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Phytoremediation of Soil Contaminated with Lead (Pb) and Zinc (Zn) Using *Chromolaena odorata* (L.) under Greenhouse Condition

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Abstract Phytoremediation is gaining popularity worldwide for its cost-effectiveness and environmentally friendly approach to removal of heavy metals from soil. This study investigated potential of weed, *Chromolaena odorata* (L.), in remediating soil contaminated with lead (Pb) and zinc (Zn). The experiment involved growing *C. odorata* as potted plants in soil with varying concentrations (0-100 mg/kg) of Pb and Zn. The survival of the seedlings was not affected by either heavy metal. Compared to the control, there was no significant effect of Pb on number of leaves at 20-60 mg/kg but significantly increased it at 80-100 mg/kg. Zn significantly reduced number of leaves at all the concentrations applied. Pb did not significantly affect stem girth while Zn led to its significant reduction at 60-100 mg/kg. Pb had no significant effect on leaf area except at 80 mg/kg where there was an increase, while it was not significantly affected by Zn. The metals decreased root length without statistical difference from the control while number of roots was not affected. Fresh and dry weight values of plant parts were higher under contamination than the control. This was significant at 80-100 mg/kg for leaf and stem, and at 80 mg/kg for root. The plant was more tolerant to Pb than Zn in growth. There were significantly higher concentrations of metals in plant parts of those grown in metal contaminated soil than the control. *C. odorata* is a potential candidate for phytoremediation of soil contaminated with Pb and Zn, and can survive up to 100 mg/kg. **Keywords** Siam weed; Heavy metals; Soil; Pollution; Remediation

1 Introduction

The soil, recognized as a vital ecosystem, assumes critical roles in sustaining life, contributing to food production, climate regulation, and supplying raw materials essential for various human activities (Atagana, 2011; Ayesa et al., 2018). Despite its paramount importance, the integrity of the global soil environment faces formidable challenges due to escalating human activities that induce pollution (FAO, 2015a, http://www.fao.org/3/a-i4324e.pdf; FAO, 2015b, http://www.fao.org/3/a-i4965e.pdf; FAO, 2015c). Soil pollution, arising from the direct or indirect discharge of extraneous substances, poses significant threats to the delicate balance of living resources, human health, and environmental well-being (Masindi and Muedi, 2018). The cumulative impact of industrial and mining activities as well as some agricultural practices exacerbates soil pollution, with heavy metals assuming a predominant role as major culprits in this environmental challenge (EEA, 2014, https://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites/progress-inmanagement-ofcontaminated-1; Cetin, 2016; Sandeep et al., 2019).

With the rapid development of economy and society, a variety of heavy metals contaminate soil which threatens the environment and public health (Lambert et al., 2012; Kapoor et al., 2021). Many of such metals including Pb and Zn are widely distributed, and persist long-term in soil environment. Soil contamination by heavy metals leads to decreasing availability of farmland as they negatively affect plant growth as well as crop yield (Yahaghi et al., 2019; Madhu and Sadagopan, 2020; Khan et al., 2023; Rashid et al., 2023). Besides, the metals may be accumulated into edible and non-edible parts of plants. Surveys have shown that continuous consumption of concentrations of heavy metals through foodstuffs lead to large accumulations of the metals in the kidney and liver of humans causing disruption of numerous body processes, leading to cardiovascular, nervous, kidney and bone diseases (Angon et al., 2024). Specifically, taking very high doses of Zn is likely unsafe and might cause stomach pain, vomiting, and many other problems. Single doses of 10-30 grams of zinc can be fatal (Herawati,

2000). When applied to the skin: Zn is likely unsafe. Using zinc on broken skin may cause burning, stinging, itching, and tingling. Similarly, Pb consumption is associated with great risk to brain development, where irreversible damage can occur. Higher levels can damage the kidney and nervous system in both children and adults. Very high lead levels may cause seizures, unconsciousness and death (Madhu and Sadagopan, 2020).

