywords: Heavy metals, *Chromolaena odorata*, growth response, morphology, phytoremediation.

1. Introduction

Studies have proved that heavy-metal pollution is gradually becoming an environmental disaster because of the alarming increase of metal presence in our environment. Apparently it devastates the ecosystems and deleteriously affects the health of plants, animals and human beings. Consequently, it is very essential to control metal pollution in our environments and remediate metal-polluted sites. The plant employed for the study was *Chromolaena odorata*; it is an invasive species that belongs to the family *Asteraceae*; its common name is Siam weed genus *Chromolaena* and the species is *odorata*.

Heavy metals (HMs) at polluted environment are introduced by anthropogenic activities such as metal- mine dumps, dumping of high metal contaminants in unsuitably secured landfills, addition of fertilizers to soils, inorganic materials, animal manures, compost, pesticides, bio-solids and atmospheric settlement (Basta et al., 2005; Khan et al., 2008; Cirlakov´a, 2009; Zhang et al., 2010).

In soil, toxic levels of HM can impede normal plant activities, disrupt metabolic processes (Hall, 2002), deterring functional groups of significant cellular molecules (Hossain et al., 2012). Heavy-metal toxicity can interrupt the functionality of pigments or enzymes which are essential biomolecules (Ali et al., 2013), adversely affecting the nature of the cytoplasmic membrane (Farid et al., 2013). This results in the suppression of vital events in plants such as respiration, photosynthesis as well as enzymatic activities (Hossain et al., 2012). Results from several analyses have confirmed that heavy metals can affect productivity level in the soil as well as the ecological geochemistry. In addition, heavy metals are constantly added to soils with the wild rate of Industrialization (Okoye, 1991).

Several processes are accessible for heavy-metal removal from the environment. Examples are chemical, physical and biological processes. Biological processes seem to be more reliable because they are environmentally friendly and retain the quality of environments during and after the remediation process. Moreover, biological methods are cheaper than physical and chemical techniques. Biological remediation of metal uses microorganisms to remediate metal-polluted environments. Plants (phytoremediation) can also do likewise (Petarca and Cioni 2011). An example of such plants is *Chromolaena odorata*. According to Omoregie et al. (2020) *Chromolaena odorata* significantly accumulated heavy

Corresponding author email: omoregie.gloria@fupre.edu.ng

metals in different plant parts (leaves, stem, and roots) with the availability of heavy metal in organic forms, implying that the plant had the capacity for heavy-metal sequestration. In phytoremediation processes, plants are grown to decrease the concentrations of heavy metals in polluted soils to recommendable levels in the environment (Henry, 2000; Zheng et al., 2002). Heavy metals can be transported to the above ground plant parts which are eventually removed when these plants are harvested from the site with traditional remediation practices (Blaylock et al., 1997). However, for a successful phytoremediation, it is necessary to have plants capable of generating high biomass and simultaneously accumulating large levels of pollutants from the soil (Tu et al., 2000; Shen et al., 1997). Previous studies have shown *C. odorata* to be capable of generating high biomass with concomitant capacities for bioaccumulation of large concentrations of heavy metals from the soil (Uyi et al., 2014; Ikhajiagbe, 2016; Ikhajiagbe and Akendolor, 2016; Anoliefo et al., 2017; Omoregie and Ikhajiagbe, 2019).

Chromolaena odorata or siam weed belongs to the Asteraceae family, and it is a recurrent and invasive shrub that is extensively distributed, and is still increasing its range. Being a shrub, sometimes it behaves like a lianascent plant. It has simple leaves lacking stipules and is opposite-decussate. The leaves are rhomboid-ovate to ovate with an acute apex and a cuneate base. Petiole is usually between 1 and 3 cm long, the lamina is between 5 and 14 cm long and between 2.5 and 8 cm wide. The plant grows in a wide range of soil pH (Gareeb, 2007). It spreads and colonizes lands in a short time while resulting in more remediating capacity (Taiwo et al., 2011). Moreover, it flourishes in disturbed areas with suitable light and temperature (Gareeb, 2007).

Different environmental stress conditions are always reflected in plant morphology. The level of concentration of heavy metals in the environment puts fort adverse effect on plants (Gorlach and Gambus, 1992; Obata and Umebayashi, 1997). Plant adaptation to stressed environmental conditions and high-contamination concentrations is a usual occurrence reflected in plant morphology (Nkongolo et al., 2008; Kraner et al., 2010). These changes are critical for understanding plant behavior as a necessary input during the selectivity of plant for specific metal-remediation strategies. The objective of this study is to investigate the plant-growth performance and any morphological changes expressed by *Chromolaena odorata* in heavy-metal polluted soil.

2. Materials and Methods

The study was carried out in a well-ventilated Screen House in the Department of Plant Biology and Biotechnology, University of Benin (Ugbowo Campus), Nigeria. The soils used in the study were top-layered garden soils (0 – 10 cm) previously collected from ten random spots in the Departmental Botanic Garden, and were pooled together to form a composite soil sample and were sun-dried to constant weight. The samples of soil used (an ultisol) were taken to the Lab for the determination of selected physical and chemical characteristics prior to use according to methods described by Bray and Kurtz (1945 a and b); Nelson and Sommers (1982); APHA (1985); Osuji and Nwoye (2007).

