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Comparative phytoremediation potential and heavy metal accumulation patterns in *Amaranthus viridis* and *Lactuca sativa*: Implications for food safety and environmental management in contaminated agroecosystems

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ABSTRACT

This study comprehensively evaluated the phytoremediation potential and physiological responses of *Amaranthus viridis* and *Lactuca sativa* to heavy metal contamination (Cd, Pb, Hg, and Zn) under controlled screen house conditions at Bayero University, Kano, Nigeria. Using a randomized complete block design with three replicates, plants were grown in both uncontaminated and contaminated sandy soil, with metal concentrations verified weekly via flame atomic absorption spectrometry (FAAS). Growth parameters (leaf number, shoot length, leaf area, fresh/dry weight, and biomass) were measured alongside heavy metal accumulation in roots, stems, and leaves, with bioconcentration (BCF) and translocation factors (TF) calculated to assess uptake and mobility. Results demonstrated *A. viridis* exhibited superior tolerance to metal stress, maintaining higher growth metrics than *L. sativa* under contamination (e.g., 8.5 vs. 4.8 leaves under Cd stress). Both species accumulated metals beyond WHO/FAO safety limits, with *A. viridis* roots showing exceptional Pb retention (35.00 mg/kg) and significant Cd/Hg translocation (TF>1), while *L. sativa* preferentially stored Hg in roots (7.30 mg/kg) but displayed unexpected Pb mobility (TF=3.81). Zinc accumulation remained within safe limits for both species. The BCF values highlighted *A. viridis* as an effective Phyto stabilizer for Pb (BCF=3.50) and *L. sativa* for Zn (BCF=1.24). Statistical analysis ($p>0.005$) confirmed species-specific responses were significant. These findings suggest *A. viridis* is suitable for phytoremediation in heavily contaminated sites, while *L. sativa* may be cultivated with caution in moderately polluted soils, provided Pb levels are monitored. The study advances the practical selection of plants for metal-polluted agroecosystems, balancing ecological restoration and food safety. Recommendations include field trials to validate these screen house observations and molecular studies to elucidate the mechanisms behind *L. sativa*'s anomalous Pb translocation.

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INTRODUCTION

Heavy metal contamination of agricultural soils has emerged as a critical environmental challenge, posing significant risks to ecosystem health, food security, and human well-being, particularly in rapidly industrializing regions (Wuana & Okieimen, 2011). The Sudan Savannah zone of Nigeria, where this study was conducted, exemplifies this growing crisis, with escalating pollution driven by artisanal mining, untreated wastewater irrigation, and improper disposal of industrial effluents (Abdussalam & Kabir, 2020). Among the most concerning contaminants are cadmium (Cd), lead (Pb), mercury (Hg), and zinc (Zn), toxic metals that persist in soils and bioaccumulate in food chains. Notably, Pb and Cd have been classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC, 2023), while Hg exposure is linked to severe neurological disorders (WHO, 2023). Even Zn, an essential micronutrient, becomes phytotoxic at elevated concentrations (White *et al.*, 2023).

Phytoremediation, the use of plants to extract, stabilize, or detoxify contaminants, has gained traction as a cost-effective and ecologically sustainable solution for metal-polluted soils (Ali *et al.*, 2023). However, its success hinges on identifying species with optimal metal uptake, tolerance, and biomass production traits. This study focuses on two contrasting but economically significant species: *Amaranthus viridis* (slender amaranth) and *Lactuca sativa* (lettuce). *A. viridis*, a C4 plant, exhibits remarkable abiotic stress tolerance, owing to its efficient antioxidant systems, metal-chelating phytochelatins, and rapid growth rate (Zhang *et al.*, 2023). These traits make it a promising candidate for phytoremediation in tropical regions. In contrast, *L. sativa*, a globally cultivated leafy vegetable, is highly sensitive to metal toxicity but serves as a critical indicator species for food safety risk assessments (Singh *et al.*, 2023). While previous studies have examined metal accumulation in these species independently (Kumar *et al.*, 2024; Rahman *et al.*, 2024), a systematic comparison of their bioconcentration (BCF) and translocation (TF) factors under identical experimental conditions remains lacking. This research aimed to: Compare the growth performance (morphological and biomass parameters) of *A. viridis* and *L. sativa* under Cd, Pb, Hg, and Zn stress; Quantify species-specific differences in metal accumulation patterns across roots, stems, and leaves; Evaluate their phytoremediation potential using BCF and TF indices; Assess compliance of edible plant parts with WHO/FAO food safety standards.

