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Successful Phytoremediation of Simulated Steel Rolling Industry Heavy Metals-Contaminated Soils Using a *Sorghum bicolor* Cultivar from Riko, Katsina, Nigeria

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ABSTRACT

The release of hazardous heavy metals (HMs) from industries and other sources threatens ecosystems in Katsina, Nigeria and beyond. Bioengineering through microbially-assisted phytoremediation (MAP) is the best innovative alternative to these industries for remediating HMs contaminated environments. *Sorghum bicolor* (L. Moench) had been reported to be efficient in heavy metals phytoremediation. This study evaluated the ability of a fast-growing local cultivar of *S. bicolor* (*rirrik'a/rirritsa/mota* in Hausa) from Riko village, Jibiya L.G.A., Katsina State, Nigeria to remediate mesocosms simulating mixed HMs contamination obtainable at the soils of the defunct DANA Steel Rolling Mills, Katsina industrial site, to residual concentrations matching USEPA/EU limits. A chronosequential, nutrient-poor phytoremediation approach was employed to study the restoration of the contaminated soils in greenhouse experiments. The bioremoval of HMs in individual (0.05-10 g/L Cr, 0.04-1 g/L Cu, 0.08-1 g/L Pb and 0.02-1 g/L Zn) and mixed mesocosms was studied over 8 weeks, in multiple replicates, with positive and negative controls. ANOVA, Mann-Whitney and Kruskal-Wallis (with Dunn's post-hoc) tests were used to statistically analyse the obtained data. The results confirmed an overall bioremoval of 66.67% of the HMs. Bioremoval rates were statistically similar across all HMs (one-way ANOVA: $p = 0.64$); with 69.48% of Zn, 67.46% of Cu, 63.34% of Cr and 58.33% of Pb bioremoved. The final residual HMs were within limits set by EPA/EU (Mann Whitney U test: $p = 0.23$). This study verified the status of the local cultivar of *S. bicolor* as a suitable agent for safe, effective phytoremediation of industrial heavy metal contaminated sites. Thus, its use is recommended for on-the-field phytoremediation of hotspots of HM contamination within the study area and beyond. The study also contributes towards sustainable and eco-friendly practices by using phytoremediation to manage environmental wastes from industrial pollution.

1. Introduction

Heavy metals (HMs) are highly electromagnetic metals occurring in the earth's crust, having a high relative atomic mass and a density of more than 5 or 7.5 g/cm^3, such as chromium, arsenic,

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copper, zinc, cadmium, lead, mercury, and manganese, among others (Mahuta *et al.,* 2021). Multiple natural (e.g. weathering of rocks, environmental disasters, etc.) and anthropogenic (mining, transportation, the use of fossil fuels, activities related to modern agriculture, and urban waste disposal practices) events lead to the daily discharge of sizeable amounts of HMs into the environment (Babar *et al*., 2021; Stofejova *et al*., 2021; Xie *et al*., 2021). Industrialisation and industrial activities, such as mining, metalwork, etc. also play a huge role in the release of heavy metals in the environment (Anbuganesan *et al.,* 2024). Moreover, HMs are among the four priority, commonest and most hazardous pollutants globally (Riko & Darma, 2024; Ortiz & Pena, 2021). In the study area (Katsina Metropolis), industries such as NAK Enterprises (formerly Dana Steel Rolling Mill, Katsina), are among the major contributors to the exacerbation of HMs issues, by the deposition of by-products related to steel slag into the environment, especially around the plant. Other sources include industrial effluents, metal works (*Yan Gwangwani* in Hausa), waste refuse dumps, automobile garages, and agricultural soils (Stathatou *et al.,* 2022; Umar *et al*., 2020b). In particular, HMs concentrations in soils of Katsina indicate slight pollution with Cu; moderate pollution with Pb and Zn and excessive pollution with Cr. These have debilitating consequences to the environment (Kinuthia *et al*., 2020; Nemati *et al.,* 2024); and their release/accumulation initiates toxicity to the living spectrum: plants, animals, and microorganisms (Umar *et al*., 2020a; Borah *et al.,* 2023). Thus, they pose a direct threat to environmental sustainability (Haque *et al.,* 2022).

Since HMs are not usually biodegradable, specific techniques were devised as solutions for HMs removal from surface/ground water and contaminated soils (Qasem *et al.,* 2021; Chen *et al.,* 2024). These include physicochemical and electro-mechanical methods, which in turn have limitations; with the best technique for HMs removal thus being bioremediation (Yunusa & Umar, 2021; Dianadar & Etemadifar, 2024), specifically Microbially-Assisted Phytoremediation (Yang *et al.,* 2020). Phytoremediation involves the utilization of plants to ‘absorb, accumulate, metabolize, volatilize, or stabilize’ environmental pollutants (Sierra *et al*., 2021). MAP remains the state of the art in terms of HMs bioremoval, as it links plant growth promotion activities of microbes to inherent remediation abilities of plants (Jain & Tembhurkar, 2024; Li *et al.,* 2024).

Previous research had highlighted the desirable characteristics possessed by *S. bicolor* which adapted it to phytoremediation in the study area. Firstly, it is indigenous and commonly cultivated in the area, in fact, Nigeria is the 2nd highest producer of *Sorghum bicolor* globally, and it produced 6.57 million tonnes of sorghum in 2020 (Shahbandeh, 2021). Secondly, it is a fast-growing plant classified as a high accumulator, capable of generating up to 120 to 150 tonnes ha^{-1} (May *et al*., 2020). Moreover, it also has many end uses, e.g. it is used to generate plant chips used in heat and electricity generation, combustion/pyrolysis, generation of biogas from silage, production of second-generation bioethanol, bioelectricity generation, and use of burnt *Sorghum* ash as fertilizer (Silva *et al.,* 2021). The lignin of sorghum is also useful chemically. It is also used in animal feeds and first-generation biofuels (De Jesus Ferreira *et al.,* 2023). Its high genetic diversity, possession of numerous cultivars and the applicability of its grain, sugar, forage, fiber and tinctorial properties), are useful for valorization research and applicable in nonfood contexts (Perlein *et al*., 2021; Liu *et al*., 2020). It also has a possibility of re-growth since its root system continues to thrive after harvest, thereby allowing the plant to regrow (May *et al*., 2020). The plant is also adaptable to and tolerates wide habitats; exhibits a dense pattern of root/shoot development; has low water and fertilizer requirements (Badamasi *et al*., 2020); tolerates various forms of stress (Balasubramanian *et al.,* 2021), including tolerance to heat, drought, and salinity (Boechat *et al.,* 2020). It can tolerate up to 6.8 and 4.5 deciSiemens per meter (dS m^{-1}) of soil and water salinity, respectively, as measured by the electrical conductivity of the dissolved ions (Yukun *et al*., 2021; Calone *et al*., 2020).

Moreover, previous researches had investigated the numerous mechanisms through which *Sorghum bicolor* can sequestrate HMs from water (phytofiltration); how its roots immobilize the HMs availability in the soil (phytostabilization); and phytoextraction – the accumulation of HMs by shoots (Osman *et al.,* 2023). The plant had also been reported to effectively carryout phytoremediation of individual HMs using both key approaches of phytostabilization (accumulation and sequestration of HMs in the root region, making their mobility or bioavailability decreased and stabilizing them in the substrate); and phytoextraction: the accumulation of HMs that had been extracted from soils, sediments or water in the shoot regions (Moreira *et al.,* 2021).