In order to determine soil pH, 20ml distilled water was added to 20g of the sieved soil sample and was allowed to stand for thirty minutes. The mixture was intermittently stirred with a glass rod. The pH was determined by inserting the pH meter (Model 238 PHS-3C), and the soil conductivity was read through a hand 239 held conductivity meter (HI 70039P, Hanna Instruments). For determination of total organic carbon (TOC), 2.5 ml of 1N $K_2Cr_2O_7$ solution was added to 0.5g of soil sample in a conical flask and was swirled gently to disperse the sample in the solution. Thereafter, 5 ml concentrated H_2SO_4 was added rapidly, into the flask and swirled gently until the sample and reagents were mixed and were finally swirled vigorously for about a minute. The flask was allowed to stand in a fume cupboard for thirty minutes. Five to ten (5 to 10) drops of the indicator were added and the solution titrated with 0.5N $FeSO_4$ to maroon colour. A blank determination was carried out to standardize the dichromate (Nelson and Sommers, 1982; Osuji and Nwoye, 2007). TOC was calculated as follows:

$$\text{TOC (\%)} = \frac{(\text{meq K2Cr2O7} - \text{meq Fe SO4}) \times 0.003 \times 100 \times 1.3}{\text{Weight of sample (g)}}$$

Where,

meq $K_2Cr_2O_7$ = 1N X 2.5 ml

meq $FeSO_4$ = 0.5 N X Volume of titrant in ml

0.03 = Milliequivalent weight of carbon

1.30 = Correction factor

During the mechanical analysis (particle size distribution) of the soil sample, 100g of soil was weighed out and placed in a one-liter-shaking bottle. To this, 50 ml Calgon solution, 3 ml of N sodium hydroxide and 200 ml of water were added. The mixture was, then, properly shaken for three hours and transferred quantitatively to the mechanical analysis cylinder. The volume was made up to the first (1130 ml.) mark with water. The cylinder was shaken by inverting it a few times, and was later placed on the bench and the time read. After 4.5 min., the hydrometer was inserted, and at after five minutes, the scale was read. Whenever there was more froth on the surface of the liquid, one or two drops of amyl alcohol were added before inserting the hydrometer. The hydrometer was then withdrawn, and the process was repeated five hours later. With 100 g of the soil sample being used for the determination, the results gave directly the percentage silt and clay (1st reading) and clay (2nd reading).

Nitrogen in the soil was determined by Kjeldahl digestion, and the resulting ammonium ion was measured calorimetrically. Elements such as iron and manganese, which may interfere in the alkaline medium during colorimetric determination, were first complexed with sodium potassium tartrate. The Ammonia was determined calorimetrically as the indophenol blue complex by reaction with alkaline sodium phenate and sodium hypochlorite.

For the determination of exchangeable acidity, 50 ml of the M KCI was added to 5 g of soil in a 150 ml plastic bottle, and was then shaken mechanically for one hour. This was filtered using Whatman filter paper No.1 into a 250 ml conical flask. Thereafter three drops of the indicator

were then added and titrated against the 0.05 M NaOH until the colorless solution turned to pink. The pink color was neutralized with 0.05 M HCI. Then 10 ml of 1 M NaF was added to restore the pink color. The set up was titrated against 0.05 m HCI until colorless.

Calculations:

Exchange Acidity = 0.05 m x Titre x 20 meq/l00 g soil

$$Al = \frac{0.05\ m \times 20 \times 26.98\ meq/l00\ g\ soil}{8.99}$$

Exchangeable bases (Na, K and Ca) were determined by weighing 5g soil into a plastic bottle. Thereafter, 100 ml of neutral 1 M ammonium acetate was added, and the mixture was shaken mechanically for thirty minutes. and filtered using No. 42 Whatman filter paper, into a 100 ml volumetric flask. This was made up with the acetate to the mark. Then, Na (589-nm wavelength) and K (766.5nm wavelength) were determined with a Flame Photometer, and Ca and Mg by the Atomic Absorption Spectrophotometer.

2.1 Soil Pollution with Metal Samples

Twenty (20) kg of the soil was filled into experimental buckets previously prepared for the study. The soils were polluted with Mn, Cd, Pb, Cu, and Zn in their respective chloride forms. The reported ecological screening values (ESV) of the metals were: 50, 4, 50, 100, and 50 mg/kg respectively (Efroymson et al., 1997). The metals were therefore divided into three concentrations each on the basis of their reported ESV as once, thrice and five times their respective ESVs. Successful soil pollution with the respective metal concentrations was achieved by dissolving each measured quantity in distilled water used to properly irrigate the soil up to its water holding capacity, which was earlier determined to be 190.3 ml/kg soil. The control soil was not amended with metal. The experimental buckets that held the soils were not perforated in order to ensure that the metals did not percolate further into the soil.

2.2 Propagation of Chromolaena odorata

Equal-sized stem cuttings of *C. odarata* (2.0 - 2.3 cm thick; 30 cm long) were obtained from a fallow land near the University of Benin Senior Staff Quarters, Ugbowo, and were propagated vertically into the soil at an angle of 45 degrees, with 15 cm of stem cutting buried into the soil.

2.2.1 Husbandry and Analyses

The plants in experimental bags were constantly weeded, and carefully irrigated every other day with 500 ml of water (pH 6.6 – 6.8) especially during dry and hot days. Care was taken to ensure that loamy soil moisture level was adequate for plant development, following procedures laid out by USDA (1998).

2.2.2 Plant parameters considered

Above- and Below-ground Parameters

The test plants were observed throughout the experiment for some plant-growth parameters including mean plant height which was measured by a tape rule, the number of leaves per plant, leaf length, petiole length, internode, as well as stem girth which was determined by a Vernier caliper. Leaf area was determined using an android application (Leaf-IT) following the methods prescribed by Julian et al. (2017). The below-ground parameters determined were the number of primary root branches and length of main root.

2.2.3 Morphological Stress Responses

Morphological measurements of the plant in response to the experimental conditions were recorded on a periodic basis. Those measurements include the color observations, color, shape, form or the appearance of the leaves and the stem of the plant as well as the positioning of the flowers and nodes. Care was also taken to ensure that the progression of chlorosis was recorded. In this case, whenever chlorosis was noted, the leaf was immediately tagged so that chlorotic progress would be followed up till the leaf became entirely chlorotic. The progress of chlorosis measured in hours was provided. The same procedure was followed to describe the progress of necrosis. The rate at which the plant lost its leaves as well as which portion of the plant lost any leaf was also taken into note. Thereupon, every plant was divided into three major plant-shoot partitions as described by Ikhajiagbe and Guobadia (2016).

According to Ikhajiagbe and Guobadia (2016), the leaves which are located in the plant part from the soil level measuring up till 45 cm above soil level, were said to be in the plant's upper partition (or the old leaves), whereas those within the plant part measuring 45 cm downwards from the apical meristem were said to be in the plant's upper partition (or young leaves). The middle partition in this study referred to the plant part in between the upper and lower partitions. Leaves herein were referred to as the intermediate leaves. Haven followed this demarcation based on partitioning, changes with regard to necrosis, senescence, leaf browning, or any other physical observation, was made and reported on time basis.

