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Eco-physiological Responses of *Saccharum spontaneum* to Chromium (Cr), Cadmium (Cd), Lead (Pb), and Mercury (Hg) in Augmented Phytoremediation Paradigm

D. G*[1]. Kever, H.O.A[1]. Oluma, C.U[1]. Aguoru, I.S[2]. Eneji and J.O[1] Olasan

[1]Botany Department, Joseph Saawuan Tarka University Makurdi, Nigeria
[2]Chemistry Department, Joseph Saawuan Tarka University Makurdi, Nigeria
*Correspondence E-mail: keversenator@gmail.com

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Abstract
Environmental pollution of the soil with heavy metals is of great concern. This tends to affect the physiological response of plant species in the tolerance of these mineral uptakes in the plant tissues. This study evaluated the physiological response of *Saccharum spontaneum* L (wild sugarcane) in the phytoremediation of Cr, Cd, Pb and Hg heavy metal contaminated soils using augmentation process in a potted experiment. Data analysis was carried out using Minitab (17.0). The results show that, augmented wild sugarcane plant performed best in the uptake of Lead (197.05) Cadmium (188.39) and Mercury (132.37) at week 10. These heavy metals were also up taken by non-augmented wild sugarcane plant but below the augmented plant. Chromium uptake was highest (85.86) under the application of augmented wild sugarcane at week 5 and 10. Heavy metal uptake index was significantly higher in week 10 than in week 5 ($T=1.99$, $P=0.05$). The relationship between heavy metal concentrations in plant tissues and growth or yield parameters of the test plants was highly positive ($R=+0.943$, $P<0.05$). The test plant is recommended for ex-situ phytoremediation of soils contaminated with Cr, Cd, Pb and Hg. This is particularly advantageous as the plant has bio-economic value and is easy to propagate. The plant can be allowed to stay for a longer time in polluted soils for a more effective phyto-extraction than the period adopted in a simulated pot experiments carried out in this study.
Key words: Eco - Physiological, Phytoremediation, Augmentation, Heavy Metals, Contaminates soil

Introduction

The contamination of soil by heavy metals is of great concern to environmentalist, as it significantly impacts both the quality and yield of plants. This pollution not only induces alterations in the composition and activity of the soil microbial community but also exerts an influence on soil enzymatic reactions by prompting shifts in the microbial community responsible for synthesizing enzymes [1, 2]. Heavy metals are widely regarded as one of the substantial environmental crisis confronted by humanity, as soil pollution poses a grave threat to both humans and the general environment [3].

Plants have very efficient mechanisms to obtain macro-nutrients from their environment, according to [4], plants' roots produce chelating agents and cause pH changes and redox reaction which enhance their uptake of nutrients from very low level in the soil. Plants uptake and translocation mechanisms are likely to be closely regulated. The heavy metals that are available for plants uptake are those that are present as soluble components in the soil or those that are easily solubilized by root exudates [5]. Though, plants require certain heavy metals for their growth, excessive amount of these metals can be toxic to the plants. According to [6], plants ability to accumulate essential metals enable them to acquire other non-essential metals. The authors further noted that metals cannot be broken down thus when the concentration within the plants exceeds optimal levels; the plant is adversely affected directly or indirectly.

In their submission, [7] identified some of the harmful effects of high concentration of metals as: inhibition of cytoplasmic enzymes and damage to plant cell structures due to oxidative stress. Also, [8, 9] in their separate views submit that, a major effect of heavy metals on plants, particularly Cd is reduction in seed germination, decrease in plant nutrient content, Chlorosis, growth inhibition, browning of root tips and death. It also reduced shoot and root length. Pb causes Reduction in the number of plant leaves, reduced plant height, decrease in plant biomass; inhibition of enzymes activity which affect CO_2 fixation and delay in seed germination [10, 11]. Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animals and human health, the absorption by plants' roots is one of the main routes of heavy metal entry into food chain [12, 13]. In the views of [14, 15], heavy metals are potentially toxic and their phytotoxicity in plants, may result in chlorosis, weak plant growth, yield depression, disorder in plants' metabolism and reduced ability to fixate molecular nitrogen. Plants