Additionally, a review of the literature highlighted numerous research gaps. Firstly, to the best of our research there was no previous study that simulated the major source of heavy metals contamination in Katsina (which is the study area chosen in this research) in mesocosm experiments, nor was there a study that chronosequentially evaluated the phytoremediation of such mesocosms to allowable heavy metals breakpoints based on EU/EPA standards. Moreover, there is a lack of studies on local cultivars from Katsina with potential phytoremediation abilities, which measure their growth performance in mixed heavy metal contaminations and their phytoremediation ability, among others.

The contributions of this research centre around the evaluation of the ability of a fast-growing local cultivar of *S. bicolor* (*rirrik'a/rirritsa/mota* in Hausa) from Riko village, Jibiya L.G.A., Katsina State, Nigeria to remediate mesocosms containing mixed HMs contaminations *in situ* matching pollution levels within a heavy-metals rich industry Katsina metropolis to residual concentrations matching USEPA/EU limits, as effectively as (or even more effectively than) allochthonous plants. The novelty of the research is in terms of the selected HMs, the chosen cultivar, the research design, and the need to solve the identified research problems by bridging the research gaps.

2. Method

2.1. Research Design, Sample Collection and Processing

The design adopted by this research is a chronosequential, nutrient-poor mesocosms approach. The ability of the *S. bicolor* seeds to be used for phytoremediation was confirmed extensively in earlier experiments (Riko *et al.,* 2022). Thereafter, mesocosms simulating the heavy metals content of the then DANA Steel Rolling Mills, Katsina (NAK Steel Rolling and Processing Mills Limited, Katsina) were constructed, and used to monitor the growth parameters of the *S. bicolor* in a chronosequential manner (biweekly), together with residual amounts and percentage removal of the selected HMs (Singh *et al.,* 2022; Gagnon *et al.,* 2020). The design is summarized in Fig. 1. and Fig. 2, and the detailed composition of each mesocosm was stated in Table 1.

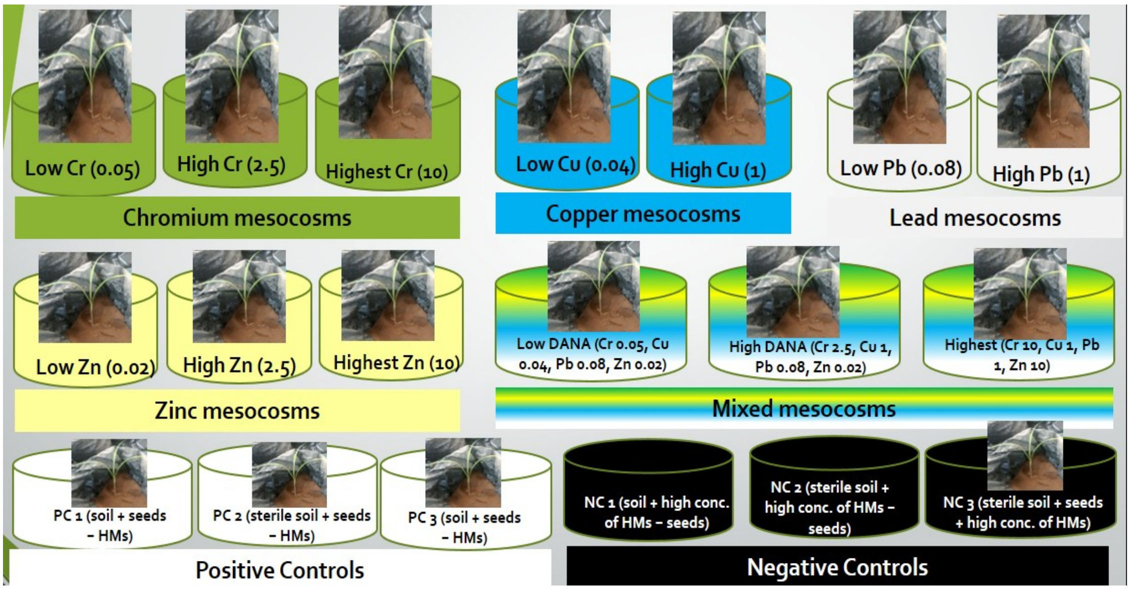

Fig. 1. Summary of the Research Design: composition of the heavy metals and control mesocosms used in the research

Table 1. Mesocosms Used in the Phytoremediation Experiments, Abbreviations and Compositions

Experimental Group	Treatment & Abbreviation	Composition
Negative control	Negative control 1/NC	Soil sample + high concentrations of heavy metals (2.5 g of Cr, 1 g Cu, 1g Pb and 2.5g Zn per Kg of mesocosm soil) but no plants
	Negative control 2/NC 2	Autoclaved soil samples + high concentrations of heavy metals ((2.5 g of Cr, 1 g Cu, 1g Pb and 2.5g Zn per Kg of mesocosm soil)) but no plants
	Negative control 3/ NC 3	Autoclaved soil sample + heavy metals (2.5 g of Cr, 1 g Cu, 1g Pb and 2.5g Zn per Kg of mesocosm soil) + plants
Positive control	Positive control 1/PC	Soil sample + plants, no heavy metals, 1st batch
	Positive control 2/PC 2	Autoclaved soil sample + plants, no heavy metals
	Positive control 3/PC 3	Soil sample + plants, no heavy metals, 2nd batch
Low Concentrations of Zn, Pb, Cr and Cu	Zn 0.02 g/L	0.02 g Zn/Kg of mesocosm soil
	Pb 0.08 g/L	0.08 g Pb/Kg of mesocosm soil
	Cr 0.05 g/L	0.05 g Cr/Kg of mesocosm soil
	Cu 0.04 g/L	0.04 g Cu/Kg of mesocosm soil
High Concentrations of Cr, Zn, Cu and Pb	Cr 10 g/L	10 g Cr/Kg of mesocosm soil
	Cr 2.5 g/L	2.5 g Cr/Kg of mesocosm soil
	Zn 10 g/L	10 g Zn/Kg of mesocosm soil
	Zn 2.5	2.5 g Zn/Kg of mesocosm soil
	Cu 1	1 g Cu/Kg of mesocosm soil
	Pb 1	1 g Pb/Kg of mesocosm soil
Mixture of the tested HMs at low concentrations	Low DANA/DANA Low	Soil containing heavy metals concentrations matching lower boundaries of concentrations of heavy metals at the DANA Steel Rolling Mill, i.e. Cr 0.05 g, Cu 0.04 g, Pb 0.08 g, and Zn 0.02g, per Kg of soil.
Mixture of the tested HMs at high concentrations	DANA High/High DANA	Soil containing heavy metals concentrations matching upper boundaries of concentrations of heavy metals at the DANA Steel Rolling Mill, i.e. Cr 2.5 g, Cu 1 g, Pb 1 g; and Zn 2.5 g, per Kg of soil.
	Highest/Highest Conc.	Soil containing maximum HMs concentrations that the *S. bicolor* seeds tolerated *in vitro*, i.e. Cr 10 g, Cu 1g, Pb 1 g, and Zn 10g, per Kg of soil

The selection of the heavy metals involved in this study and their concentrations (Chromium, Copper, Lead and Zinc, at concentrations of 0.05-10, 0.04-1, 0.08-1, and 0.02-1 g/L, respectively) is based on a number of criteria, including: the concentrations/level of pollution of these heavy metals at the chosen model site (i.e. the steel rolling mill) and other areas containing high levels of heavy metals contamination in Katsina, and how the degree of soil contamination compares to international standards, and the toxicity of the metals Cr and Pb/their roles as plant nutrients, i.e. Cu and Zn (Darma & Riko, 2022).