Care was taken to ensure that the total number of leaves that folded, curled or showed signs of foraging were taken into consideration and as such were counted and presented as the percentage of the total number of leaves that appeared in the plant at any given time. These were therefore presented in the result sections as the percentage of folded leaves, the percentage of curled leaves as well as the severity of leaf foraging. For the latter, a severity score chart was developed. For a severity score of 5, it implied that more than 20% of plant total leaves showed signs of foraging. A severity score of 4 meant that 10 – 20% of plant total leaves showed signs of foraging. A 5 – 10% occurrence equaled a severity score of 3. A severity score of 2 meant that 1 – 5% of the plant total leaves showed signs of foraging. When there were no signs of foraging, a severity score of zero sufficed.

2.2.4 Refoliation

In the present study, because it was possible that when plants lost their leaves, they refoliated, the researcher decided to tag defoliated nodes and followed up with the time in hours taken for every defoliated node to eventually regrow. Therefore, refoliation percentage or recovery percentage as herein referred to was presented in the result section to mean the time in hours taken between defoliation of a node and the re-emergence of a bud at the node position.

2.2.5 Progression of Necrosis and Chlorosis

In order to appreciate the presentation of chlorotic and necrotic symptoms in treated and control plants, the total number of leaves, that showed both chlorotic and necrotic symptoms, was counted on a weekly basis. And then presented as graphs.

2.2.6 Statistical Analyses

A complete randomized experimental design was adopted for the study. A single-factor analysis of variances (ANOVA) was used to analyse data having assumed the homogeneity of the entire experimental plot when soils were pooled before use. Least significant differences (LSD) were used to separate treatment means at a 95% confidence limit. Statistical analyses were performed using the SPSS® version 23 as well as the PAST® version 2.17c according to Hammer et al. (2001) where necessary.

3. Results

The physical and chemical properties of the soil before pollution are presented in Table 1.

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

Parameters	Mean (n = 5)
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/100g)	0.22 ± 0.08
Na (meq/100g)	10.90 ± 2.11
K (meq/100g)	1.48 ± 0.62
Ca (meq/100g)	14.32 ± 3.10
Mg (meq/100g)	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
Pb (mg/kg)	0.03 ± 0.01
Cu (mg/kg)	3.93 ± 0.01
Zn (mg/kg)	30.12 ± 3.06

The impact of heavy metals on the selected above-ground parameters of *Chromolaena odorata* after six months of planting and the application of treatment showed highly significant changes in the average plant height as well as the number of plant root ($P<0.01$) (Table 2). In regard to plant height, there were significant decreases compared to the control. Although plant height in the control was 142.2 cm in the Mn-exposed plant, it ranged from 84.44 to110.30 cm. Similarly, in the Cu-exposed plants, the highest plant height was obtained in Cu +1ESV (ht = 98.43cm). There was a significant increase in the number of leaves per plant in the metal-exposed plants when compared to the control. As for leaf length, there were no significant changes in the number of leaves per plant in both control and metal-exposed plants, although these changes differed among metals. For example, increases were recorded for Cu-exposed plants but not recorded for Pb, Zn, and Cd-exposed plants. Leaf length ranged from 5.21 to 7.85 cm. There were significant changes in leaf area when compared between control and metal-exposed plants. Leaf area in the control plant was 18.87cm. This significantly increased in Mn-exposed plants (21.49 to 23.96 cm^2) as well as in Zn-exposed plant (18.89 to 23.32cm^2). The changes in Pb Cu and Cd-exposed plant were minimal ($P>0.05$). There were significant changes in the average number of primary branches by plant as affected by Cd. However, changes were only minimal ($P>0.05$) in pants exposed to Pb, Mn, and Cu.

As presented in Table 3, the percentage of foliar folding ranged from 3.8 – 9.4 % ($p>0.05$) in both experiments and control plants in the upper-plant partition. Although foliar folding was not reported in the control plants, as well as in Mn and Pb-exposed plants, over 5 % of the leaves of the Cu and Zn-exposed plants showed signs of foliar folding. These folded leaves eventually recovered from this folding characteristic. There was no evidence of foliar folding at the lower-plant portions. Leaves of the upper partition were more curled (< 53 %) than those of the intermediate partition (< 36 %). Leaves of the Cd-exposed plants were more curled (16.6 – 36.3 %); Mn-exposed leaves (11.5 – 25 %). The results eventually showed that leaves in the upper partition of both control and HM-exposed plants lost less than 10 % of foliage to the foragers. The least foraging occurred in the older plant partition.

Generally, all plants showed signs of curling. However, most of the curled cleaves in both control and metal-affected plants recovered from the curling anomaly, herein referred to as recovery time. Plants therefore differed significantly in their recovery period (Figure 1). All curled leaves recovered from the anomaly within twelve days, which was not statistically different from the control implying that these heavy metals may not have accelerated foliar curling. However, Cd-exposed leaves showed a significant delay ($p < 0.05$) (< 19 days).

Some morphological disposition of the leaves of the metal-exposed plants showed somewhat similar manifestations of metal toxicity. In the Mn-exposed leaves, these appeared first on the leaf's left margin and on the right (but not always prominent), and it progressed throughout the entire leaf (Figure 2). Cu-exposed leaves predominantly showed foliar scorching beginning from the lower right margin of the leaf (Figure 3), whereas in the Pb-exposed leaf, scorching progressed mainly from the upper right margin of the leaf (Figure 4). Scorching in the Zn-exposed leaves progressed from the left leaf margin (Figure 5), whereas for the Cd-exposed leaves, burning was mainly restricted to foliar tips (Figure 6).

Table 2. Effects of treatment on above-ground parameters of the test plant after 6 months of sowing.