The development and production of plants depend on several nutrients such as Mg, Cu, Mn, Zn, Fe, Ca, Mo and Ni. Some of these are micronutrients that can improve a variety of cellular processes in plants, including pigment biosynthesis, ion homeostasis, gene regulation, respiration, enzyme activity, sugar metabolism, photosynthesis, nitrogen fixation, etc., at relatively low concentrations (Tiwari and Lata, 2018). However, they can negatively impact plant growth, development and reproduction when they are accumulated at concentrations above their optimal levels (Rashid et al., 2023; Angon et al., 2024). Arsenic, cadmium, zinc and lead are among the prominent heavy metals identified as common pollutants, exerting adverse effects on the intricate interplay between soil health, plant vitality, and the overall well-being of human and animal populations (Herawati et al., 2000). Studies have shown that these heavy metals can persist in nature for more than twenty years (Kapoor and Singh, 2021), and the only solution is to remove them from soil to a permissible level for plants.

According to Madhu and Sadagopan (2020), a lot of studies have been conducted on remediation techniques for heavy metal polluted soil, including in-situ remediation techniques (surface capping, encapsulation, electrokinetic extraction, soil flushing, chemical immobilization, phytoremediation or bioremediation) and ex-situ remediation techniques (landfilling, soil washing, solidification or vitrification). Although these methods have high performance, most of them are expensive, harmful to the environment and time-consuming. Phytoremediation has been identified for cost-effectiveness and environmentally friendly approach to removal of heavy metals from soil. It emerges as a widely accepted solution, utilizing the inherent capabilities of plants to degrade or remove pollutants from the soil environment (Haq et al., 2020; Islam et al., 2024). There are advantages of using phytoremediation. It is economically feasible as it is an autotrophic system powered by solar energy; it is simple to manage; and the cost of installation and maintenance is low. Also, it is eco-friendly as it can reduce exposure of the pollutants to the people and environment particular underground water.

Chromolaena odorata (L.), commonly known as Siam weed is a fast-growing perennial, diffuse and scrambling shrub. It is an exotic weed that has become aggressively invasive in Nigeria. It forms dense stands and is a problem in agricultural land and commercial plantations causing great economic and biodiversity losses. It is found in disturbed areas, abandoned and waste lands, where there can be potentially high soil heavy metal contamination (Srirueang et al., 2022). It has been recorded to grow naturally in waste sites, hence it was hypothesised that it has some inherent ability for survival under toxic environment, which might include ability to absorb and sequester heavy metals without negative symptoms. The objective of the study was to assess the potential of *C. odorata* for phytoremediation of soil contaminated with lead (Pb) and zinc (Zn). This was hoped to contribute to the ongoing discourse surrounding sustainable and effective environmental remediation strategies.

2 Materials and Methods

2.1 Collection of soil used for planting

Samples of the soil used for planting were analysed to know its physical and chemical properties. The samples were shade-dried, passed through a 2-mm sieve, and analyzed for physical and chemical properties using standard methods of the Association of Official Analytical Chemists (AOAC, 1990).

2.2 Experimental set up

Uniform young seedlings of *C. odorata* were collected from a plantation site in Akure, Ondo State, Nigeria, and transplanted into polyethylene pots filled with top soil mixed with 0, 20, 40, 60, 80 and 100 mg ZnSO₄ or PbSO₄ to 1 kg soil (mg/kg Pb or Zn). The targeted concentrations fell within and above the permissible level for Pb (85 mg/kg) and Zn (50 mg/kg) in soil according to World Health Organization, WHO (1996). The experiment was conducted at the screen house of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. During the experiment, the screen house had an average morning



temperature and humidity of 26.50 °C-28.30 °C and 80.40%-87.60% respectively while that of afternoon was 30.60 °C-34.90 °C and 69.40%-75.40%. There was one seedling per pot with each treatment replicated five times in a completely randomized design, and it lasted for 3 months.

2.3 Data collection

Shoot length was measured using a meter rule from the soil level to the apical bud. Stem girth was determined using a digital vernier caliper (model 0-200 mm) at 10 cm point from the base of the stem. Leaf area was measured with leaf area meter (model LI-COR 300) of the first three fully expanded leaves. Leaves and roots on each plant were counted, and root length measured using a meter rule. Biomass was assessed through fresh and dry weight determinations.