The *S. bicolor* cultivar (a fast growing, high-yielding cultivar known in Hausa as *rirrik'a/rirritsa/mota*) and the soil samples used in this study were collected through composite sampling (Perez-Rodriguez *et al*., 2020) in late September, 2021, from a sorghum farm (sampling location obtained from GoogleMaps: Latitude = 13.199489 °N and Longitude = 7.624501 °E) in the outskirts of Riko Village, Jibiya L.G.A., Katsina State. The village is located at ~ Latitude 12.9772 °N, and Longitude 7.4353 °E (Fig. 3.) at an elevation of 414 feet above sea level (Elevationmap.net, 2021). Both the rhizospheric soil and the sandy soil predominant in the study area were collected in the ratio 1:3 (~ 15:5 kg), from 0-15 cm depths, using a clean hand trowel, in two instalments (Kinuthia *et al.,* 2020). The ears of the sorghum were dried, thrashed, chaffed, the seeds separated from them, collected and placed in a clean polythene bag (Stofejova *et al.,* 2021), and taken to the microbiology laboratory, Umaru Musa 'Yar'adua University, Katsina, for further analyses. Similarly, the soil samples were dried, mixed thoroughly, sieved using a metal sieve of 0.5 mm pore diameter, and immediately transported to the lab for further analyses. Additionally, the pH of the collected soil samples was measured by preparing a slurry in a 1:10 solid to liquid phase ratio of (2g of soil in 20 mL distilled water), shaken thoroughly and measured using calibrated electrometric pH meter (ExTech) after the meter had stabilized (Kicinska *et al.,* 2021).

Fig. 2. Research Design (continued). Top left = measuring the growth parameters of the plants during the phytoremediation, centre = measuring the stem girth of the plants; c = digesting the soil samples for AAS; bottom left = AAS machine, right = SpectrAA software for AAS analyses

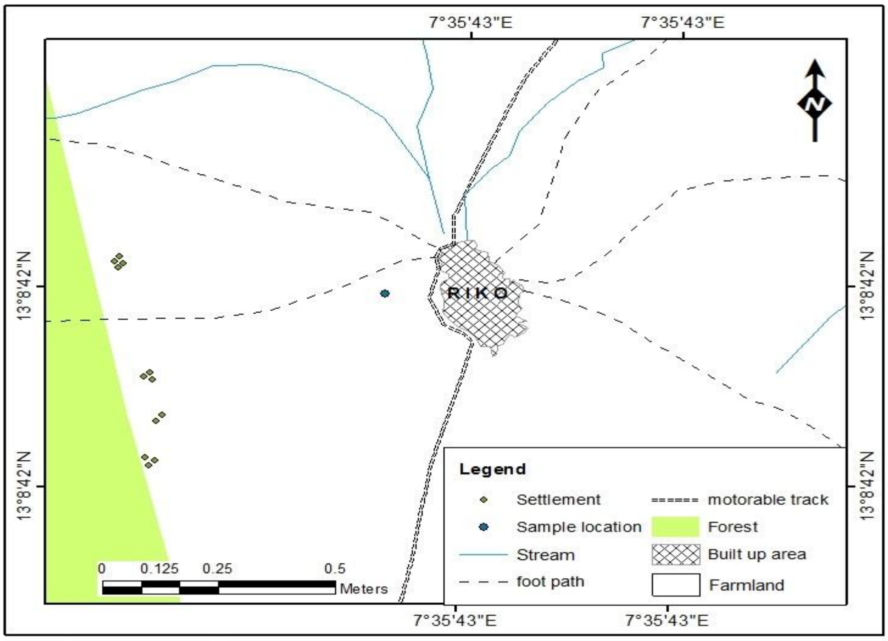

Fig.1; Map of Riko Vlage Showing Sample Location
Source:- National Aeronoutic and Space Administration Spot image 2021

Fig. 3. Map of Riko village, from where the cultivar and soil samples were collected

2.2. Phytoremediation Experiments and Measurements of Growth Rate, Shoot Tolerance Index and Stem Girth

The phytoremediation experiments were carried out in a greenhouse constructed using transparent light blue polythene bags and mosquito nets (to facilitate transpiration and allow for the regulation of temperature) at the Botanical Garden, Department of Biology, UMYU, Katsina, from December, 2021-February, 2022, as adapted modified from the protocols of Ahmad *et al.* (2022) and Bulgakov *et al.* (2022). The greenhouse has the following dimensions: 270 (length) × 118 (breath) × 59 (height). Viable seeds (confirmed using specific gravity/floatation method), that had been surface-sterilised seeds and subjected to dormancy breakage through hydropriming were germinated in sterile distilled water for 48 hours, and then sown in experimental mesocosms comprising of double layers of polybags (Viva, Decent Polybag, Kano) measuring ~ 50 × 40 cm, in which 2 kg of a soil mix (1500 g rhizospheric soil: 500 g sandy soil) was added. The soil was mixed with solutions of heavy metals salts containing concentrations matching those selected for the heavy metals described in the research design section. Double layers were used to prevent leaching of the heavy metals solution, and breakage of the bag when the plants matured. Those seeds were sown in duplicates of 8 seeds per experimental microcosm. The average temperature of the mesocosms was measured using a thermometer (Bulgakov *et al.,* 2022), and was determined to be 35 ± 1.73 °C. Furthermore, autoclaved sandy soil was used in some controls, to study the effects of minimising microbial effects on the phytoremediation (Kabeer *et al*, 2021). The mesocosms used and their compositions were as earlier stated in table 3.1. Each mesocosm was watered regularly (at 48 hour intervals) till the conclusion of the research period (2 months).

The parameters used to monitor the plant growth include the growth rate, stem girth and shoot tolerance index. These were calculated using the formulae below:

$$GR = \frac{Length\ of\ the\ plant\ at\ termination\ of\ experiment\ (56\ days) - Length\ of\ plant\ at\ the\ initiation\ of\ experiment}{Duration\ of\ the\ experiment\ (56\ days)} \times 100 \tag{1}$$

$$STI = \frac{Average\ shoot\ length\ of\ metal\ treated\ plants}{Average\ length\ of\ shoot\ in\ distilled\ water\ treated\ plants\ i.e.\ control} \times 100 \tag{2}$$

$$Stem\ Girth = MS + LC \times VSR \tag{3}$$

Where: GR = Growth Rate; STI = Shoot Tolerance Index; MS = Main Scale Reading of the Vernier calipers, LC = Least Count of the Vernier calipers (= 0.1 cm/10 = 0.01 cm) and VSR = Vernier Scale Reading.

The growth rate was calculated as modified from Chen *et al.* (2022), the shoot tolerance index from Kokavcová *et al.* (2023), as whereas the stem girth/width of the plants was determined using vernier callipers (Perez-Rodriguez *et al*., 2020).

2.3. Measuring the Residual Heavy Metals Contents: Sample Digestion and Atomic Absorption Spectrometry

In accordance with the chronosequential nature of this research, soil samples from the mesocosms were collected at two weeks-intervals (i.e. 2 weeks, 4 weeks, 6 weeks and 8 weeks) and used to determine the residual heavy metals contents (Singh *et al.,* 2022). Composite soil samples (0.5 g) were collected from each experimental treatment, and digested using the commonest wet digestion process optimized for high recovery of metals from soil samples, as described by Zhao *et al.* (2023), involving nitric and perchloric acids (HNO_3:$HClO_4$), at a ratio of 2:1, and concentrations of 65% and 70%, respectively, were used, and they were heated in a hot-air oven until yellow and white fumes were observed. Briefly, to the beaker containing the soil sample, 5 mL of HNO_3 and 2.5ml of $HClO_4$ were added, and the mixture was boiled till the appearance of fumes. Thereafter, 10 mL of distilled water was added to the mixture, and refluxed (to boiling), until all the fumes were released and a clear liquid was observed. The solution was allowed to cool at room temperature, and particulate matter was filtered using WhatMan No. 1 filter paper. The final filtrate volume was bought to 50 mL by washing the inner sides of the beakers with distilled water, in a 60 mL plastic reagent bottle. The bottles were stored at 4°C before analysis using Atomic Absorption Spectrometry (Ullah *et al.,* 2022). The contents of the selected heavy metals (Cr, Cu, Pb and Zn) in the digested soil samples were evaluated using Agilent 55B Spectrometer equipped with SpectrAA software (version 5.0), according to the protocols of Yahaya *et al.* (2021); and Ramírez *et al.* (2024).