Treatment	Average Plant height(cm)	*No. of Leaves/ Plant	Leaf length (cm)	Leaf Area (cm^2)	Petiole length (cm)	Internodal distance (cm)	*No. of primary nodes	*Average No of pry. Branches	Stem girth (mm)
Control	142.2±1.00	252±10	5.71±1.2	18.87±0.51	2.01±0.11	8.02±0.03	4±0	7±0	23.01±1.00
Mn+1ESV	84.44±1.10	261±10	5.52±1.5	21.49±0.82	1.82±1.00	7.92±1.59	4±1	5±0	23.02±1.00
Mn+3ESV	100.1±1.40	240±11	5.45±0.5	21.96±2.40	2.03±1.00	9.31±0.53	3±1	7±1	12.03±1.01
Mn+5ESV	110.3±1.05	256±11	7.33±0.64	23.96±3.68	1.84±1.13	7.73±1.28	2±1	6±1	20.04±0.12
Cd+1ESV	112.2±1.00	295±14	6.85±0.7	21.92±1.45	2.03±1.1	6.42±0.72	3±0	8±1	18.01±0.99
Cd+3ESV	103.1±3.50	303±13	5.55±0.11	18.45±1.34	2.04±.056	7.82±0.31	4±0	8±1	22.51±0.56
Cd+5ESV	110.3±1.20	274±14	5.82±0.51	17.71±1.11	1.82±0.62	7.61±0.67	3±1	9±0	23.02±0.44
Pb+1ESV	104.2±0.5	268±5	5.05±0.68	16.74±1.35	1.82±0.77	7.04±0.06	3±0	5±0	17.01±1.0
Pb+3ESV	79.12±1.52	359±6	6.65±0.71	17.93±2.40	1.81±0.56	7.52±0.90	3±1	6±0	17.04±1.00
Pb+5ESV	85.33±1.95	285±5	5.55±0.4	24.80±5.20	2.01±0.88	9.01±0.09	4±1	5±0	20.03±2.12
Cu+1ESV	98.43±1.01	319±8	7.85±0.70	20.41±3.4	2.53±0.10	8.02±0.34	4±1	5±1	17.03±0.97
Cu+3ESV	93.22±0.61	297±11	6.75±0.41	19.88±4.2	2.51±0.94	6.53±0.36	3±1	6±0	18.51±0.34
Cu+5ESV	87.43±0.88	335±12	6.65±0.18	16.21±3.4	2.04±0.33	7.21±0.93	4±1	5±1	15.02±0.12
Zn+1ESV	88.21±0.98	298±13	5.12±0.46	18.89±1.56	1.83±0.92	7.54±0.06	4±0	6±1	20.03±0.01
Zn+3ESV	111.4±0.91	248±11	5.15±0.45	23.32±1.30	1.84±0.12	8.93±0.41	3±0	4±0	20.04±1.00
Zn+5ESV	96.22±0.10	313±17	5.21±0.50	23.32±1.32	2.51±0.23	9.72±0.55	3±1	6±1	21.02±0.55
p-values	0.000	0.000	0.091	0.042	0.075	0.001	0.021	0.018	0.031
LSD(0.05)	16.3	11.7	2.66	2.46	1.66	2.00	1.6	1.67	2.60

**Expressed to the nearest integer. **Results expressed as Mean ± SEM 0.05*

Table 3. Foliar morphological changes during 10 – 25-week periods of *Chromolaena odorata* growth.

Conc. of contaminant in soil	Percentage of folded leaves			Percentage of curled leaves			Severity of foraging signs on leaves (see severity score key)		
	UP	IP	OP	UP	IP	OP	UP	IP	OP
Control	4.0±0.2	0.0±0.0	0.00	0.79±1.3	0.0±0.0	0.0±0.0	3.22±1.20	3.61±0.38	0.21±0.05
Mn+1ESV	0.38±0.1	0.0±0.0	0.00	0.77±3.1	1.15±1.9	0.0±0.0	3.83±1.21	3.22±1.50	0.12±0.55
Mn+3ESV	0.83±0.7	0.0±0.0	0.00	4.17.±13.4	2.50±5.5	0.06±0.04	0.63±0.11	1.24±1.00	0.02±1.51
Mn+5ESV	0.44±0.2	0.0±0.0	0.00	0.88±4.2	0.44±1.1	0.0±0.0	0.94±0.11	0.24±0.01	0.23±0.04
Cd+1ESV	0.52±1.4	0.0±0.0	0.00	5.42±2.4	1.69±1.6	0.08±0.07	0.11±0.21	0.73±0.94	0.14±0.02
Cd+3ESV	0.66±0.9	0.0±0.0	0.00	1.65±9.1	0.66±2.5	0.0±0.0	3.23±2.20	3.61±0.88	0.12±0.02
Cd+5ESV	0.72±0.4	0.36±0.9	0.00	7.30±6.5	0.36±7.1	0.0±0.0	3.42±2.1	2.51±1.56	0.03±0.21
Pb+1ESV	0.75±1.2	0.0±0.0	0.00	0.75±5.2	0.61±0.3	0.0±0.0	3.44±2.54	3.34±2.10	0.44±0.00
Pb+3ESV	0.84±0.2	0.0±0.0	0.00	2.79±9.8	1.11 ±2.8	0.0±0.0	3.04±1.11	3.64±0.31	0.12±0.00
Pb+5ESV	0.85±0.2	0.0±0.0	0.00	2.98±12.2	1.28±4.3	0.0±0.0	3.23±1.23	2.12±0.57	0.01±0.00
Cu+1ESV	0.94±0.3	0.0±0.0	0.00	1.25±9.9	0.31±0.3	0.0±0.0	3.31±2.11	3.14±2.10	0.01±0.00
Cu+3ESV	0.63±0.1	0.51±1.8	0.00	0.40±4.2	0.64±1.1	0.0±0.0	2.34±1.56	2.42±0.11	0.01±0.00
Cu+5ESV	0.00±0.0	0.60±3.1	0.00	00.0±0.0	0.84±0.0	0.0±0.0	0.82±0.01	0.03±0.00	0.13±0.01
Zn+1ESV	1.01±0.8	0.75±0.8	0.00	2.013±9.3	0.0±0.0	0.0±0.0	3.23±2.1	3.22±1.48	0.24±0.12
Zn+3ESV	0.81±0.8	0.90±0.6	0.00	2.82±12.3	0.0±0.0	0.0±0.0	2.81±1.45	2.51±0.80	0.02±0.01
Zn+5ESV	1.60±9.0	0.64±1.2	0.00	0.96±5.4	0.0±0.0	0.0±0.0	2.74±1.3	2.12±1.50	0.03±0.01
Significance	P>0.05	P<0.05	NA	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05
(LSD (0.05)	8.3	0.42	NA	11.7	9.8	NA	0.67	0.51	0.11