By addressing these objectives, the study provides actionable insights for environmental managers and policymakers. For instance, *A. viridis's* ability to tolerate and translocate metals (e.g., TF>1 for Cd and Hg) suggests its utility in phytoextraction systems, whereas *L. sativa*'s restricted metal translocation (except for Pb) may inform safer cultivation practices in marginally contaminated soils. Furthermore, the findings contribute to the broader framework of "Phyto management," which seeks to balance ecological restoration with agricultural productivity in polluted landscapes (Pandey *et al.*, 2023). Future research directions include field validations of these screen house observations and molecular investigations into the mechanisms driving *L. sativa*'s anomalous Pb translocation (TF=3.81), a phenomenon with implications for food chain contamination.

MATERIALS AND METHODS

Study Area/Experimental Site

The research was conducted at the screen house of the Department of Plant Biology and Biotechnology, Bayero University, Kano, situated at the old Campus. The site is located between Latitude 11.2333 and Longitude 12.3833 in the Sudan Savannah ecological zone of Nigeria.

Analytical Procedures for Soil Analysis

Determination of soil pH

The study adopted the method reported by Ifenna & Osuji (2013) without modification. In this method, a 20.0 g soil sample was mixed with 40.0 mL of distilled water in 1:2 ratios. The suspension was stirred intermittently with a glass rod for 30 minutes and was left for one hour. The probe of the pH meter was inserted into the supernatant for two minutes, and the pH was recorded.

Determination of soil temperature

A mercury-in-glass thermometer was used to determine the temperature of the soil. The thermometer was calculated according to the manufacturer's instructions to ensure accuracy. The thermometer probe was inserted into the soil at the desired depth (usually 5-10 cm) and location. The thermometer was allowed to equilibrate with the soil temperature for a minimum of 30 minutes. A temperature reading was taken from the thermometer. The temperature reading, along with the date, time, and location, was recorded (Baver *et al.*, 1972).

Determination of electrical conductivity

The method described by Wagh *et al.* (2025) was adopted for the determination of electrical conductivity of a soil sample. This was determined using an Equiptronics digital electrical conductivity bridge, for which 20.0 g of soil was added to 40.0 mL of distilled water. The suspension was stirred intermittently for half an hour and was kept for 30 minutes without any disturbances for complete dissolution of soluble salts. The soil was allowed to settle down, and the conductivity cell was inserted in the solution, and the EC values were read and recorded.

Determination of organic carbon (OC)

A representative soil sample was collected from the desired location and depth. The soil sample was air-dried and ground to pass through a 2-mm sieve (Nelson & Sommers, 1996). The soil sample was treated with 1N hydrochloric acid (HCl) to remove inorganic carbon (Walkley & Black, 1934). The organic carbon in the soil sample was oxidized using potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4) (Walkley & Black, 1934). The excess potassium dichromate was titrated with ferrous sulfate ($FeSO_4$) to determine the amount of organic carbon oxidized (Walkley & Black, 1934). The organic carbon content in the soil was calculated using the following formula:

Organic Carbon (%) = (A x B x C)/(D x E)

Where:
A = Volume of potassium dichromate used (mL)
B = Normality of potassium dichromate
C = Equivalent weight of carbon
D = Weight of soil sample (g)
E = 1000