The results of the AAS analysis (ppm) were converted to rates of heavy metals removal day, by dividing the total removal by the experimental days, and the % HM bioremoval was calculated by dividing the difference of the residual heavy metals at the time of testing to the initial heavy metal concentration by the initial heavy metals concentrations, and multiplying by 100.

2.4. Statistical Analyses

All values were stated correct to 2 decimal places, or approximated to two significant figures, where necessary. Statistical analyses were performed using SPSS, version 23.0, AnalyStat (version 1.6.50) and MicrosoftExcel (2016) was used to calculate the heavy metal removal rates (ppm/day) and the percentage heavy metal removal rates. Welch test (unpaired t-test assuming unequal SD) was used for comparing sandy and rhizospheric soil pH; two-tailed paired t-test was used to compare efficiency of phytoremediation in the treatment mesocosms and the negative controls; and one-way ANOVA was used to analyse three or more dataset means. Individual means were separated using Tukey's Honest Significant Difference (HSD) test (Bukar *et al.*, 2021). All post-hoc tests indicating pairwise comparison of means (Boechat *et al.*, 2020) were reported using the compact letter display technique, where means not sharing any letter are significantly different (Schneider, 2020).

3. Results and Discussion

3.1. Features of the Collected Samples

The descriptive features of the site of collection of the samples used in this study were presented in Table 2. The seeds of the sorghum cultivar were creamy white, and weighted, on average, 0.018 ± 0.0050 g (10 replicates). The average time it takes for the seeds to germinate was 3 days (72 hours), and the plant can reach up to 214 cm when fully grown. The pH of the rhizospheric soil sample where the plant was grown was neutral (7.55) and differs from the pH of the sandy soil (7.40) when compared using Welch test/unpaired t-test assuming unequal SD for the compared samples ($p = 0.02$, $t_{cal} = 8.60$, $t_{crit} = 4.97$), even though sandy soil was dominant in the area.

Table 2. Descriptive Features of the Sample Collection Site and Collected Samples

S/No	Characteristic	Description
1	Coordinates of soil and seeds collection site	Latitude = 13.199489 °N Longitude = 7.624501 °E
2	pH of collected rhizospheric soil	7.40 ± 0.021^a
3	pH of collected sandy soil	7.55 ± 0.014^b
4	Dimensions of the *S. bicolor* cultivar from which seeds and rhizosphere soil samples were collected	Roots = 30cm Shoots = 214cm
5	Colour of the *S. bicolor* seeds	Creamy white
6	Average weight of each *S. bicolor* seed (10 replicates)	0.018 ± 0.0050 g
7	Timeframe for sample collection	September-October, 2021
8	Average time for maximal germination of the seeds	3 days (72 hours)

Key: Different letters between the means indicate statistically significant difference when compared using Welch test (unpaired t-test assuming unequal SD for the compared samples) (for pH, $p = 0.02$, $t_{cal} = 8.60$, $t_{crit} = 4.97$).

These characteristics mirrored what was reported in previous literature. For instance, the pH of the soil samples from the study area ranged from 7-4 to 7.55, and the pH of bulk soil for growing *S. bicolor* can reach up to 7.85-7.91 (Lu *et al.,* 2022). These soil profiles however do not conform with the dominant edaphic structures reported by Abdullahi *et al.* (2021), from soils in Katsina State, in that the soils were described as acidic, with pH values ranging from 6.00-6.76, nonetheless, they reported that pH values are highly variable, depending on elevation of the sample collection site, and Riko village is located at an elevation of 414 feet above sea level, as earlier stated. The Time it takes for the seeds to grow (3 day) was higher than other plants, e.g. wheat, radish and alfalfa, which, on average, take 7, 6 and 4 days to germinate, respectively (Francis *et al.,* 2022), and this indicates the fast-growing nature of the cultivar.

3.2. Growth Parameters of the *S. bicolor* Cultivar during the Phytoremediation: Growth Rate, Shoot Tolerance Index and Stem Girth

Table 3 showed the growth rates (GR) of the plants in the various mesocosms during the phytoremediation experiment. Amongst the controls, the highest average GR was obtained from PC (6.51), while the NC3 had an average GR value of 5.17 mm/day. Amongst the treatments, the highest average GR was obtained from Cr 0.05 g/L (3.71 mm/day), while the lowest was obtained from Cu 1 (0.61 mm/day). Some treatments also had negative growth rates, ranging from -0.33 mm/day in Cr 10 g/L to -3.11 mm/day in Cr 2.5 g/L, indicating that the toxicity of the heavy metals was so high that it leads to retardation and ultimately death. This observation is supported by what López -Bucio *et al.* (2022) earlier said regarding chromium leading to oxidative stress, destabilizing absorption of nutrients by plants, interfering with photosynthesis, upsetting plant metabolism, and impeding growth and development; even though Cr had been shown to, at very low concentrations, enhance root organogenesis.

In general, the growth rates tend to increase with time (even though there was an amount of variability in this trend), and the higher the concentration, the lower the growth rate. One-Way ANOVA confirmed that the duration does not significantly affect the growth rate ($p = 0.25$, $F_{cal} = 0.86$, $F_{crit} = 2.75$); however, the type of heavy metal/control treatment ($p < 0.0001$, $F_{cal} = 15.76$, $F_{crit} = 2.36$) and the concentrations of the heavy metals in the individual mesocosms ($p < 0.0001$, $F_{cal} = 20.16$, $F_{crit} = 1.91$), significantly affected the growth rate.

These observations could be understood in light of the generally known rules that high concentrations of heavy metals exert deleterious effects on plants which may lead to a reduction in their biomass/growth rate. This may occur in form of the production of free radicals and reactive oxygen species, excessive oxidation and chain reactions with key cellular macromolecules such as fats and oils, nucleic acids and proteins, oxidative stress and cellular damage, undesirable metabolic transformations, decreased growth and ultimately lower biomass (Goyal *et al.,* 2020). The fact that the duration does not significantly affect the growth rate can be linked to the fast-growing nature of the cultivar (May *et al.,* 2020) and the fact that the experiment was short (2 months) such that the growth rates of the sorghum plants had not yet peaked. Moreover, the negative growth rates that increased with time, as seen in some Cr and Zn treatments, are indicating the slow build-up of toxicity (Turkovskaya *et al.,* 2020). The small variation observed in the growth rates of PC and PC 3 may be explained by the variable pollution index between them (0.0000006 and 0.00015, for PC3 and PC, respectively), a comprehensive measure of the extent of heavy metal pollution in an environment (Krishnakumar *et al.,* 2021), and highlights the importance of replication to capture the range of possible outcomes in phytoremediation experiments.