with high tolerance rate to withstand or accommodate these in a metal contaminated environment are thus good for phytoremediation. Wild sugarcane (*Saccharum spontaneum*) is a fast-growing tolerant plant species that has good potentials for effective in phytoremediation of heavy metals [16]. However, the eco-physiological responses of wild sugarcane to multiple heavy metals like Cr, Cd, Pb and Hg in an augmented phytoremediation paradigm using cow dung are not well undertaken in research. In this research, we present a study that investigates the responses of wild sugarcane to these heavy metals (Cr, Cd, Pb, and Hg) in an augmented phytoremediation. The study specifically aims to provide insights into the eco-physiological mechanisms underlying the phytoremediation potential of wild sugarcane and to inform the development of effective phytoremediation strategies for contaminated environments. The study builds on existing literature that has investigated the challenges and opportunities in the phytoremediation of heavy metals contaminated soils [16, 17] and the accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential [16, 18].

Materials and Methods

Study Area

The study area is Makurdi, the State Capital of Benue State. The experimental site is within the Joseph Saawuan Tarka University (the defunct Federal University of Agriculture) Makurdi (latitude 7°38' N-7°50' N, and longitude 8°24'E-8°38'E) [19]. The nursery barn of the Forestry Department, Joseph Saawuan Tarkaa University Makurdi, situated behind the water works Unit of the University and enclosed by a fence, serves as a designated study area for soil preparation, treatment, and planting of test plants. This location provided a controlled environment for the conduct of the research.

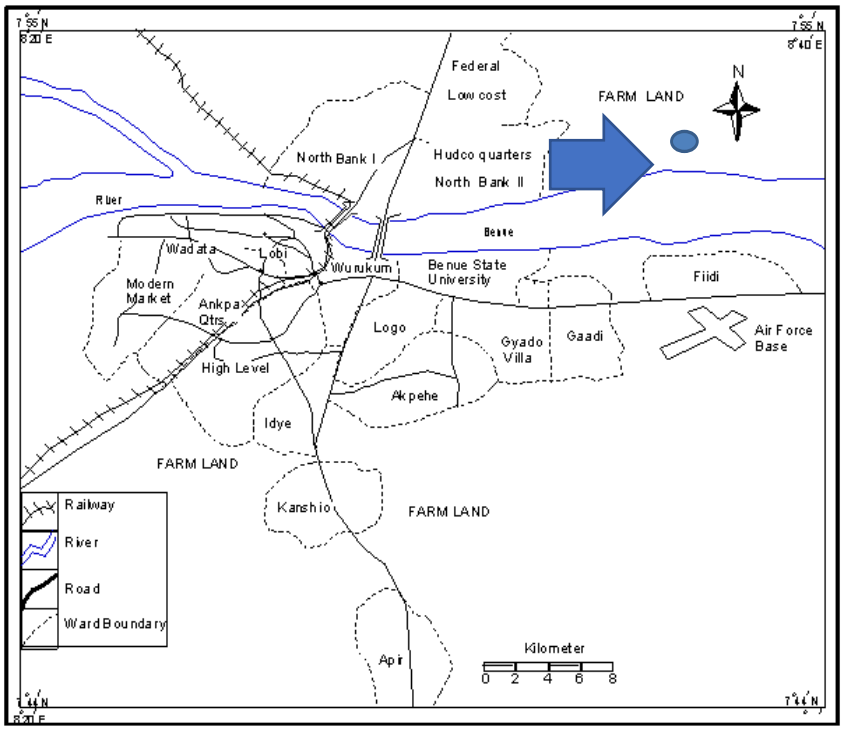

Figure 1: Map of Makurdi Local Government, showing the study area

Experimental Design

The study employed the Completely Randomized Experimental Design to ensure comparability among treatment groups and attribute any observed differences in the response variable specifically to the treatments, rather than other factors. This approach facilitates proper statistical analysis, hypothesis testing, and the estimation of treatment effects along with confidence intervals, [20]. The experimental design utilized the Complete Randomized Design (CRD) in conjunction with the General Full Factorial design (5x3x2) X 3 structure, with the Minitab 16.0 software's tool function playing a crucial role in the implementation.