Determination of nitrogen in soil

The nitrogen content in the soil was determined using a combination of extraction and analysis techniques. A representative soil sample was collected from the desired location and from the top 0-20 cm depth (Peech, 1965). The soil sample was then air-dried and ground to pass through a 2-mm sieve (Nelson & Sommers, 1996). The nitrogen was extracted from the soil using 2M potassium chloride (KCl) for ammonium-N (NH_4+-N) and nitrate-N (NO_3-N) (Keeney & Nelson, 1982), and digestion with sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) for total nitrogen (TN) (Bremner, 1996). The extracted nitrogen was then analyzed using colorimetry for NH4+-N and NO3-N (Keeney & Nelson, 1982), and Kjeldahl digestion and titration for TN (Bremner, 1996). The nitrogen content in the soil was calculated using the formula: N (%) = (N concentration in extract x extract volume)/soil weight.

Phosphorus in the soil

The determination of phosphorus in the soil sample was done using Olsen's Method (ASTM, 2007). Exactly 2.00 g of air-dried soil sample (passed in a 2 mm sieve) was weighed into a 125 mL Erlenmeyer flask, and 5.00 mL of 18.0 M of sulphuric acid was added with 0.400 g of ammonium persulfate and boiled until a final volume of about 10.0 mL was reached. The solution was filtered and made up with distilled water to 40.0 mL. And 5.00 mL of Antimony Molybdate was added to the solution, followed by the addition of 2.00 mL of ascorbic acid. The blank and standard solutions were subjected to the same treatment as above. After about 10-20 minutes, the absorbance of the sample, standard and blank solutions were measured with a violet spectrophotometer at a wavelength of 680 nm. The calibration curve was obtained for a standard solution of 1.00, 2.00, 3.00, 4.00, and 5.00 ppm phosphate, and the concentration of the samples was obtained from the calibration curve using the absorbance of the samples.

Determination of potassium (K) in the soil

A soil sample was collected from the desired location and depth. The soil sample was air-dried and ground through a 2-mm sieve. The potassium was extracted from the soil using 0.5N hydrochloric acid (HCl). The extract was filtered through filter paper. The potassium concentration was extracted using atomic absorption spectroscopy (AAS). The potassium content in the soil was calculated using the formula: K (mg/kg) = (K concentration in extract x extract volume)/soil weight. The potassium content in the soil was expressed as milligrams per kilogram (mg/kg) (Jackson, 1958).

Source of Seeds for Terrestrial Plants

The *Amaranth* seeds and Lettuce seeds were obtained from the National Institute of Horticultural Research (NIHORT), Bagauda, Kano.

Sowing

The seeds were sown on the 7th of January 2024, by broadcasting method and lightly covered with soil and mulched to avoid losing seeds by wind or during watering (Hassan *et al.*, 2021).

Fertilizer Application

NPK 20:10:10 was applied at the rate of 10g/pot by side placement three weeks after planting (Hassan *et al.*, 2021).

Irrigation

A Watering can was used to irrigate the plants at a day interval throughout the duration of the study (Hassan *et al.*, 2021).

Preparation of the Standard Heavy Metal Solution (Stock Solution)

The stock solutions (1000 mg/L) of Pb, Cd, Hg, and Zn were prepared using analytical-grade reagents (Pb(C□H□O□)□·3H□O, CdCl□, HgCl□·2½H□O, and ZnSO□·7H□O; BDH, England) in ultrapure distilled water (resistivity 18.2 MΩ· cm at 25 °C) to minimize impurities. To ensure accuracy, all glasswares were pre-soaked in 10% HNO□ for 24 hours and rinsed with deionized water before use. Working standards were prepared daily by serial dilution, with concentrations verified using FAAS (PerkinElmer AAnalyst 400) against NIST-traceable reference materials (recovery rates: 95-102%) (Fatma *et al.*, 2022).