2.4 Laboratory procedure and data analysis

Dried plant samples were milled into fine powder and digested with 10 ml of 20% sulfuric acid. Pb and Zn concentrations were determined using spectrophotometer according to AOAC (1990). One-way Analysis of Variance was used for statistical analysis, and Tukey HSD test was performed to separate means where there were differences using the Statistical Package for Social Sciences (SPSS) version 24.0. This was used as it offered reliable and fast answers; effective in data management; didn't require a lot of effort to use the software; and easy to use for quantitative data analysis.

3 Results

3.1 Soil used for planting

The soil used for planting had 5.60 pH, 6.19% clay, 4.29% silt, 89.7% sand, 2.89% C, 0.14% N, 9.02 mg/100 g P, 6.24 mg/100g Ca, 1.84 mg/100 g Mg, 0.34 mg/100 g Na, 0.23 mg/100 g K, 0.20 mg/100 g H, 8.86 mg/100 g CEC, 0.14 mg/kg Pb and 3.56 mg/kg of Zn.

3.2 Plant survival and growth

All plants survived the heavy metal concentrations applied, and there was no significant effect of Pb on number of leaves at 20-60 mg/kg but significantly increased it at 80-100 mg/kg compared to the control (Table 1). Number of leaves of plants grown in Zn-contaminated soil was significantly reduced at all the concentrations applied, in comparison to the control. Statistical analysis revealed that stem girth of plants grown with Pb contamination was not significantly impacted compared to the control. However, Zn contamination led to a significant reduction of stem girth at 60-100 mg/kg. Soil contamination with Pb did not significantly affect leaf area except at 80 mg/kg where an increase was recorded relative to the control. Leaf area of plants grown under Zn contamination did not, however, differ significantly from those grown in soil free of heavy metals. Pb and Zn decreased root length without statistical difference from the control while number of roots was not significantly impacted by the metals.

Growth	Heavy	Heavy metal concentration in soil (mg/kg)							
parameter	metal	0	20	40	60	80	100		
Number	Pb	47.50+9.26 ^b	37.10+3.42 ^b	40.00+1.00 ^b	50.50+3.62 ^b	107.70+17.66ª	76.20+11.85ª		
of leaves	Zn	43.70+1.47 ^a	29.70+2.98 ^b	28.00+1.31b	28.00+1.41 ^b	22.70+1.00 ^b	19.50+2.73°		
Plant	Pb	82.07+4.95 ^b	96.00+12.43 ^b	79.03+4.09b	92.85+4.17 ^{ab}	125.30+8.21ª	110.70+10.54 ^a		
height (cm)	Zn	84.16+4.84 ^b	98.00+11.32 ^b	81.03+4.09 ^b	94.74+4.06 ^{ab}	127.30+8.10 ^a	112.70+10.43ª		
Stem	Pb	14.10+1.35 ^a	12.64+2.0.37 ^a	13.22+0.57ª	15.70+0.55ª	16.00+1.94ª	18.30+1.03ª		
girth (cm)	Zn	16.30+0.72 ^a	17.00+2.06ª	17.20+1.27 ^a	11.80+0.34 ^b	10.48+0.91 ^b	10.88+0.39 ^b		
Leaf	Pb	54.50+11.12 ^b	33.10+3.37 ^b	58.00+5.46 ^b	52.00+15.29 ^b	107.00+13.52ª	87.00+11.00 ^{ab}		
area (cm ²)	Zn	49.50+3.45ª	48.70+3.41ª	55.30+1.49 ^a	48.70+4.01ª	38.00+1.60 ^{ab}	37.00+2.63 ^{ab}		
Root	Pb	39.22+14.21ª	28.70+2.76ª	26.10+4.63ª	29.50+4.63ª	34.10+4.61ª	24.40+3.51ª		
length (cm)	Zn	57.44+18.43 ^a	46.91+4.59ª	44.30+6.85ª	47.70+3.77 ^a	51.30+6.82ª	42.60+4.73ª		
Number	Pb	5.44+0.44 ^a	5.10+0.35ª	4.10+0.34 ^a	5.30+0.82 ^a	5.50+0.57ª	5.00+0.71ª		
of roots	Zn	6.79+2.23ª	$6.20 + 0.48^{a}$	5.30+0.47 ^a	6.50+1.03 ^a	6.70 ± 0.79^{a}	6.00+0.93ª		