The results of the shoot tolerance index (STI) of the plants were shown in Table 4, and they showed that plants in the PC mesocosm an average STI value of 0.55, while the NC plants had an average STI value of 0.57. Amongst the treated plants, the highest average STI was obtained from Zn 0.02 g/L (1.03), while the lowest was obtained from Cu 1 g/L (0.11). By and large, the STI values tend to decrease as the time (weeks) increase, and the higher the concentration, the lower the STI. One-way ANOVA revealed that the time (weeks) and the type of heavy metal had no significant influence on the STI ($p = 0.08$, $F_{cal} = 2.34$, $F_{crit} = 2.77$; and $p = 0.91$, $F_{cal} = 0.30$, $F_{crit} = 2.31$, respectively). However, the concentration of the heavy metals in the individual mesocosms significantly affected the STI ($p = 0.0001$, $F_{cal} = 20.16$, $F_{crit} = 1.91$).

Table 3. Growth Rates of the *Sorghum bicolor* Plants in the Presence of Various Concentrations of the Heavy Metals

S/No	Experimental Treatments	Heavy Metal Concentrations (g/L)	Average growth rate of the *Sorghum bicolor* plants ± Standard Deviation (mm/day)				
			2 weeks	4 weeks	6 weeks	8 weeks	Average
1	Controls ^{a}	PC ^{a}	6.27 ± 0.16	6.55 ± 0.21	7.69 ± 0.79	5.55 ± 1.40	6.51 ± 0.89
		PC 2 ^{a}	4.43 ± 0.06	4.10 ± 0.29	5.59 ± 1.36	11.28 ± 3.85	6.35 ± 3.35
		PC 3 ^{a}	5.40 ± 0.31	5.17 ± 0.21	6.30 ± 1.06	6.31 ± 1.56	5.79 ± 0.60
		NC 3 ^{ab}	6.37 ± 0.74	5.15 ± 0.38	4.00 ± 0.00	-	5.17 ± 1.18
2	Mixed HM Contamination ^{b}	Highest conc. ^{fg}	-1.00 ± 0.11	-1.04 ± 0.22	-	-	-1.02 ± 0.03
		DANA High ^{cdef}	0.75 ± 0.03	0.79 ± 0.05	1.09 ± 0.12	2.21 ± 0.92	1.21 ± 0.68
		DANA Low ^{bcd}	2.82 ± 0.22	2.95 ± 1.56	2.40 ± 0.21	2.97 ± 0.14	2.78 ± 0.27
3	Chromium ^{b}	Cr 0.05 ^{abc}	2.97 ± 0.17	2.68 ± 0.10	2.90 ± 0.25	6.30 ± 2.69	3.71 ± 1.73
		Cr 2.5 ^{g}	-1.66 ± 1.20	-4.56 ± 1.18	-	-	-3.11 ± 2.05
		Cr 10 ^{efg}	-0.55 ± 0.04	-0.34 ± 0.14	-0.21 ± 0.01	-0.23 ± 0.03	-0.33 ± 0.16
4	Copper ^{b}	Cu 0.04 ^{bcd}	2.95 ± 0.05	2.55 ± 0.65	2.38 ± 0.23	2.77 ± 0.50	2.66 ± 0.25
		Cu 1 ^{def}	0.59 ± 0.07	0.63 ± 0.04	0.58 ± 0.13	0.63 ± 0.13	0.61 ± 0.03
5	Lead ^{b}	Pb 0.08 ^{cdef}	1.62 ± 0.10	1.71 ± 0.14	1.28 ± 0.66	0.87 ± 0.08	1.37 ± 0.38
		Pb 1 ^{bcdef}	1.74 ± 0.02	1.82 ± 0.06	2.22 ± 0.19	2.87 ± 0.19	2.16 ± 0.52
6	Zinc ^{b}	Zn 0.02 ^{bcde}	2.68 ± 0.47	2.21 ± 0.06	1.72 ± 0.08	2.43 ± 0.67	2.26 ± 0.41
		Zn 2.5 ^{efg}	-0.76 ± 0.16	-0.79 ± 0.09	-0.50 ± 0.26	-0.35 ± 0.07	-0.60 ± 0.21
		Zn 10 ^{fg}	-0.48 ± 0.02	-0.53 ± 0.02	-0.58 ± 0.04	-0.88 ± 0.18	-0.62 ± 0.18

Key: days = days after transplantation, - = plant death, hence no measurement. Values were stated as average ± standard deviation of eight replicates. All mesocosm abbreviations were as described in Table 1. Down the experimental treatment and heavy metals concentration columns, treats not sharing a superscript alphabet were significantly different (Tukey's post-hoc: $p < 0.05$).

The shoot tolerance index is a measure of the shoot growth of a treatment compared to the control (Kokavcová *et al.,* 2023). The lower tolerance indexes involved in the controls may be explained by sterilization of the soil, and the neutralization of the microbial legacy effect of the resident microbiome in the rhizosphere, which lowered the ability of the seeds to grow or survive heavy metals stress, as the case may be; as the role of the rhizobacteriome in plant growth promotion and alleviation of heavy metals stress had been established in several studies before (Muratova *et al.,* 2020; Chen *et al.,* 2024; Mazzon *et al.,* 2024). The reason for Zn having a shoot tolerance index of >1, which indicates that the shoots of the plants in this mesocosm grew more than the control, can be traced to its role as an essential micronutrient in plants. However, even heavy metals serving as micronutrients exert deleterious effects in excess (Chrysygyris *et al.,* 2022), as seen in the toxicity exerted by Zn in the 2.5 and 10 g/L treatments, where the tolerance index drastically reduced to 0.50 and 0.38 respectively. The highest toxicity, as manifested in the lowest shoot tolerance index, exhibited by copper can be explained by the multiple mechanisms through which it exerts deleterious effects to *S. bicolor* seeds, including growth reduction, chlorophyll loss, and alterations in plant morphology and function, leading to a decrease in chlorophyll, total biomass and growth (Subramanian *et al.,* 2020).

Similarly, the observation that shoot tolerance index reduces with increase in time of the experiment, and that higher concentrations lead to lower STI values are all in agreement with the known fact that the exertion of toxicity effects by heavy metals in sorghum plants is gradual, leading to progressively reduced growth, chlorophyll loss, and overall adverse impacts on the plant's health and development (Mishra *et al.,* 2021). This also supports the chrnosequential nature of phytoremediation, and supports the use of chronosequence approaches in studying phytoremediation, as they explore temporal variations as they happen (Singh *et al.,* 2022; Gagnon *et al.,* 2020). Additionally, it had been established that higher concentrations of heavy metals lead to lower growth and tolerance in sorghum plants, by affecting various factors including antioxidant defense, shoot length, fresh and dry weights, chlorophyll content, relative water content, and overall biomass (Hassan *et al.,* 2020). This also explains why the type of heavy metal had no statistically significant effect on the tolerance, as the mechanisms for tolerating heavy metals in plants can be generic (Goyal *et al.*, 2020); but the concentration has, as explained before.