Experimental Set Up

Amaranthus viridis and *Lactuca sativa* were grown in 8 kg of sandy soil (collected from Kano River) that had been pre-tested for baseline heavy metal content Pb (0.1±0.3 mg/kg),Cd (0.4±0.1 mg/kg), Hg (0.1±0.02 mg/kg), Zn (3.2±2.1 mg/kg). The experiment followed a Randomized Complete Block Design (RCBD) with three biological replicates per treatment (n=24 pots per species) and included negative controls (0 mg/L metals) irrigated with distilled water only. Pots were fitted with drainage holes and pre-irrigated for 48 hours to stabilize soil conditions. Plants were exposed to heavy metal treatments at concentrations of Cd (0, 2.0, 4.0, 6.0, 8.0 mg/L), Pb (0, 1.0, 5.0, 10.0, 20.0 mg/L), Hg (0, 5.0, 10.0, 15.0, 20.0 mg/L), and Zn (0, 10.0, 15.0, 20.0, 30.0 mg/L). Treatment verification was performed weekly by sampling irrigation water and analyzing metal concentrations via FAAS to confirm consistency (±5% deviation from targets). Plants were monitored every 14 days for morphological changes and harvested after 56 days. Roots, stems, and leaves were separately processed using trace-metal-clean techniques (agate mortar, acid-washed containers) to prevent cross-contamination during heavy metal analysis (Hassan *et al.*,2021).

Extraction of Heavy Metals from Plants

The extraction of heavy metals from plant tissues followed a standardized acid digestion protocol to ensure complete dissolution of metal constituents. After harvesting, plant

samples (roots, stems, and leaves) were thoroughly washed with deionized water to remove soil particles and surface contaminants. The samples were then oven-dried at 80 °C for 48 hours to achieve constant weight. Dried plant tissues were ground to a fine powder using an agate mortar and pestle to ensure homogeneity.

For acid digestion, approximately 0.5 g of each powdered sample was weighed into 50 mL digestion tubes. A mixture of concentrated nitric acid (HNO□, 65%) and hydrogen peroxide (H□O□, 30%) in a 4:1 ratio was added to each sample. The digestion process was conducted using a block digester at 120 °C for 2 hours until a clear solution was obtained. After cooling, the digestate was filtered through Whatman No. 42 filter paper and diluted to 50 mL with deionized water.

Metal concentrations (Cd, Pb, Hg, and Zn) in the digestates were quantified using flame atomic absorption spectrometry (FAAS, PerkinElmer Analyst 400) with appropriate calibration standards and quality control measures (Priyanka *et al.*, 2021; Fatma *et al.*, 2022).

Bioconcentration Factor

Bioconcentration factor was used as an index to determine the quantity of metal extracted by the plant from soil and water (Mellem *et al.*, 2009). The Bioconcentration factor (BCF) is a measure of the plant's ability to absorb and accumulate metal from its growth medium, which in this case is soil and water. The BCF index (Bianconi *et al.*, 2013) can be calculated using the formula,

$$BCF = \frac{\text{Concentration of metal in plant root}}{\text{Concentration of metal in water / soil}}$$

Translocation Factor

Translocation factor (TF) is a value that indicates the ability (or inability) of a plant to move metal from the root to the shoot region of the plant. This ratio (Yoon *et al.*, 2006) is given by the following relation.

$$TCF = \frac{\text{Concentration of metal in shoot}}{\text{Concentration of metal in root}}$$

Statistical Analysis

The data obtained from the research was subjected to two-way analysis of variance (ANOVA) using Microsoft Excel spreadsheet and Statistical Analysis Software (SAS).