Sources of Heavy Metals

The following heavy metal salts used in the work were procured from a standard commercial laboratory: $CdCl_2$ (Cadmium Chloride), $K_2Cr_20_7$ (Potassium dichromate), $PbCl_2$ (Lead II Chrolide) and $Hg(NO_3)_2$ (Mecury (II) Nitrate) for the preparation of Chromium (Cr), Lead (Pb), Mercury (Hg), and Cadmium (Cd) salts.

Soil Sample Collection (Pre – Experiment)

Soil samples were collected from an undisturbed area behind the Botany Department of the University using the soil auger at the depth of 0-15 cm for pre-soil analysis. Samples were collected in sterilized polythene bag and taken to the laboratory for pre-experiment of soil.

Preparation of heavy metal stock solutions for treatment

Cadmium stock solution

In Cadmium, 18.33 g of $CdCl_2$ (Cadmium Chloride), was dissolved in 200 cm^3 of distilled water in a 1000 cm^3 volumetric flask and diluted to mark to give a solution of 1000 mg/L stock solution of Cadmium and stored [21].

Chromium stock solution

In Chromium, 1084.14 g of $K_2Cr_20_7$ (Potassium dichromate), was dissolved with 200.00 cm^3 of deionized water in a 1000 cm^3 volumetric flask and diluted to mark with water to give a 1000 mgL-1 stock solution of Chromium and stored [21].

Lead stock solution

In Lead, 27.81 g of $PbCl_2$ (Lead II Chrolide) was dissolved in 50.00 cm3 of deionized water in a 1000 cm^3 volumetric flask. The solution will be diluted to mark with distilled water to give a 1000 mgL-1 stock solution of Lead and stored [21].

Mercury stock solution

To prepare 1000 mL of a 0.1 mol/L solution of Mercury (II) nitrate we have to dissolve 32.46 g of $Hg(NO_3)2$ (100 % purity) in deionized or distilled water. After the solid is completely dissolved, dilute the solution to a final volume with deionized (distilled) water. The solution was transferred to a clean container and stored [21].

Plant Sample Collection and Authentication

Whole Plant samples of wild sugarcane (*Saccharum spontaneum L.*) was collected and authenticated at the farm village of the forestry Department Joseph Sarwuan Tarka University, with further confirmation using the flora of West Africa album.

Procedures of treatment application and planting of test plants

The soil in the pots were spiked with 50 ml of the different heavy metals based on the experimental design and mixed thoroughly using a spatula, except in the control pot without treatments. For pots receiving manure treatments, the soil was mixed with 0.4 kg of cow dung and allowed to stand for four weeks before planting. This was to ensure that the organic matter in the cow dung undergoes decomposition and integrates with the soil, enhancing its overall fertility and quality. This integration period also allows for better nutrient release and transformation, creating a favorable environment for plant growth. The test wild sugarcane (*Saccharum spontaneum L.)* was planted in the experimental pots filled with 5 kg of pre-determined unpolluted soil. The method described by [22] was employed with modifications.

Sample Preparation

Collection of samples was done at weeks 5 and 10 after planting. The whole plant samples were carefully collected in a Ziplock bag, labeled, and conveyed to the laboratory. Plant samples were thoroughly washed with tap water and rinsed with distilled water to remove soil debris. The stems were separated from the roots and cut into smaller pieces. All plant samples were oven dried using GNLAB Mino economy oven of model MINO/75 at 105 ^0C to a constant weight and crushed using wooden mortar and pestle [23]. Porcelain mortar and pestle were also used to crush the soil samples to a homogenized state. The Porcelain mortar and the wooden mortar and pestles were rinsed with distilled water and dried after each sample ground to avoid cross contamination [24]. Each sample was passed through a sechi standard test sieve of 2mm; the fine powder of the samples was stored in airtight plastic containers with lid for analysis [25].