Table 4. Shoot Tolerance Indexes of the *Sorghum bicolor* Plants Growing under Various Concentrations of the Heavy Metals

S/No	Experimental Treatments	Heavy Metal Concentrations (g/L)	Shoot tolerance index of the *Sorghum bicolor* plants (Average ± Standard Deviation) × 100				
			2 weeks	4 weeks	6 weeks	8 weeks	Average
1	Controls	PC 2 ^{bcd}	0.49 ± 0.18	0.66 ± 0.12	0.68 ± 0.02	0.38 ± 0.32	0.55 ± 0.14
		NC 3 ^{bcd}	0.18 ± 0.02	0.69 ± 0.12	0.84 ± 0.03	-	0.57 ± 0.35
2	Mixed HM Contamination	Highest conc. ^{d}	0.35 ± 0.04	0.10 ± 0.04	0.03 ± 0.00	-	0.16 ± 0.17
		DANA High ^{d}	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.00
		DANA Low ^{ab}	0.86 ± 0.20	0.82 ± 0.05	0.72 ± 0.01	0.59 ± 0.07	0.75 ± 0.07
3	Chromium	Cr 0.05 ^{abc}	0.79 ± 0.18	0.71 ± 0.10	0.68 ± 0.01	0.55 ± 0.08	0.68 ± 0.10
		Cr 2.5 ^{bcd}	0.77 ± 0.20	0.44 ± 0.25	0.10 ± 0.00	-	0.44 ± 0.33
		Cr 10 ^{d}	0.44 ± 0.07	0.15 ± 0.06	0.07 ± 0.01	0.04 ± 0.01	0.17 ± 0.19
4	Copper	Cu 0.04 ^{ab}	0.87 ± 0.05	0.68 ± 0.06	0.75 ± 0.01	0.61 ± 0.06	0.73 ± 0.11
		Cu 1 ^{d}	0.11 ± 0.06	0.13 ± 0.10	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.00
5	Lead	Pb 0.08 ^{abcd}	0.87 ± 0.09	0.57 ± 0.06	0.54 ± 0.06	0.45 ± 0.07	0.61 ± 0.18
		Pb 1 ^{bcd}	0.25 ± 0.07	0.26 ± 0.04	0.27 ± 0.02	0.27 ± 0.02	0.26 ± 0.01
6	Zinc	Zn 0.02 ^{a}	1.39 ± 0.31	1.06 ± 0.11	0.95 ± 0.02	0.72 ± 0.11	1.03 ± 0.28
		Zn 2.5 ^{cd}	0.50 ± 0.16	0.19 ± 0.20	0.07 ± 0.03	0.01 ± 0.02	0.20 ± 0.22
		Zn 10 ^{d}	0.38 ± 0.03	0.17 ± 0.03	0.09 ± 0.02	0.04 ± 0.02	0.17 ± 0.15

Key: days = days after transplantation, - = plant death, hence no measurement. Values were stated as average ± standard deviation of eight replicates. All mesocosm abbreviations were as described in Table 1. Down the heavy metals concentrations column, individual treatments not sharing a superscript alphabet were significantly different ($p \le 0.05$) when the means were separated using Tukey's post-hoc.

The stem girth/width of the plants in the various mesocosms during the phytoremediation experiments was shown in Table 5. Amongst the controls, the highest final stem girth was obtained in the PC 3 treatment (18.5 mm), while the lowest (0.75 mm) was obtained from NC 3. Amongst the treated plants, the highest final stem girth was obtained from Cr 0.05 g/L (9.5 mm), while the lowest was obtained from Zn 10 g/L (0.75 mm). Generally, the stem girth increased with increase in time, and decreased with increase in the concentration of the HM. Statistically, the stem girth did not differ significantly with regards to the duration of the experiment/weeks ($p = 0.88$, $F_{cal} = 0.22$, $F_{crit} = 2.73$), however, the experimental groups significantly affected the stem girth ($p = 0.0003$, $F_{cal} = 9.23$, $F_{crit} = 3.12$), likewise the concentrations of the heavy metals in the individual treatments ($p < 0.0001$, $F_{cal} = 16.63$, $F_{crit} = 1.79$).

These observations can be understood in the light of the following: the positive control had the highest stem girth because of the absence of heavy metal toxicity, whereas the negative control had the lowest because of the absence of the rhizobacteriome to enhance plant growth, coupled with heavy metal stress, as explained earlier. The high stem girth in the chromium treatments is also linked to the fact that Cr exerts toxicity effects typically at higher concentrations (Kumar, 2020), but at lower doses, it may stimulate plant growth, as a previous study had stated that exposure to chromium at lower levels (0.5-1 mg/L induces hormesis as manifested in increased biomass and larger leaves (Christou *et al.,* 2020). The increase in stem girth as the experiment progresses is an obvious indicator of anabolism/synthesis of organic matter, growth, increase in biomass, and to a lower extent, heavy metl accumulation. However, the increase in stem girth over time was not statistically significant, and this can be explained by the reduction in the ability of the crops to grow uninhibitedly, due to the heavy metals presence, nevertheless, the type and concentration of heavy metals affected the stem girth, which points at the different ways through which individual heavy metals exert toxicity on plants (Goyal *et al.,* 2020). Generally, Ameh *et al.* (2020) had reported that heavy metals lead to lower stem girths in plants.

Furthermore, the lower shoot heights and stem girths in this study can be explained by both the nature of the cultivar used (known to be usually short), the high concentrations of heavy metals used, which exceed the concentrations found in agricultural soils, and matched the very high concentrations of the industrial site chosen for the study and the short duration of the study. The plasticity of height in *S. bicolor* had been submitted before (Lu *et al.,* 2022). Additionally, dwarfism in *Sorghum* had a genetic basis: traditionally, sorghum plant height was tied to about five dwarfing (Dw) genes, namely: *Dw 1-5* (Habyarimana *et al*., 2020); and recent experiments had reported a fifth gene, *Dw5*. Furthermore, some other genes interfering with plant height had been confirmed in previous literature, including genes controlling for brassinosteroid and gibberellin metabolism (Yu *et al.*, 2022). Height in sorghum is a complexly regulated process that is controlled by about 42 SNPs (single nucleotide polymorphisms) found at 7 chromosomes (Habyarimana *et al.*, 2020).

Table 5. Stem Girth/Width of the Plants Exposed to Various Concentrations of Heavy Metals During the Phytoremediation Experiments

Experimental Treatments	Heavy Metals Concentrations (g/L)	Duration of the experiments			
		Week 2	**Week 4**	**Week 6**	**Week 8**
		Stem girth/width of the plants (mm)			
Control ^{b}	PC	7.14 ± 0.19	10.50 ± 3.54	11.50 ± 3.53	12.50 ± 3.19
	PC 2	7.50 ± 3.54	12.50 ± 3.24	8.50 ± 2.12	7.50 ± 2.12
	PC 3	10.50 ± 2.75	15.50 ± 4.19	17.50 ± 3.35	18.50 ± 3.53
	NC 3	2.75 ± 0.35	2.75 ± 0.35	1.75 ± 0.35	0.75 ± 0.25
High ^{c}	Highest concentration	3.05 ± 0.35	2.75 ± 0.53	0 ± 0.00	0 ± 0.00
	DANA High	4.00 ± 0.33	3.00 ± 0.19	2.70 ± 0.33	1.75 ± 0.35
	Cr 10	4.25 ± 1.06	4 ± 0.71	3.50 ± 2.02	3 ± 1.41
	Zn 10	1.75 ± 0.19	1.25 ± 0.23	0.75 ± 0.35	0.75 ± 0.35
	Cr 2.5	1.50 ± 0.35	2.75 ± 0.53	2.75 ± 0.35	3.75 ± 0.35
	Zn 2.5	1.45 ± 0.30	1.75 ± 0.25	2.25 ± 1.06	2.75 ± 0.35
	Cu 1	4.25 ± 1.06	3.75 ± 1.77	2.5 ± 1.41	1.60 ± 0.33
	Pb 1	8.50 ± 3.54	2.75 ± 0.35	2.25 ± 0.35	1.45 ± 0.55
Low ^{a}	DANA Low	2.10 ± 0.35	2.75 ± 0.35	7.5 ± 0.71	5.50 ± 2.12
	Zn 0.02*	15.50 ± 3.32	11.50 ± 2.45	8.50 ± 2.12	4.5 ± 2.02
	Cu 0.04*	7.50 ± 2.12	6.50 ± 2.01	4 ± 1.41	3.25 ± 0.35
	Pb 0.08*	8.50 ± 3.54	7.50 ± 3.14	4.50 ± 2.01	4.25 ± 1.76
	Cr 0.05*	10.50 ± 3.19	8.50 ± 3.19	8.50 ± 3.53	9.5 ± 3.12

Key: Values were stated as average ± standard deviation of duplicate measurements. All mesocosm abbreviations were as described in Table 1. Down the experimental treatments column, treatments not sharing a superscript alphabet were significantly different ($p < 0.05$); and down the heavy metal concentrations (g/L) columns, treatments followed by an asterisk were not significantly different from PC ($p = 0.076$) when means were separated using Tukey's post-hoc.