Table 1 Effect of soil contamination with Pb and Zn on growth parameters of Chromolaena odorata

Note: Values are mean + standard error of 5 replicates. Means with the same letter(s) in superscript on the same row are not significantly different at p>0.05 (Tukey HSD Test)



3.3 Plant biomass

Values of fresh and dry weights of plant parts were higher in those exposed to Pb and Zn than the control with the highest values recorded at 80 mg/kg (Table 2). Meanwhile, statistical analysis showed that the increase was significant at 80-100 mg/kg for leaf and stem, and at 80 mg/kg only for root.

Table 2 Effect of soil contamination with Pb and Zn on biomass parameters of Chromolaena odorata

Biomass	Heavy	Plant	Heavy metal concentration in soil (mg/kg)						
parameter	metal	part	0	20	40	60	80	100	
Fresh weight (g) Pb		Leaf	38.90+5.21 ^b	55.72+2.30 ^b	51.03+17.09 ^b	65.47+21.72 ^b	141.31+65.48ª	77.84+39.09ª	
		Stem	14.82+3.32 ^b	25.39+5.68 ^b	17.11+4.32 ^b	60.27+31.77 ^b	124.51+92.74ª	29.32+10.04b	
		Root	15.56+7.56 ^b	26.57+4.13 ^b	15.81+6.26 ^b	$50.40 + 34.51^{ab}$	$70.41 + 48.07^{a}$	23.06+8.50 ^b	
	Zn	Leaf	$36.89 + 4.10^{b}$	53.61+2.57 ^{ab}	49.92+16.98 ^{ab}	63.36+20.61 ^{ab}	83.20+29.03ª	75.63+28.18 ^a	
		Stem	$11.71 + 2.21^{b}$	22.28+4.57 ^b	15.00+3.24 ^b	57.16+21.64ª	75.40+31.63ª	44.20+9.13ª	
		Root	13.45+6.45 ^b	24.46+3.02 ^b	14.70+5.15 ^b	48.39+33.40ª	68.30+37.19 ^a	21.95+7.49b	
Dry weight (g)	Pb	Leaf	15.62+7.64 ^b	32.57+6.69 ^b	33.21+13.51 ^b	$20.07 + 8.36^{b}$	69.63+24.55ª	60.15+31.84 ^a	
		Stem	$6.00 + 3.40^{b}$	14.35+5.11 ^b	5.74+2.47 ^b	12.83+4.42 ^b	34.07+21.34ª	13.24+7.16 ^b	
		Root	$5.97 + 0.55^{b}$	13.65+2925 ^b	4.71+1.81 ^b	23.49+16.00ª	39.29+30.38ª	13.60+5.43 ^b	
	Zn	Leaf	14.51+6.53 ^b	29.46+5.85 ^b	30.10+12.40 ^b	20.41+7.25 ^b	66.52+21.42ª	57.04+28.73ª	
		Stem	$5.02 + 2.32^{b}$	11.24+4.00 ^{ab}	4.63+2.06 ^b	18.72+3.33ª	22.96+10.23ª	12.13+6.05 ^{ab}	
		Root	5.65+0.51 ^b	12.54+2.62 ^b	6.68+1.98 ^b	20.36+11.03ª	36.18+20.27 ^a	10.50+4.32 ^b	

Note: Values are mean + standard error of 5 replicates. Means with the same letter(s) in superscript on the same row are not significantly different at p>0.05 (Tukey HSD Test)

3.4 Pb and Zn accumulation in plant parts

Table 3 reveals that there were significantly higher concentrations of Pb and Zn in parts of plants grown in metal contaminated soil compared to the control treatment. According to the data, Pb in the leaf (28.50-54.50 ppm), stem (5.50-26.00 ppm) and root (5.50-26.50 ppm) were significantly more than their respective control values of 3.00, 0.01 and 1.50 ppm. Likewise, Zn concentrations were significantly higher in the leaf (35.00-36.50 ppm), stem (11.00-16.00 ppm) and root (19.00-27.50 ppm) than the control values at 3.00, 2.00 and 2.00 ppm respectively. Metal concentrations in plant parts increased with increasing level of soil contamination, with more accumulation in the leaf than other plant parts.