Heavy Metal Analysis in Plants and soil

Digestion of samples was done in advanced research laboratory, Joseph Saawuan Tarka University Makurdi. The method of [23] was adopted in digesting the heavy metals. 0.5 g of plant samples were weighed into a clean flat bottom flask of 250 ml using a scale of model AR2130 Ohaus Corporation China. 5 ml of concentrated Nitric per Chloric acid (HNO_3 / $HCLO_4$) in the ratio of 2:1 was added to the sample. The plant samples were allowed to stay for two minutes before been placed on the hotplate of model ES-3615, Everest China in a fume cupboard. This was heated gently until a clear solution was obtained which signified a complete digestion. The crushed plant material was allowed to cool to room temperature (25 ^oc). Clean crucibles were used for soil samples digestion; 5 ml of concentrated Nitric per Chloric acid (HNO_3 / $HCLO_4$) in the ratio of 2:1 was added to each soil sample and shook for proper mixing. Soil samples in the crucibles were placed in the fume cupboard and allowed to stay for 24 hours before being filtered. Both plant and soil samples

were filtered using Whatman no. 1 Filter Paper. The filtrates were diluted with deionized water to 25 ml mark and transferred into clean plastic bottles with lid and labeled accordingly for heavy metals analysis.

Analysis of selected heavy metals (Cd, Pb, Cr, Hg) were done using Atomic Absorption Spectrophotometer AAS in mgl-1 of Model ICE 3000 Series, Thermo Scientific, USA at the Chemistry Advanced Research Centre (CARC), Sheda Science and Technology Complex (SHESTCO) Abuja.

Result and Discussions

Growth of Test Plant:

Table 1 gives the effects of the various heavy metal treatments (Cadmium-Cd, Lead-Pb, Chromium-Cr and Mercury-Hg) on the growth of wild sugarcane plant tested for remediation. At week 4, plant shoot grew to the highest height of 11.23±6. 10 cm in augmented Pb treatment (J-Pb-cowd); the second tallest shoot (10.53±4.43cm) was measured in augmented Hg pot while the shortest shoot was measured in Cd pot (6.60±2.29cm). The observed differences in wild sugarcane shoot length at week 4 under different treatments were insignificant (F=0.7, P> 0.05).

At week 8, test plant in augmented Hg pot recorded the tallest shoot (31.43±9.88cm) those without heavy metal but augmented with cow dung (ws-cowd) were the shortest plant (8.23±2.25cm). The observed differences in wild sugarcane shoot length at week 8 under different treatments were significant (F=2.72, P<0.05).

The shoot growth rate of wild sugar cane in different heavy metal applications is shown in figure 2. Shoot growth increased from week 4 to week 8 across all treatments. Test plant in augmented Hg pot showed the fastest growth rate of 5.23 while slowest rate was observed in pots treated with Hg only (0.43) or cow dung only (0.41).

Table 1: Effects of heavy metal treatments on shoot length of wild sugarcane (Ws)

Wild sugarcane based treatment	Shoot length @ 4 weeks (cm) Mean ± SD	Shoot length @ 8 weeks (cm) Mean ± SD
Ws only	9.73±5.60[a]	17.33±12.71[b]
Ws-Cd	6.60±2.29[a]	11.50±6.06[b]
Ws-Pb	7.00±4.36[a]	9.67±2.08[b]
Ws-Cr	8.03±4.61[a]	12.67±5.01[b]
Ws-Hg	9.80±1.48[a]	11.53±3.27[b]
Ws-cowd	6.60±2.29[a]	8.23±2.25[b]
Ws-Cd-cowd	7.77±4.65[a]	14.67±5.03[b]
Ws-Pb-cowd	11.23±6.10[a]	19.07±10.66[b]
Ws-Cr-cowd	8.67±4.62[a]	17.67±3.51[b]
Ws-Hg-cowd	10.53±4.43[a]	31.43±9.88[a]
F-statistics	F=0.70, P=0.698 (P>0.05)	F=2.72, P=0.030 (P<0.05)
Fisher LSD	5.93	9.81

Legend: cowd = cow dung; Cd=Cadmium; Pb=Lead; Cr= Chromium; Hg= Mercury; Ws=Wild sugarcane