3.3. Heavy Metals Bioremoval in the Phytoremediation Mesocosms: Percentage Bioremoval, Bioremoval Rates and Ability of the Plants to Remediate the Soils to USEPA/EU Limits

The results of the heavy metals bioremoval were presented in Table 6 and Table 7. Table 6 showed the biweekly percentage bioremoval of the HMs in the mesocosms. The highest final percentage bioremovals in the controls, for Cr, Cu, Pb and Zn were obtained from the PC 3 (84.92%), NC 3 (99.13%), PC (76%) and PC 3 (87%) mesocosms, respectively; while the lowest were obtained from NC 2 (33.43%), PC (61.82%), PC (39.13) and NC (33.00%), respectively.

In the HM treated mesocosms, the highest bioremoval percentages for Cr, Cu, Pb and Zn were obtained from the Cr 10 g/L (92%), Cu 0.04 g/L (90.5%), Pb 0.08 g/L (80.94%) and Zn 10 g/L (Zn 95%), mesocosms; while the lowest were obtained from Cr 2.5 g/L (36%), Cu 1 g/L (17.40%), and Low DANA (46% and 58.57%), respectively.

Table 6. Biweekly Percentage Bioremoval of the Heavy Metals in the Phytoremediation Mesocosms

Treatments	Percentage Heavy Metal Removal															
	2 Weeks ^{c}				4 Weeks ^{b}				6 Weeks ^{ab}				8 Weeks ^{a}			
	Cr	**Cu**	**Pb**	**Zn**	**Cr**	**Cu**	**Pb**	**Zn**	**Cr**	**Cu**	**Pb**	**Zn**	**Cr**	**Cu**	**Pb**	**Zn**
NC	30.00	20.87	9.00	18.00	40.33	78.26	13.24	22.00	49.67	79.13	18.14	30.00	57.67	84.35	60.24	33.00
NC 2	1.43	23.00	6.73	36.00	5.71	66.00	8.27	36.00	9.71	70.40	32.33	44.00	33.43	96.60	47.33	50.00
NC 3	17.11	64.35	44.10	70.00	41.44	79.13	58.81	72.00	48.00	84.35	62.19	78.00	53.11	99.13	69.90	81.00
PC	17.85	38.18	2.85	32.00	26.31	54.55	13.55	54.00	29.23	60.00	33.90	64.00	77.38	61.82	76.00	78.00
PC 2	1.71	50.00	2.33	13.33	17.86	67.50	21.80	13.33	37.71	82.00	32.33	20.00	50.71	99.00	39.13	33.33
PC 3	6.46	56.00	1.81	77.00	12.92	74.00	28.26	79.00	24.31	88.67	30.32	86.00	84.92	93.33	50.65	87.00
Highest Conc.*	7.67	33.04	20.00	63.00	7.67	33.04	57.67	63.00	15.89	35.65	60.05	69.00	80.22	40.87	60.05	79.00
High DANA *	19.56	8.48	37.90	77.00	27.11	49.70	45.48	77.00	39.89	56.36	47.33	81.00	80.22	66.06	52.43	82.00
Low DANA *	25.29	30.67	5.88	24.29	41.29	45.33	38.00	37.14	42.71	56.00	39.88	50.00	52.71	64.00	46.00	58.57
Cr 0.05	6.71	N	N	N	28.71	N	N	N	60.57	N	N	N	61.14	N	N	N
Cr 2.5	2.80	N	N	N	6.00	N	N	N	16.00	N	N	N	36.00	N	N	N
Cr 10	80.00	N	N	N	82.00	N	N	N	87.00	N	N	N	92.80	N	N	N
Cu 0.04	N	42.50	N	N	N	76.25	N	N	N	88.75	N	N	N	90.50	N	N
Cu 1 *	N	4.00	N	N	N	11.00	N	N	N	15.00	N	N	N	17.40	N	N
Pb 1	N	N	40.15	N	N	N	40.63	N	N	N	40.85	N	N	N	61.11	N
Pb 0.08	N	N	16.29	N	N	N	22.71	N	N	N	40.47	N	N	N	80.94	N
Zn 0.02	N	N	N	21.43	N	N	N	37.14	N	N	N	68.57	N	N	N	72.86
Zn 2.5	N	N	N	52.00	N	N	N	72.40	N	N	N	78.80	N	N	N	84.00
Zn 10	N	N	N	87.20	N	N	N	88.30	N	N	N	92.10	N	N	N	95.00

Key: Weeks indicate time after transplantation, N = not applicable. Values were stated as averages of duplicate measurements. Treatment abbreviations were as described in Table 1. Across the four heavy metals (row), treatments not sharing a superscript alphabet were significantly different ($p < 0.05$) when separated using Tukey's posthoc test. Down the treatments column, treatments followed by an asterisk were not significantly different from the NC 3 mesocosm when separated using Tukey's posthoc ($p = 0.63$).

Generally, the % bioremoval increased with increase in time, however, the trend for percentage bioremoval differs with increase in concentration, in Cu, it decreased, but in Zn, Pb and Cr, it increased, with slight variations. One-way ANOVA demonstrated that statistically, the duration of the experiment (weeks) significantly affected the % bioremoval ($p = 0.0002$, $F_{cal} = 6.82$, $F_{crit} = 2.63$), likewise the concentration of HMs in the individual mesocosms ($p < 0.0001$, $F_{cal} = 8.14$, $F_{crit} = 1.6$.

Similarly, the rates of the heavy metals bioremoval (ppm/day) were presented in Table 7. The results showed that the highest removal rate for Cr, Cu, Pb and Zn were obtained from the mesocosms: Cr 10 g/L (0.066), PC 2 (0.071), Pb 0.08 g/L (0.058) and Zn 10 g/L (0.068) ppm/day, respectively, while the lowest were obtained from the NC 2 (0.024), Cu 1 (0.012), PC 2 (0.028) and NC/PC2 (0.024 each) ppm/day, respectively. In general, the removal rate increased with increase in time, and, by and large, decreased with increase in concentration.

Table 7. Biweekly Percentage Bioremoval of the Heavy Metals in the Phytoremediation Mesocosms