RESULT

Table 1 below presents a comparative analysis of the performance of *Amaranthus viridis* and *Lactuca sativa* grown in control (uncontaminated) and contaminated soil samples, assessing various growth parameters in response to different heavy metals (Cd, Pb, Hg, and Zn). *A. viridis* exhibited higher values across all parameters compared to *L. sativa*, indicating greater tolerance to heavy metal contamination. For instance, in control soil, *A. viridis* had a higher number of leaves (11.20±1.00 under Cd) compared to *L. sativa* (5.30±0.62 under Cd), a trend that persisted in contaminated soil (8.50±0.60 vs. 4.80± 0.78 under Cd). Similar patterns were observed for shoot length, leaf area, fresh weight, dry weight, and plant biomass, with *A. viridis* consistently outperforming *L. sativa*. Contaminated soil generally reduced growth metrics in both species, though the decline was more pronounced in *L. sativa*. For example, *A. viridis* dry weight decreased from 0.69±0.01 (control) to

Table 1: Performance of *Amaranthus viridis* and *Lactuca sativa* in control and contaminated soil samples at the end of the experiment

S. No.	Plant Sample	Parameter	Soil Sample	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	Zn (mg/kg)
1	*Amaranthus viridis*	Number of Leaves	Control	11.20±1.00	10.20±0.90	9.20±0.90	8.10±0.50
			Contaminated	8.50±0.60	7.80±0.70	7.20±0.80	6.30±0.40
		Shoot Length (cm)	Control	7.10±0.40	7.10±1.23	6.40±0.80	6.80±0.71
			Contaminated	6.50±0.50	6.20±0.60	5.80±0.70	5.90±0.80
		Leaf Area (cm^2)	Control	25.30±1.20	24.80±1.10	23.50±1.00	22.70±0.90
			Contaminated	20.10±1.00	19.80±0.90	18.50±0.80	17.20±0.70
		Fresh Weight (g)	Control	15.70±0.33	14.80±0.10	13.20±0.10	12.90±0.12
			Contaminated	14.50±0.50	14.20±0.30	13.00±0.20	12.50±0.40
		Dry Weight (g)	Control	0.69±0.01	0.71±0.21	0.66±0.11	0.65±0.15
			Contaminated	0.62±0.05	0.60±0.04	0.55±0.03	0.50±0.04
		Plant Biomass (g)	Control	16.39±0.34	15.51±0.31	13.86±0.21	13.55±0.27
			Contaminated	15.12±0.55	14.80±0.34	13.55±0.23	13.00±0.44
2	*Lactuca sativa*	Number of Leaves	Control	5.30±0.62	4.10±1.00	4.00±1.30	4.20±0.61
			Contaminated	4.80±0.78	3.30±1.00	3.30±1.41	3.10±1.30
		Shoot Length (cm)	Control	3.80±1.26	3.70±0.61	3.60±0.36	3.50±0.40
			Contaminated	3.50±0.50	3.20±0.40	3.00±0.30	2.80±0.20
		Leaf Area (cm^2)	Control	12.50±0.80	11.80±0.70	10.90±0.60	10.20±0.50
			Contaminated	9.80±0.60	9.20±0.50	8.50±0.40	7.90±0.30
		Fresh Weight (g)	Control	17.30±0.00	17.80±0.30	15.10±0.72	15.20±0.33
			Contaminated	15.30±0.21	15.00±0.21	14.30±0.11	14.80±0.23
		Dry Weight (g)	Control	0.77±0.30	0.79±0.10	0.15±0.52	0.76±0.30
			Contaminated	0.36±0.10	0.34±0.21	0.29±0.01	0.20±0.10
		Plant Biomass (g)	Control	18.07±0.30	18.59±0.40	15.25±1.24	15.96±0.63
			Contaminated	15.66±0.31	15.34±0.42	14.59±0.12	15.00±0.33

0.62±0.05 (contaminated) under Cd, while *L. sativa* showed a sharper reduction from 0.77±0.30 to 0.36±0.10. Statistical significance ($p>0.005$) suggests these differences are not due to random variation, reinforcing *A. viridis* as a more resilient species under heavy metal stress.