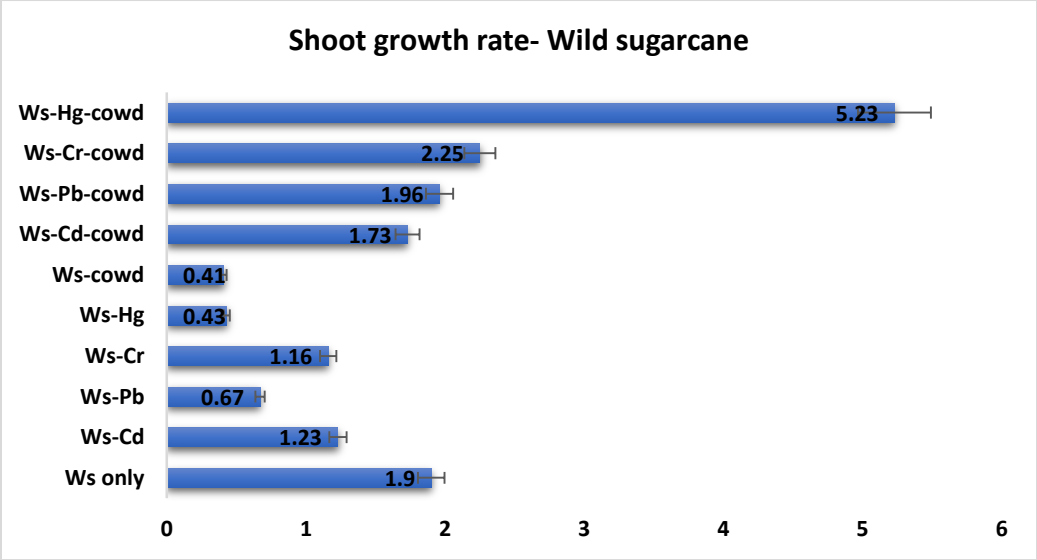

Figure 2: Shoot growth rate of wild sugarcane in different treatments

Number of Leave Produced

Table 2 explains the effects of the various heavy metal treatments (Cadmium-Cd, Lead-Pb, Chromium-Cr and Mercury-Hg) on the number of leaves produced by wild sugarcane plant tested for remediation. At week 4, average number of leaves varied from 5 leaves in augmented wild sugarcane (Ws-cowd) pot to 9 leaves in Pb treated pots, although the differences were insignificant (F=0.42, P>0.05). At week 8, average number of wild sugarcane leaves varied from 7 leaves in the control (test Plant only) and Cr pots to 10 leaves in Cd pots, with statistically insignificant differences among the treatment means (F=0.42, P>0.05). Number of leaf increased from week 4 to week 8 across the treatments except in two cases. Rate of leaf production was fastest in augmented Cr potted (0.67) where 5.67-8.33 leaves were produced in week 4-8 respectively whereas a static change in leaf production was observed in non-augmented Hg and Cr pots (Figure 3).

Table 2: Effects of heavy metal treatments on number of leaves produced by wild sugarcane (Ws)

Wild sugarcane treatment	Number of leaf @ 4 weeks Mean ± SD	Number of leaf @ 8 weeks Mean ± SD
Ws only	6.33±2.89[a]	7.00±1.73[a]
Ws-Cd	8.33±2.52[a]	9.67±1.53[a]
Ws-Pb	8.67±3.06[a]	9.00±2.65[a]
Ws-Cr	7.00±2.00[a]	7.00±2.00[a]
Ws-Hg	7.33±2.08[a]	7.33±2.08[a]
Ws-cowd	5.33±2.31[a]	7.33±3.06[a]
Ws-Cd-cowd	7.00±1.73[a]	7.67±0.58[a]
Ws-Pb-cowd	7.00±3.46[a]	7.67±2.31[a]
Ws-Cr-cowd	5.67±4.62[a]	8.33±2.52[a]
Ws-Hg-cowd	6.33±2.52[a]	8.33±3.51[a]
F-statistics	F=0.42, P=0.912 (P>0.05)	F=0.43, P=0.901 (P>0.05)
Fisher LSD	3.96	3.26

Legend: cowd = cow dung; **Cd**=Cadmium; **Pb**=Lead; **Cr**= Chromium; **Hg**= Mercury; **Ws**=Wild sugarcane