	Heavy Metal Removal Rates (ppm/day)															
Treatments	**2 weeks**				**4 weeks**				**6 weeks**				**8 weeks**			
	Cr	**Cu**	**Pb**	**Zn**	**Cr**	**Cu**	**Pb**	**Zn**	**Cr**	**Cu**	**Pb**	**Zn**	**Cr**	**Cu**	**Pb**	**Zn**
NC	0.021	0.015	0.006	0.013	0.029	0.056	0.009	0.016	0.035	0.057	0.013	0.021	0.041	0.060	0.043	0.024
NC 2	0.001	0.016	0.005	0.026	0.004	0.047	0.006	0.026	0.007	0.050	0.023	0.031	0.024	0.069	0.034	0.036
NC 3	0.012	0.046	0.031	0.050	0.030	0.057	0.042	0.051	0.034	0.060	0.044	0.056	0.038	0.071	0.050	0.058
High DANA	0.014	0.006	0.027	0.055	0.019	0.035	0.032	0.055	0.028	0.040	0.034	0.058	0.057	0.047	0.037	0.059
Low DANA	0.018	0.022	0.004	0.017	0.029	0.032	0.027	0.027	0.031	0.040	0.028	0.036	0.038	0.046	0.033	0.042
Cr 0.05	0.005	N	N	N	0.021	N	N	N	0.043	N	N	N	0.044	N	N	N
Cr 2.5	0.002	N	N	N	0.004	N	N	N	0.011	N	N	N	0.026	N	N	N
Cr 10	0.057	N	N	N	0.059	N	N	N	0.062	N	N	N	0.066	N	N	N
Cu 0.04	N	0.030	N	N	N	0.054	N	N	N	0.063	N	N	N	0.065	N	N
Cu 1	N	0.003	N	N	N	0.008	N	N	N	0.011	N	N	N	0.012	N	N
Pb 1	N	N	0.029	N	N	N	0.029	N	N	N	0.029	N	N	N	0.044	N
Pb 0.08	N	N	0.012	N	N	N	0.016	N	N	N	0.029	N	N	N	0.058	N
Zn 0.02	N	N	N	0.015	N	N	N	0.027	N	N	N	0.049	N	N	N	0.052
Zn 2.5	N	N	N	0.037	N	N	N	0.052	N	N	N	0.056	N	N	N	0.060
Zn 10	N	N	N	0.062	N	N	N	0.063	N	N	N	0.066	N	N	N	0.068
PC	0.013	0.027	0.002	0.023	0.019	0.039	0.010	0.039	0.021	0.043	0.024	0.046	0.055	0.044	0.054	0.056
PC 2	0.001	0.036	0.002	0.010	0.013	0.048	0.016	0.010	0.027	0.059	0.023	0.014	0.036	0.071	0.028	0.024
PC 3	0.005	0.040	0.001	0.055	0.009	0.053	0.020	0.056	0.017	0.063	0.022	0.061	0.061	0.067	0.036	0.062
Highest	0.005	0.024	0.014	0.045	0.005	0.024	0.041	0.045	0.011	0.025	0.043	0.049	0.057	0.029	0.043	0.056

Key: Weeks indicate the time after transplantation, N = not applicable. Values were stated as averages of duplicate measurements. Treatment abbreviations were as described in Table 1.

In this research, the use of greenhouse pot experiments and uniform soil of the same texture in the research design helped ensure that the effects of soil texture and other soil parameters such as initial pH, electrical conductivity, texture etc. were reduced to the barest minimum in the research (Ahmad *et al.,* 2022).

The bioremoval results are understandable in the context of the composition of the individual phytoremediation mesocosms. The controls lacking heavy metal treatments tend to have higher removal rates, likewise mesocosms bereft of microbial activity tend to have lower rates. This further supports the role of microorganisms in the phytoremediation process as described before. The statistically significant impacts of heavy metal concentration and time (chronosequence) on the phytoremediation efficiency had also been discussed previously.

The increase in % bioremoval with increase in concentration of the heavy metals can be related to the bioavailability of the metals when a high concentration of the particular metal is used, and the high accumulation ability of the sorghum biomass (Turkovskaya *et al.,* 2020). Similarly, the low bioremoval rates seen in the controls had to do with absence of heavy metals, plants or microorganisms, as explained earlier.

Generally, the indices of phytoremediation efficiency in this research tend to show no significant differences with regards to the heavy metal treatment mesocosms and the duration of the experiment i.e. weeks (e.g.). Thus, the concentration of HMs and not their type was the most influential variable in phytoremediation. This indicates the phytoremediation ability of the plants was not restricted to a particular heavy metal/concentration; the plant can remediate soils contaminated with a variety of heavy metals; and that the phytoremediation proceeds at approximately the same pace biweekly. Numerous researches had shown that sorghum can be used in the phytoremediation of an array of heavy metals, including Al, As, Cu, Cd, Cr, Fe, Mn, Ni, Pb, Zn, etc. (Boechat *et al.,* 2020; Shaltout *et al.,* 2021; Riko *et al.,* 2022; Pasichna *et al.,* 2024). Additionally, Li *et al.* (2021) had shown that Cd bioremoval in sorghum proceeds at a relatively constant pace.

When the success of the phytoremediation experiments was evaluated based on the ability of the plants to bring the final residual HM concentrations to within limits set by the USEPA/EU (Al Disi *et al.,* 2022), it was found out that at the conclusion of the experiments, 4/12 treatments (33.33%) in the negative control were successfully remediated to values within EPA/EU standard, compared to 8/12 in the positive control (66.67%) and 12/22 (54.55%) in the treatments. Thus, the HM treatment mesocosms more efficiently remediated the soils compared to the negative controls (two-tailed paired t-test: $p = 0.0002$, $T_{cal} = 4243.0$, $T_{crit} = 12.71$). Statistical analysis using one-way ANOVA showed that the success of the phytoremediation (ability to remediate soil HM concentrations to within EPA/EU limits) depended on the type of HM ($p < 0.0001$, $F_{cal} = 14.00$, $F_{crit} = 2.73$) but not the concentrations ($p = 0.70$, $F_{cal} = 0.80$, $F_{crit} = 1.79$). This supports the use of the plants to remediate the chosen heavy metals at the high concentrations expected to be found at the industrial site simulated in this study. This also shows that the plants can remediate low, moderate or high levels of the HMs, but the toxicity of the individual metals delimits the phytoremediation efficacy. Previously, Osman *et al.* (2023) had demonstrated the ability of sorghum to phytoremediate Taken together, these results proved that phytoremediation using the plant is associated with less toxicity and higher efficiency (Mendy *et al*., 2021).

The high percentage bioremoval observed in the PC 3 mesocosm indicate the natural role of *S. bicolor* as a phytoremediator even in the absence of high HM stress, and this is related to the microbial legacy effect, since the collected soil samples habour autochthonous microbiota from the *Sorghum* farm that had been perennially in association with the plants (Hannula *et al.,* 2022).

At the end of the experiment, the mesocosms demonstrated similar average bioremoval of the heavy metals in all the treatments, specifically 63.34% for Cr, 67.46% for Cu, 58.53% for Pb and 69.48% for Zn ($p = 0.64$, $F_{cal} = 0.56$, $F_{crit} = 2.83$). The phytoremediation experiment is considered successful as Mann-Whitney U test confirmed that the final average residual level of HMs in the mesocosms did not differ with the baseline of EPA/EU acceptable limits ($p = 0.23$, $U_{cal} = 20.0$, $U_{crit} = 13.0$). This observation supported the potential of the plant to be used in remediating soils with high polyelement anomalies (Muratova *et al.,* 2020), such as the simulated industrial site.

4. Conclusion

This study demonstrated the use of the fast-growing local cultivar of *S. bicolor* obtained from Riko village, Jibia L.G.A., Katsina State, Nigeria, in remediating the simulated poly-heavy metals (Cr, Cu, Pb and Zn) contaminated soils obtainable at the DANA Steel Rolling Mills, Katsina, to within USEPA/EU tolerable limits for heavy metals in soils. On the whole, 66.67% of the HMs (69.48% of Zn, 67.46% of Cu, 63.34% of Cr and 58.33% of Pb) were successfully remediated to residual limits below or matching the said limits (Mann Whitney U test: $p = 0.23$), with bioremoval rates being statistically similar across the HMs tested (one-way ANOVA: $p = 0.64$). Therefore, this research verified the status of this crop as a suitable agent for safe, effective phytoremediation of industrial heavy metal contaminated sites. Its use in on-the-field phytoremediation of hotspots of HM contamination within the study area and beyond, including industries, is recommended, towards sustainable and eco-friendly management of environmental wastes from industrial pollution through phytoremediation.

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