Table 2 presents the mean heavy metal concentrations (Cd, Pb, Hg, Zn) in roots, stems, and leaves of *A. viridis* and *L. sativa* grown in control and contaminated water samples, compared with maximum allowable limits (WHO/FAO/FSSAI). For *A. viridis* under control conditions, Cd (0.89 mg/kg) and Hg (2.25 mg/kg) were detected only in roots, while Pb was undetected (ND) across all plant parts, and Zn was highest in roots (4.78 mg/kg) but negligible stems and leaves. Under contamination, roots accumulated the highest levels of Pb (35.00 mg/kg) and Zn (5.00 mg/kg), while stems showed elevated Hg (3.50 mg/kg) and Cd (2.80 mg/kg), and leaves contained lower but detectable levels of all metals (e.g., Cd: 1.80 mg/kg; Pb: 4.50 mg/kg), suggesting partial translocation from roots. For *Lactuca sativa* in control samples, only Hg (3.69 mg/kg) and Zn (18.34 mg/kg) were detected in roots, with no traces in stems or leaves. Under contamination, roots accumulated the highest Zn (24.70 mg/kg) and Hg (7.30 mg/kg), while stems retained moderate Pb (5.60 mg/kg) and Hg (2.30 mg/kg), and leaves showed minimal metal uptake (e.g., Cd: 0.40 mg/kg; Pb: 0.50 mg/kg), indicating restricted translocation compared to *A. viridis*. Both species exceeded permissible limits (Cd: 0.2; Pb: 2.5; Hg: 0.05 mg/kg) in contaminated samples, particularly for Pb (*A. viridis* roots: 35.00 mg/kg) and Hg (*L. sativa* roots: 7.30 mg/kg), while Zn levels remained within safe limits (99.4 mg/kg). Notably, *A. viridis* demonstrated higher metal accumulation in edible parts (leaves), posing potential health risks, whereas *L. sativa* retained most metals in roots, suggesting safer consumption if leaves are the primary harvest. With *p*>0.005, the data robustly highlights species-specific uptake patterns: *A. viridis* favors metal translocation to shoots, while *L. sativa* restricts metals to roots. These findings are critical for risk assessment in agricultural pollution, emphasizing *A. viridis*'s suitability for phytoremediation and *L. sativa*'s relative safety as a leafy vegetable in mildly contaminated systems, adhering to WHO/FAO standards for environmental and public health relevance.

Table 3 presents the Bioconcentration Factor (BCF) and Translocation Factor (TF) for *A. viridis* and *L. sativa* grown in control and contaminated soil conditions, providing insights into their heavy metal uptake and translocation capabilities. For *A. viridis* under control conditions, the BCF values were relatively low for Cd (0.15), Hg (0.15), and Zn (0.24), while Pb was not detected (ND), and TF values were zero for all detected metals, indicating minimal translocation from roots to shoots. Under contaminated conditions, *A. viridis* exhibited higher BCF values for Pb (3.50) and Cd (0.75), suggesting significant root accumulation, particularly for Pb. The TF values for Cd (1.02) and Hg (3.13) exceeded 1.0, demonstrating efficient translocation to aerial parts, while Pb (0.27) and Zn (0.80) showed more restricted movement.

For *L. sativa* in control conditions, only Hg (BCF: 0.25) and Zn (BCF: 0.92) were detected in roots, with no translocation (TF: 0.00), while Cd and Pb were undetected. Under contamination, *L. sativa* showed moderate BCF values for Cd (0.63), Hg (0.49), and Zn (1.24), with Zn demonstrating the highest root accumulation. Notably, Pb exhibited a TF of 3.81, indicating substantial translocation despite a low BCF (0.16), while other metals had TF values below 1.0 (Cd: 0.45; Hg: 0.36; Zn: 0.08), suggesting limited shoot translocation.

The data ($p>0.005$) highlights key species-specific differences: *A. viridis* efficiently translocated Cd and Hg to shoots under contamination, making it suitable for phytoremediation but potentially risky for consumption, whereas *L. sativa* showed preferential Pb translocation despite lower root uptake. Both species accumulated Zn effectively, though *L. sativa* had a higher BCF (1.24), indicating stronger root retention. These findings underscore the importance of species selection for metal-contaminated sites. *L. airidis* for extraction purposes and *L. sativa* for safer cultivation with monitored Pb levels. The results align with phytoremediation and food safety strategies, providing actionable insights for environmental management.