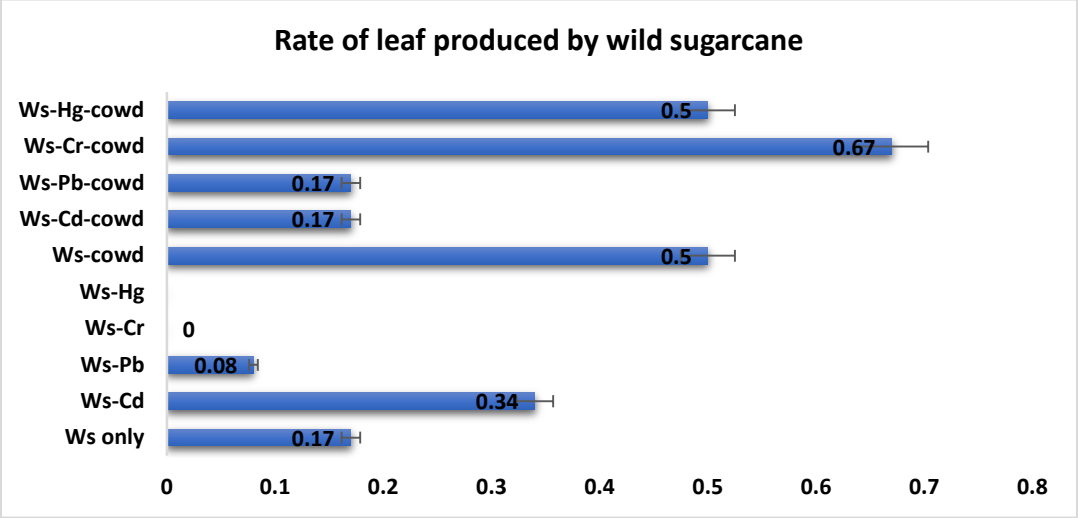

Figure 3: Rate of leaf produced by wild sugarcane in different treatments

Leaf area

Table 3 explains the effects of the various heavy metal treatments (Cadmium-Cd, Lead-Pb, Chromium-Cr and Mercury-Hg) on the leaf area of wild sugarcane plant tested for remediation. At week 4, results showed that most of the treatments exerted the same effect on the leaf area ($F=0.83$, $P>0.05$). However, the effects of treatments that produced the minimum and maximum leaf areas were significantly different ($P<0.05$). Hence, the smallest leaf area was measured in augmented pots of test plant without heavy metal (19.00±14.00 cm^2) while the largest leaf area was measured in augmented Lead pot (63.80±40.70 cm^2).

At week 8, results also showed that most of the treatments exerted the same effect on the leaf area ($F=0.72$, $P>0.05$). However, the effects of treatments that produced the minimum and maximum leaf areas were significantly different ($P<0.05$). Hence, the smallest leaf area was measured in Cd pot (30.13±10.34) while the largest leaf area was measured in augmented Pb pot (68.20±33.30 cm^2).

All but augmented Hg pot showed positive changes (increment) in leaf area from week 4 to 8. The largest change in leaf area was observed in Ws-cowd (wild sugarcane and cowdung only) pot at of rate of 4.4 and it was followed by Ws-Hg (wild sugarcane and Hg) pot at 3.38 rate and wild sugarcane control pot at 3.12 rate (Figure 4).

Table 3: Effects of heavy metal treatments on leaf area of wild sugarcane (Ws)

Wild sugarcane treatment	Leaf area @ 4 weeks (cm^2) Mean ± SD	Leaf area @ 8 weeks (cm^2) Mean ± SD
Ws only	31.91 ± 2.70^{ab}	44.40 ± 11.20^{ab}
Ws-Cd	25.57 ± 6.76^{ab}	30.13 ± 10.34^{b}
Ws-Pb	37.7 ± 30.7^{ab}	41.1 ± 27.2^{ab}
Ws-Cr	35.92 ± 16.44^{ab}	39.80 ± 20.9^{ab}
Ws-Hg	34.42 ± 11.92^{ab}	47.93 ± 17.16^{ab}
Ws-cowd	19.00 ± 14.00^{b}	36.6 ± 19.30^{ab}
Ws-Cd-cowd	45.80 ± 20.60^{ab}	47.60 ± 17.50^{ab}
Ws-Pb-cowd	63.80 ± 40.70^{a}	68.20 ± 33.30^{a}
Ws-Cr-cowd	34.90 ± 39.20^{ab}	40.60 ± 36.50^{ab}
Ws-Hg-cowd	36.54 ± 4.11^{ab}	30.87 ± 4.23^{ab}
F-statistics	F=0.83, P=0.601 (P>0.05)	F=0.72, P=0.682 (P>0.05)
Fisher LSD	32.02	30.77