** Mean ± S.EM 0.05*

Key:
Foliar partitions - UP Upper foliar partition, IP intermediate partition, OP older leaf partition

Percentage of plant total leaves showing signs of foraging	Severity score	Percentage of plant total leaves showing signs of foraging	Severity score
>20%	5	1 – 5%	2
10 - 20 %	4	<1%	1
5 - 10%	3	None	0

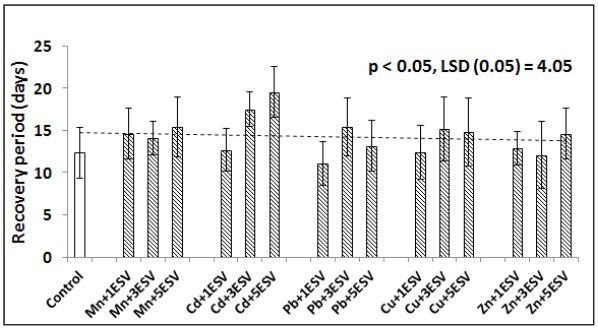

Figure 1. Period required for foliar recovery from curled symptoms.

Figure 2. Presentation of foliar scorching in Mn-exposed leaves.

Figure 3. Cu-exposed leaves showing scorching symptoms.

Figure 4. Pb-exposed leaves presenting position of scorching in leaves.

Figure 5. Scorching in Zn-exposed leaves.

Figure 5. Scorching in Zn-exposed leaves.

Table 4 presents the percentage of chlorosis and necrosis after three months of exposure to heavy metals. The results show that chlorosis and necrosis were significantly elevated by the HM presence in the soil. This incident was mostly pronounced in the plant's lower partition, where older leaves usually existed (13 – 30 %), compared to both intermediate (3 – 14 %) and upper-plant partitions (1 – 6 %) respectively in that order. There was neither chlorosis nor necrosis at the upper-plant partition. The results also showed that the occurrence of chlorosis in the control plant were on the sixth day (Table 4), whereas necrosis occurred two days later. Generally, as observed, foliar necrosis usually occurred two days following the confirmation of foliar chlorosis (Table 4).

Table 4. Percentage of Chlorosis and necrosis 3 months after sowing according to foliar partitions. (Mean ± SEM).

Conc. of contaminant in soil	Plant partition/Chlorisis (%)			Total necrotic leaves (%)	Chlorosis period (days)	Necrosis period (days)
	Lower	Middle	Upper			
Control	9.09±0.15	3.64±0.55	0±0.00	11.92±4.72	6.32±2.22	8.12±٤,٠٠
Mn+1ESV	19.61±4.42	7.84±1.45	3.92±1.16	29.86±2.89	4.14±1.09	6.03±3.91
Mn+3ESV	31.25±2.16	10.42±4.27	4.17±1.23	43.18±5.27	4.21±1.34	6.41±3.88
Mn+5ESV	23.07±5.31	9.62±0.60	1.92±0.08	35.02±8.67	4.33±1.54	6.04±3.92
Cd+1ESV	16.67±2.88	6.67±2.89	0±0.92	24.11±5.16	4.43±1.87	5.22±2.27
Cd+3ESV	29.09±8.67	5.45±0.77	3.64±1.29	37.63±3.15	4.22±1.67	5.31±2.91
Cd+5ESV	17.31±2.67	3.85±0.75	1.92±0.98	24.16±9.87	3.02±0.85	5.42±2.99
Pb+1ESV	21.82±3.15	7.27±2.36	0.00±0.00	27.11±8.67	4.33±1.99	6.13±3.92
Pb+3ESV	22.95±5.10	14.75±0.45	0.00±0.00	32.88±1.21	4.14±1.21	5.22±3.27
Pb+5ESV	20.41±3.09	10.26±5.51	6.12±2.08	33.02±5.59	4.43±1.71	6.31±3.81
Cu+1ESV	24.56±5.05	8.77±3.64	0±0.00	31.66±0.57	5.11±2.00	7.41±4.02
Cu+3ESV	22.60±6.05	7.55±2.50	3.77±2.21	33.03±1.39	4.24±1.77	6.04±4.22
Cu+5ESV	16.18±2.04	2.94±0.91	2.94±1.19	21.67±0.10	5.03±3.21	7.12±4.19
Zn+1ESV	15.87±1.50	3.17±0.29	1.59±0.54	19.18±1.09	5.01±3.49	6.33±4.71
Zn+3ESV	13.79±4.32	8.62±1.51	3.45±2.12	24.93±5.21	3.24±2.10	5.24±4.53
Zn+5ESV	13.73±4.41	9.83±0.29	1.96±1.23	26.81±5.07	4.11±2.72	6.03±5.23
Significance	0.018	0.024	0.015	0.000	0.029	0.038
(LSD(0.05)	7.2	6.12	3.24	8.63	1.17	2.26

Table 4. Percentage of Chlorosis and necrosis 3 months after sowing according to foliar partitions. (Mean ± SEM).

After six months, the presentation of chlorosis and necrosis was similar to that presented after three months (Table 4). Although the occurrence of both chlorosis and necrosis was enhanced by HM- pollution, the percentage of total plant leaves reported to be chlorotic during the sixth month was less compared to the results obtained during the third month (Tables 4 and 5); whereas 2.19 % of the lower-partition control-plant leaves were chlorotic, 3.39 – 12.56 % of the same leaves were chlorotic when-exposed to HM. The most prominent region of necrosis occurrence is reported in Table 5. In the control, this was observed as burning patches, which expanded to occupy the entire leaf. However, for the Cd-exposed leaves, necrosis was observed to spread from leaf tips, and it was accompanied by similar burning patches. In the Zn-exposed plants, foliar necrosis was mainly restricted to the left leaf margin.