Table 3 Concentrations (ppm) of Pb and Zn in plant parts of Chromolaena odorata grown in soil contaminated with the heavy metals

Heavy metal	Plant part	Heavy metal concentration in soil (mg/kg)							
		0	20	40	60	80	100		
РЪ	Leaf	3.00 ^b	28.50ª	35.50 ^a	37.00 ^a	46.00 ^a	54.50ª		
	Stem	0.01°	5.50 ^b	17.50 ^a	19.50 ^a	20.50 ^a	26.00 ^a		
	Root	1.50°	5.50 ^b	8.50 ^b	19.00 ^a	22.50 ^a	26.50ª		
Zn	Leaf	3.00 ^b	35.00ª	26.50 ^a	25.50 ^a	37.50 ^a	36.50 ^a		
	Stem	2.00 ^b	11.00ª	10.00 ^a	8.50 ^a	10.50 ^a	16.00 ^a		
	Root	2.00 ^b	19.00ª	31.00 ^a	22.50 ^a	20.00 ^a	27.50 ^a		

Note: Values are mean + standard error of 3 replicates. Means with the same letter(s) in superscript on the same row are not significantly different at p>0.05(Tukey HSD Test)

4 Discussion

The soil was relatively free of heavy metal contamination as the baseline values were 0.14 mg/kg Pb and 3.56 mg/kg Zn before addition of heavy metals to the soil. The values correspond with records on Pb and Zn concentrations in uncontaminated soils from South West, Nigeria according to Adeyi and Babalola (2017) as well as Patinvoh et al. (2021). The presence of Pb and Zn in the soil stimulated growth of *C. odorata*. For instance, according to Islam et al. (2024), zinc is an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Growth and development would stop if specific enzymes were not present in plant tissue. Carbohydrate, protein, and chlorophyll formation is significantly reduced in zinc-deficient



plants. Where growth reduction was recorded at high concentrations, Zn was generally not significant compared to the control. This is an indication that negative impact of heavy metals on *C. odorata* growth was minimal, demonstrating remarkable tolerance to lead and zinc contamination, though more in Pb than Zn. This was evident in plant height, stem girth, leaf area, number of leaves as well as number of roots and root length. This resilience aligns with findings by Lambert et al. (2012), indicating that plant responses to heavy metals vary, with some possessing natural remediation abilities. Tolerance of plants to toxicity depends on the species and tissue, element type, and duration of exposure to stress (Ayesa et al., 2018; Tiwari and Lata, 2018). Heavy metals, when occurring at low concentrations, are involved in redox reactions, electron transfers, nucleic acid metabolism, and as an integral part of several enzymes. Some heavy metals such as Cu, Fe, Mn, Zn, and Ni are components of some enzymes and proteins, thereby essential for plant growth and metabolism.