Legend: cowd = cow dung; **Cd**=Cadmium; **Pb**=Lead; **Cr**= Chromium; **Hg**= Mercury; **Ws**=Wild sugarcane

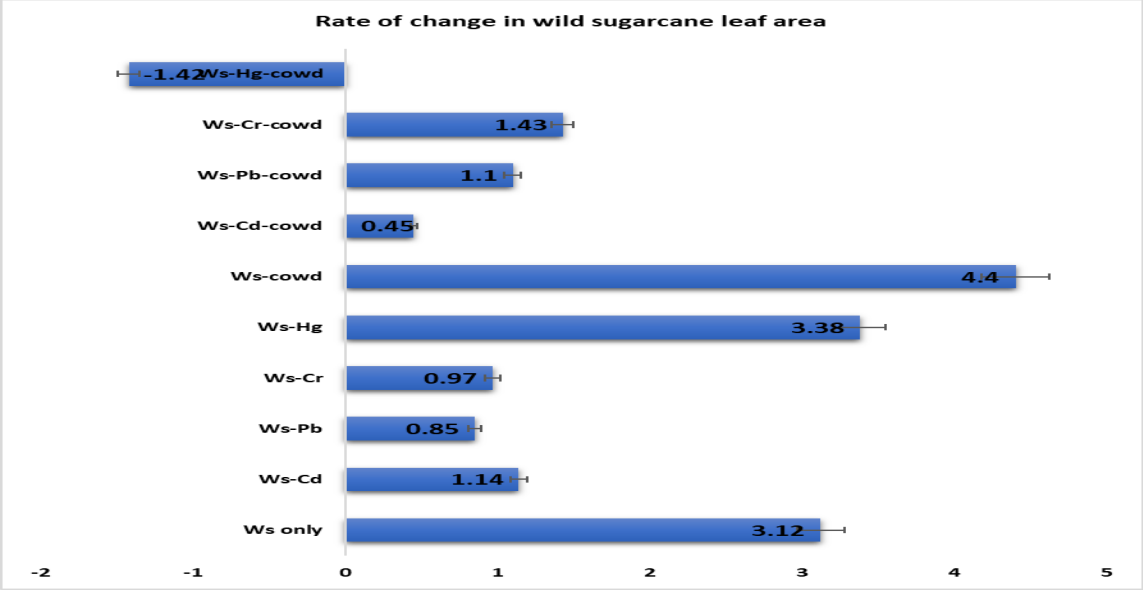

Figure 4: Rate of change in leaf area of wild sugarcane in different treatments

Plant vigor

Wild sugarcane vigor was 100% (week 4 and 8) in all treatments except in augmented Pb pot where it was 66.7% at week 4 and 100% at week 8 as shown in figure 17. Sunflower vigor was 100% (week 4 and 8) in all but three treatments. This plant had poor vigor (0%) at week 4 in augmented Cd, Pb and Cr pots but vigor was regained to 100% level in the respective pots at week 8 as shown in figure 5.