Figure 7 presents the percentage of leaf senescence six months after sowing. A significantly higher percentage of the HM-exposed leaves detached from the parent plant, compared to the control. Cd-exposed leaves were the most senesced (4.63 – 16.74 %), compared to 5.45 % reported for the control leaves (Figure 7).

The time taken for refoliation of defoliated nodes is provided in Figure 8. It took ninety-three hours for the control plants to produce leaves at defoliated nodes. However, this refoliation time delayed when the plant was-exposed to Cd (123 hrs.).

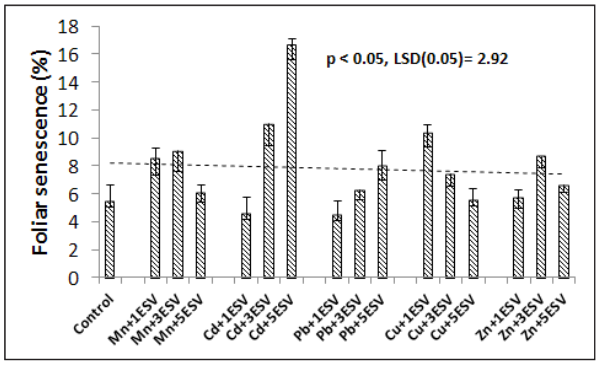

Figure 7. Percentage of leaf senescence 6 months after sowing.

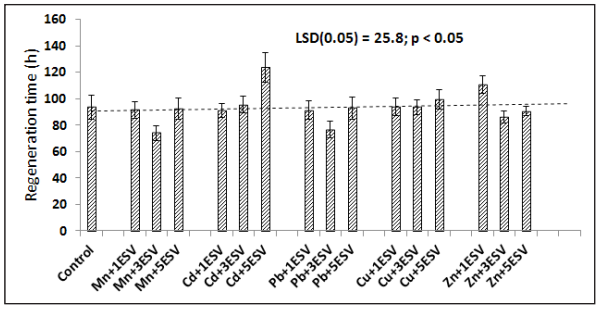

Figure 8. Time taken for the replacement of a new leaf from a defoliated node during the post-flowering period of plant development.

Table 5. Percentage of chlorosis and necrosis 6 months after sowing according to foliar partitions. (Mean ± S.E.M).

Conc. of contaminant in soil	Plant partition/Chlorosis			Total necrotic leaves (%)	Chlorosis period (days)	Necrosis period (days)	Most prominent region of necrosis
	Lower	Middle	Upper				
Control	2.19±0.32	3.17±0.29	2.35±0.60	8.71±1.45	7.05±1.23	9.12±3.23	Burning patches, expands to occupy entire leaf
Mn+1ESV	5.81±1.40	3.75±1.29	1.53±1.29	11.09±2.60	5.25±2.12	7.32±0.22	Similar to control, marginal and patches
Mn+3ESV	8.03±1.47	4.02±0.03	0.81±0.53	11.68±3.40	5.05±2.12	7.33±3.34	
Mn+5ESV	8.31±0.31	5.54±0.93	1.33±0.54	14.02±1.45	5.23±2.34	8.12±4.34	
Cd+1ESV	7.79±0.41	5.08±0.13	2.03±0.05	13.96±3.60	4.31±1.23	5.44±0.23	Spread from leaf tips accompanied with similar burning patches
Cd+3ESV	6.62±1.54	3.96±0.06	0.33±0.05	11.01±1.45	4.31±2.34	6.02±1.11	
Cd+5ESV	8.28±0.05	4.38±0.65	0.36±0.05	13.62±1.12	4.22±3.23	5.11±0.12	
Pb+1ESV	8.58±1.72	5.62±1.07	2.24±0.41	15.69±4.60	4.14±1.20	6.32±3.23	Progression from the right leaf margin inwards
Pb+3ESV	8.62±1.45	2.51±0.88	1.00±0.06	11.92±0.56	5.41±3.65	7.22±0.09	
Pb+5ESV	11.49±1.69	8.09±0.15	3.41±0.71	22.98±7.89	4.01±2.00	6.33±2.76	
Cu+1ESV	3.39±0.90	2.19±0.32	0.94±0.91	8.12±0.33	4.22±1.02	7.24±1.23	Progression from the right leaf margin inwards
Cu+3ESV	6.07±1.12	4.86±1.48	0.81±0.05	11.42±2.11	6.03±4.77	8.22±2.12	
Cu+5ESV	8.28±0.46	2.69±1.19	0.33±0.05	10.63±1.90	5.44±2.4	8.11±0.12	
Zn+1ESV	4.69±0.10	4.36±0.62	1.00±0.73	9.06±1.99	4.22±1.90	6.41±0.34	Majorly restricted to the left leaf margin
Zn+3ESV	8.87±0.90	4.34±0.58	0.81±1.12	13.99±2.45	4.11±2.16	7.22±2.23	
Zn+5ESV	12.56±0.05	2.88±1.54	1.64±0.11	16.42.03±3.35	4.23±2.47	7.32±4.33	
Significance	0.031	0.027	0.018	0.029	0.033	0.028	NA
LSD(0.05)	1.63	1.43	1.10	3.06	0.55	2.57	NA

** Results above are for chlorosis, but as observed herein, all chlorotic leaves turned necrotic*

Table 6 shows bivariate correlation among the selected morphological characteristics of the test plant. Plant leaf number negatively correlated with the percentage of foliar chlorosis/necrosis; implying therefore that a reduction in plant leaves may have been a result of the former ($r = 0.66$, $p<0.01$). The percentage of the occurrence of chlorosis was significantly correlated with the occurrence of necrosis ($r = 1.00$, $p<0.01$); indicating that nearly every chlorotic leaf turned necrotic.

Table 6. Bivariate correlation among selected morphological characteristics of the test plant.