Reduction in number of leaves and stem girth by Zn, and reduction in biomass by Pb confirm that heavy metals can negatively impact plant growth, development and reproduction when they are accumulated at concentrations above their optimal levels (Rashid et al., 2023). This can, therefore, be ascribed to metal accumulation beyond permissible level. Pb is an example of potential heavy metal that is neither an essential element nor have any role in the process of cell metabolism but is easily absorbed and accumulated in different parts of a plant. High concentration of heavy metals such as lead can cause a number of toxic symptoms in plants that may be retardation in growth (stunted growth), negative effects on photosynthesis (chlorosis), blackening of roots and different other symptoms. Lead has the ability to inhibit photosynthesis, disturb mineral nutrition and water balance, changes in hormonal status and affects membrane structure and permeability (Nas and Ali, 2018). This corresponds to reports of heavy metals affecting plant growth, causing degradation in crop quality, soil health and yield because of their accumulation in crops (Shah and Daverey, 2020; Wang et al., 2020). Many researchers have also confirmed negative effects of heavy metals on plants. For instance, lead and zinc were reported to have negative impact on seedling growth of alfalfa (Medicago sativa L.) by Yahaghi et al. (2019). Also, cadmium, chromium and lead had negative impact on growth and development of cultivated plants (Madhu and Sadagopan, 2020). Similarly, Nickel (Ni) was found to negatively affect vegetative and reproductive growth parameters of Nigella sativa (Khan et al., 2023). Reduction of fresh and dry weights of plant parts particularly by Zn at 100 mg/kg is similar to the findings of Kekere et al. (2020) who explained biomass reduction in *Tithonina diversifolia* due to the variations depending on the quantity of metal. Plants, when grown in soils contaminated with heavy metals, are often faced with some changes at physiological levels which include nutrient accumulation, respiration, and gaseous exchange. Heavy metals at high concentrations also affect plant metabolism and physiological events, reduce growth, and contaminate the environment. Heavy metals also cause oxidative stress in plants mainly through excessive production of reactive oxygen species (ROS). Excessive production of ROS increases production of unsaturated lipid peroxidation, fatty acids and disturbs cell membrane function. Cell membrane damage can cause an imbalance in enzymatic activities, disrupts the normal redox balance of the cell, and causes oxidative damage that affects cell metabolism (Wang et al., 2020). Metals accumulation in plants can significantly affect plant viability, carbohydrate level, and respiratory rates.

Interestingly, high concentrations of the heavy metals had a positive impact on some plant growth indices, supporting Masindi and Muedi (2018) findings of increased growth rate in *Tithonia diversifolia* due to heavy metal effects. The rapid growth of *C. odorata* seedlings, even under high metal concentrations, also aligns with Kupper et al. (2002) suggestion that such growth could result from cell division processes, respiration, and protein synthesis. The experiment results indicated that the overall performance of *C. odorata* was not severely hampered by increased heavy metal concentrations. Even at high levels of metals, the plant exhibited improved growth with minimal negative effects. This re-echoes the findings of Ayesa et al. (2018) on *Tithonia diversifolia*, reinforcing the notion that certain plants can thrive in heavy metal-contaminated environments. Srirueang et al. (2021) earlier confirmed this result with studies showing that certain plants succeed in the accumulation of multiple heavy metals in the presence of high concentrations. Plants have developed some mechanisms against heavy metal stress. These mechanisms include immobilization; exclusion of plasma membranes; restriction of absorption and transport; synthesis of specific heavy metal carriers; induction of stress proteins; chelation; and sequestration by certain ligands (Kumar et al., 2019; Yu et al., 2019; Madhu and Sadagopan, 2020; Wang et al., 2020; Islam et al., 2024).



Accumulation of heavy metals in plant parts might have contributed to negative effect of zinc on *C. odorata* at high concentration of 100 mg/kg. High level of heavy metal in plant tissues might inhibit uptake of essential nutrients for growth and development. In an earlier research, cadmium was found to hinder nutrient transport in rice (Huybrechts et al., 2021). However, the plant's ability to absorb and accumulate high heavy metal in shoot and root is an indication that it has the potential to clean up heavy metal contaminated soil. This has been confirmed by Islam et al. (2024) that plants with capacity for absorption and sequestration of pollutants from soil can be used for phytoremediation of such contaminants.

5 Conclusion and Recommendations

Chromolaena odorata is tolerant to Pb and Zn contamination as it was able to accumulate high concentrations of the metals on its leaf, stem and root with minimal negative effect. Therefore, the plant is recommended for removal of excess Pb and Zn from contaminated soils. However, experiments with plants in pots can be beset by several often-unrecognized shortcomings. There is therefore, a need for future research to investigating the mechanisms of Pb and Zn absorption and tolerance in field experiments by imposing higher concentrations of the metals.

Authors' Contributions

O. Kekere was the experimental designer, and O. M. Ajayi was the executor of this study; O. M. Ajayi completed data analysis and wrote the first draft of the paper; O. M. Ajayi participated in experimental design and analysis of experimental results; O. Kekere was the project conceptualizer and leader, guiding experimental design, data analysis, paper writing, and revision. All authors read and approved the final manuscript.

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