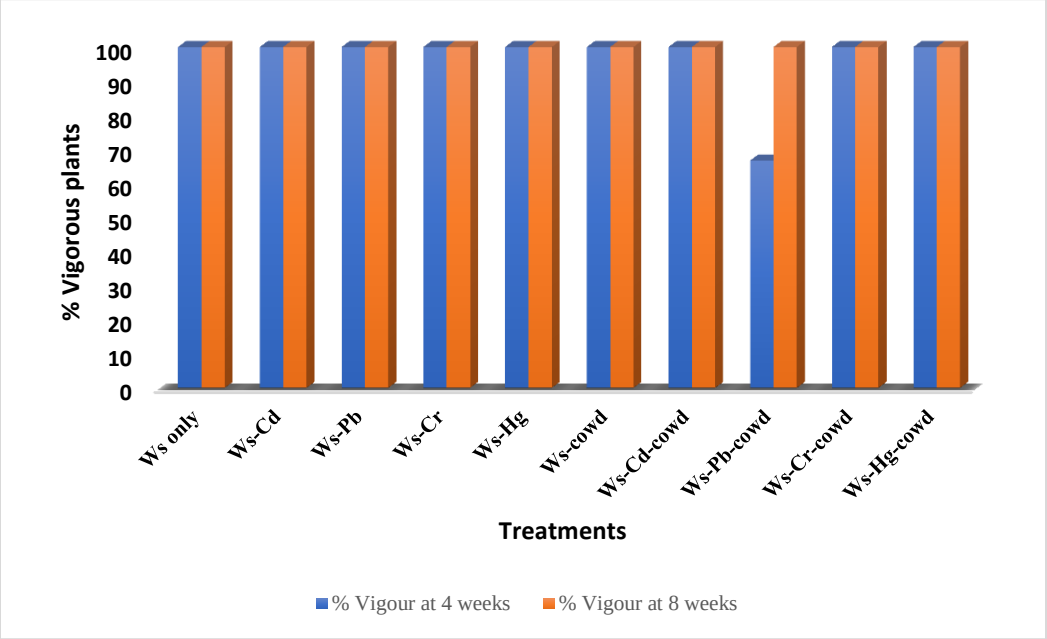

Figure 5: Plant vigor of wild sugarcane in heavy metal treatments

Table 4: Pearson's correlation matrix of heavy metal concentration and growth parameters of wild sugarcane

	Root HM conc	Stem HM conc	Wet mass	Dry mass
Stem HM conc	R=0.943 P<0.05			
Wet mass	R=0.632 P=0.05	R= 0.504 P>0.05		
Dry mass	R= 0.584 P>0.05	R= 0.489 P>0.05	R= 0.949 P<0.05	
Root length	R= 0.745 P<0.05	R= 0.752 P<0.05	R= 0.436 P>0.05	R= 0.419 P>0.05

Legend: HM conc= Heavy metal concentration, R= Correlation coefficient, P= Probability

Discussion

Heavy metals affected all growth parameters of the test plants except the percentage vigor. Cd and Pb reduced the leaf area of wild sugarcane. Cr, Cd and Pb negatively affected root sizes of the test plant at the initial state, importantly with the addition of cow dung. Despite the observed negative effects of heavy metals reported, test plants showcased perfect vigor at later stages of development.

Soil augmentation can improve soil quality, promoting better nutrient availability, or reducing heavy metal toxicity, which could stimulate shoot growth and increase leaf number. Similarly, the methane gas released by cow dung at early stages of decomposition may affect the growth parameters of the test plants. Heavy metals might affect the physiological process of the plant parts. This result disagrees with findings of [9] who reported a drastic reduction in the number of plant leaves, plant height and plant biomass accompanied in selected plants growing on heavy metal contaminated soils. Pb, Cr, Cd and Hg toxicity has been shown to induce adverse effects on morphology, physiology, germination, and early crop growth in a variety of crops [26- 29].

The outcome of the correlation studies shows that the high concentration of heavy metals in the root caused a proportional increase in the heavy metal concentration in the stem as found in wild sugarcane plant. This could be attributed to plant species and uptake mechanism. This agrees with [30] whose study shows the translocation of these heavy metals to aerial parts of the plant like the stem. This emphasizes the role of species-specific uptake mechanisms and resonates with recent findings regarding the translocation of these metals to aerial plant parts.

Conclusion

The findings of the present research showed the impact of heavy metals on the test plant, particularly, Cadmium (Cd) and lead (Pb) adversely affected the leaf area, while Chromium (Cr), Cd, and Pb notably compromised root size of the test plant, particularly in pots where cow dung was introduced. The positive aspect however was, the adverse effects did not translate to vigor component of the test plant during the latter stages of plant development. Overall, these findings highlight the importance of understanding the complex interactions between environmental factors, plant physiology, and species-specific mechanisms to develop effective bioremediation techniques for heavy metal-contaminated soils.

Conflict of interest

The authors declare no conflict of interest. **However this is a reversed version of this article already published in FUAMJPAS Vol. 4 No. 1, June, 2024**

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