	PLHT	NLVS	LVA	NCR	CHL	RCV	SEN	REGT	RTBR
PLHT	1	-0.22	-0.08	-0.02	-0.02	0.017	0.172	0.129	0.406
NLVS	-0.224	1	-0.52*	-0.66**	-0.66**	0.207	-0.07	0.028	-0.1
LVA	-0.076	-0.52	1	0.469*	0.469*	0.074	0.259	-0.34	0.153
NCR	-0.015	-0.66**	0.469*	1	1	-0.22	0.085	-0.19	0.099
CHL	-0.016	-0.66**	0.469*	1**	1	-0.22	0.085	-0.19	0.099
RCV	0.0165	0.207	0.074	-0.22	-0.22	1	0.355	-0.35	0.117
SEN	0.1721	-0.07	0.259	0.085	0.085	0.355	1	-0.18	0.316
REGT	0.1289	0.028	-0.34	-0.19	-0.19	-0.35	-0.18	1	0.005
RTBR	0.4059	-0.1	0.153	0.099	0.099	0.117	0.316	0.005	1
RTLN	-0.028	0.038	-0.23	-0.27	-0.27	-0.13	0.027	0.447*	-0.09

**Correlation is significant at 0.05, **Correlation is significant at 0.01*
PLHT - Plant height, NLVS - No of leaves per plant, LVA - Leaf area, NCR - Total foliar percentage necrosis, CHL - Total foliar percentage chlorosis, RCV - Plant recovery from curling, SEN - Incident of Senescence, REGT - Foliar regeneration time, RTBR - No of pry root branches, RTLN - Root length.

Figure 9 shows that the plant characteristics including the number of leaves, chlorosis occurrence, necrosis occurrence, and leaf area were loaded on component 1, and these were basically related to the plant leaf. This implied that the plant leaf was the most disposed factor responsible for determining the effects of HM on morphology. The impact on foliar abundance was mostly associated with copper pollution. With a Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy, being a factor of 0.51 (Figure 9), and a highly significant Bartlett's Test of sphericity ($p<.0.01$), the results of the Principal component analysis (PCA) was deemed reliable. The results of cluster analyses showed a presentation of two groups; one group was predominantly Mn and Pb, and the other was predominantly Cd and Cu (Figure 10). The control was a standout in the first group with Mn and Pb as predominates. Plant responses in soils polluted with Mn at ecological screening value (ESV) was more closely related to those exposed to soils polluted with Cu at three times the ESV compared to other plants in their respective metal-exposed conditions.

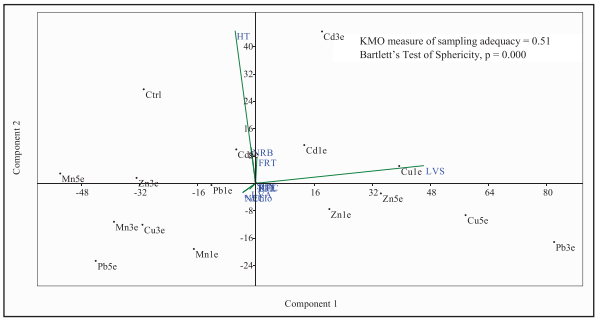

Figure 9. Principal component analysis (PCA) biplot showing association between selected morphological characteristics of the test plant (letters in blue) and their respective soil heavy-metal concentrations (letters in black).

Key:
Soil metal concentrations: Cu1e – plant response in soil polluted with Cu at 1 ESV; Cd3e – plant response in soil polluted with Cd at 3 ESV, Mn5e – plant response in soil polluted with Mn at 5 ESV, respectively and so on.
Plant morphological characteristics: HT - Plant height, NLV - No of leaves per plant, LVA - Leaf area, NEC - Total foliar percentage of necrosis, CHL - Total foliar percentage of chlorosis, REC - Plant recovery from curling, SEN - Incident of Senescence, FRT - Foliar regeneration time, NRB – No. of pry root branches, RTL - Root length.

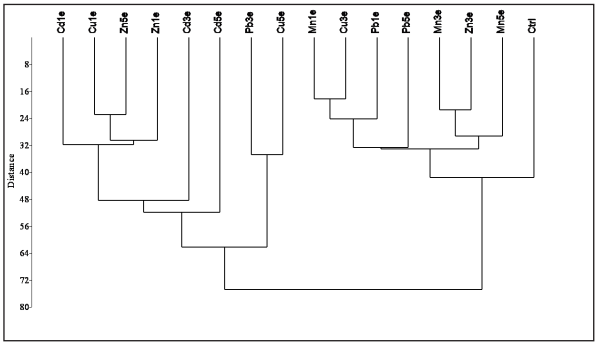

Figure 10. Dendrogram from cluster analyses of showing association among plants exposed to various heavy-metal concentrations.

Key:
Cu1e – Plant response in soil polluted with Cu at 1 ESV; Cd3e – plant response in soil polluted with Cd at 3 ESV, Mn5e – plant response in soil polluted with Mn at 5 ESV, respectively and so on.

4. Discussion

Heavy metals available for plant uptake are in soluble forms which are easily solubilized by root exudates in the soil. Even though plants need soluble heavy metals for their growth and development, extreme concentrations can become injurious to plants. Metals cannot be broken down when they exceed maximum concentrations in plants as such has an adverse effect on plants, such effect could be the inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress or growth inhibition. The toxic effects of heavy metals lead to a drop in plant growth which from time to time results in the death of plants. (Jadia and Fulekar, 2009; Schaller and Diez, 1991). Decline in the growth parameters of plants growing on heavy metal-polluted soil can be credited to the reduction in photosynthetic activities, activities of some enzymes and plant mineral nutrient (Kabata-Pendias and Pendias, 2001)

The effects of heavy-metal (HM) pollution on plant morphological features were different for each plant part; whereas there was a growth suppression in some parts (e.g. height), there was enhancement in others (e.g. leaf number). This has pointed to compensatory growth responses. In this study, growth parametric compensation refers to the ability of the test plant to enhance the development of certain parameters when other parameters have been growth-sup by the presence of the stressor provided that the parameter in question has similar contribution to plant growth and development processes. This perhaps may not be unconnected to a possible ability by the plant for metal exclusion (Hossain et al., 2009). As reported in the study, the capability for the test plant to survive in HM-polluted soil is a clear indication for tolerance, which is notably its capacity for compensatory growth in the face of HM-induced stress (Peralta-Videa et al., 2004).

The aerial parts of the plant show visual symptoms of leaf curling in response to heavy-metal pollution, cadmium toxicity also causes leaf curling and stunted growth as reported by Alloway and Ayres (1997); Fontes and Cox (1998); Moreno et al. (1999); Kabata-Pendias and Pendias (2001); Emamverdian et al. (2015). Leaf curling was reported as a prominent morphological feature in the study. It was difficult to link leaf curling with metal pollution in the upper-plant partition since it also significantly appeared in the control. However, leaf folding, and curling symptoms were HM-associated in the intermediate partition. The mechanism behind this could not be explained. It is therefore suggested that this may just be an indicative characteristic that plant biologists might use to suggest HM toxicity. Although in very minute concentrations, some heavy metals like Zinc are important mitopromotors for cell division, Pandey and Upadhyay (2010); Saha (2015) have both showed that heavy metals in high concentrations were mitodepressive, and prompted a multiplicity of chromosomal abnormalities. Consequently, the development of cell and cellular organelles becomes negatively impaired thus leading to impaired plant structure, leaf curling, and rolling in the young leaves.

From the morphological observation, it was clear that chlorosis, necrosis, and wilting are visual signs of metal toxicity (Sanitá di Troppi and Gabbrielli, 1999; Pandey and Upadhyay, 2010; Kekere et al., 2011; Ikhajiagbe and Chijioke-Osuji, 2012; Ikhajiagbe et al., 2013; Saha, 2015). Zenginand and Munzuroglu (2005); Pandey and Singh (2009); Pandey and Upadhyay (2010); Ikhajiagbe (2016); Ikhajiagbe and Ogwu (2020) reported the inhibition of chlorophyll occasioned by heavy metal accumulation in plant leaves. Pandey and Singh (2009); Saha (2015) also reported suppression in the activities of protease and RNase, which decreased photosynthetic pigments, changed chloroplast structure, and decreased enzyme activities for the assimilation of carbon(IV)oxide. Chlorosis may also arise partly from an induced iron (Fe) deficiency as hydrated Zn^{2+} and Fe^{2+} ions have similar radii (Marschner, 1986). Excess in Zn can also give rise to Mn and Cu deficiencies in plant shoots. There have been general chlorosis and necrosis in HM-exposed plants irrespective of the type of metal. Although this morphological anomaly occurred also in the control, but the magnitude of chlorosis and necrosis in the HM-exposed leaves was significant. The presentation of leaf browning was reported, but it was difficult to link this to HM toxicity, as this same anomaly was reported in the control, although with minimal differences in magnitude. Also,

the leaves which showed browning symptoms eventually recovered at some point or another. According to previous studies, it is evident that soils polluted with Pb, Zn, Mn, Cu, and Cd showed the same peculiar symptoms such as leaf chlorosis, necrotic lesions, reduction in *C. odorata* height and leaf area (Zhu and Alva, 1993; Taylor and Foy, 1985, Guo et al., 2008; Wojcik and Tukiendorf, 2004, WHO, 1992, Lee et al., 1996; Baryla et al., 2001, Gupta and Gupta, 1998; Elamin and Wilcox, 1986; Bachman and Miller, 1995, Harmens et al., 1993; Fontes and Cox, 1998).

Leaves of *C. odorata* showed common patterns of display of foliar scorching. In the control, burning patches appeared which expanded eventually to occupy the entire leaf. Although burning patches appeared all over the leaves in the Mn-exposed plant, the majority of this phenomenon, however, predominantly occurred from the left leaf margin and was accompanied by burning patches scattered on the surface of the leaf. Scorching in Zn-exposed leaves was mainly restricted to the left leaf margin. For those plants exposed to Cd pollution, the majority of leaf scorching spread from leaf tips accompanied with similar burning patches. This occurrence was similar for Cu and Pb-exposed leaves, which progressed from the left leaf margin inwards. No possible ecophysiological explanation has been attributed to this phenomenon; however, the fact that these leaves also showed diversity of presentation in leaf scorching or burning patterns implied a genetic or environmental influence. Yadav (2010) proposed that genetic manipulations of plant antioxidant systems can help plants ameliorate toxic effects of heavy metals and as such enable the plants to present a huge diversity of morphological features in response to metal toxicity. Generally, however, metal toxicity induces deficiency of essential ions causing leaf discolorations, with the upper and lower leaflets turning brown or purple before they die (Reichman, 2002; Asati et al., 2016; Van Assche and Clijsters, 1990; Meharg, 1994).

There was a significant leaf loss/senescence in HM-exposed plants compared to the control, with Cd initiating the highest foliar loss. However, the capacity for each plant to refoliate at defoliated nodes was reported; Mn-exposed plants refoliated better than others (in approx. < 75 hrs), while Cd-exposed plants were slowest to refoliate (approx. >120 hrs). Plants adjust to conditions of defoliation and the associated reduction in whole-plant photosynthetic rates by altering resource allocation patterns and reducing relative growth rates. In contrast, a transient period of modified physiological function frequently accompanies the plant defoliation followed by a recovery of steady-state plant function (Bhandal and Malik, 1988). A large decrease in the photosynthesis/transpiration ratio of the canopy (i.e., water-use efficiency) is also associated with this pattern of plant defoliation (Hopkins, 1999). Therefore, when plants begin to show quick signs of refoliation, it becomes a plus. In terms of plant refoliation capacity, information on the time, which a defoliated bud takes to refoliate, can be a very useful tool in presenting plant's survival capability with net assimilation.

5. Conclusions

Plants grown in heavy metal-polluted soil expressed several morphological changes including stunted growth, leaf distortions, chlorotic and necrotic lesions. These were common features exhibited by *C. odorata* in this study. Although a significant presentation of morphological anomalies due to exposure to elevated amounts of heavy metals has been reported in the study, the capacity of the plant to subsist has also been reported. Despite the exposure of plants to toxic levels of metals, these plants have developed a very potential mechanism to combat such adverse environmental heavy-metal toxicity problems. These capacities lie in their metabolism, physiology or in their ability for morphological adaptation. Minimal differences in morphology between metal-impacted and control plants indicated that the test plant might be a tolerant plant to heavy metals and thus, can be used for phytoremediation studies.

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