Table 2: Mean heavy metal concentration (mg/kg) in roots, stems and leaves of *Amaranthus viridis* and *Lactuca sativa* from control and contaminated water samples compared with standards values

S. No.	Plant Species	Treatment	Plant Part	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	Zn (mg/kg)
1	*A. viridis*	Control	Root	0.89±0.03	ND	2.25±0.05	4.78±0.07
			Stem	0.00±0.00	ND	0.00±0.00	0.01±0.00
			Leaves	0.00±0.00	ND	0.00±0.00	0.00±0.00
		Contaminated	Root	4.50±0.10	35.00±0.20	1.50±0.05	5.00±0.10
			Stem	2.80±0.08	5.00±0.12	3.50±0.08	2.50±0.08
			Leaves	1.80±0.06	4.50±0.10	1.20±0.05	1.50±0.06
2	*L. sativa*	Control	Root	ND	ND	3.69±0.08	18.34±0.20
			Stem	ND	ND	0.00±0.00	0.00±0.00
			Leaves	ND	ND	0.00±0.00	0.00±0.00
		Contaminated	Root	3.80±0.08	1.60±0.04	7.30±0.12	24.70±0.25
			Stem	1.30±0.04	5.60±0.10	2.30±0.05	1.20±0.04
			Leaves	0.40±0.02	0.50±0.02	0.30±0.02	0.80±0.03
	Maximum Allowable Limits in vegetables (mg/kg) A			0.2	2.5	0.05	99.4

A = WHO/FAO/FSSAI (Abdussalam & Kabir, 2020), ND = Not Detected

Table 3: Bioconcentration Factor (BCF) and Translocation Factor (TF) for *Amaranthusviridis* and *Lactuca sativa* exposed to control and contaminated soil conditions

S. No.	Plant Species	Treatment	Heavy Metal	BCF	TF
1	*A. viridis*	Control	Cd	0.15	0.00
			Pb	ND	ND
			Hg	0.15	0.00
			Zn	0.24	0.00
		Contaminated	Cd	0.75	1.02
			Pb	3.50	0.27
			Hg	0.10	3.13
			Zn	0.25	0.80
2	*L. sativa*	Control	Cd	ND	ND
			Pb	ND	ND
			Hg	0.25	0.00
			Zn	0.92	0.00
		Contaminated	Cd	0.63	0.45
			Pb	0.16	3.81
			Hg	0.49	0.36
			Zn	1.24	0.08

DISCUSSION

The present findings demonstrate significant differences in heavy metal accumulation and physiological responses between *Amaranthus viridis* and *Lactuca sativa* under contaminated conditions, providing important insights for phytoremediation and food safety applications. The growth performance data (Table 1) reveal that *A. viridis* exhibited greater tolerance to heavy metal stress compared to *L. sativa*, with less pronounced reductions in morphological parameters across all metal treatments. This observation aligns with recent studies documenting the remarkable stress tolerance of Amaranthus species, attributed to their efficient antioxidant systems and metal chelation capacity (Zhang *et al.*, 2023; Kumar *et al.*, 2024). The maintenance of relatively high growth rates in *A. viridis* under contamination suggests this species possesses superior physiological mechanisms for coping with metal toxicity, possibly through enhanced production of phytochelatins and metallothioneins as reported by Wang *et al.* (2023).

The heavy metal distribution patterns (Table 2) show distinct species-specific accumulation strategies. *A. viridis* accumulated exceptionally high Pb concentrations in roots (35.00 mg/kg), exceeding WHO/FAO safety limits by 14-fold, while maintaining substantial translocation to aerial parts. This finding corroborates recent work identifying *Amaranthus* species as effective Phyto stabilizers for Pb-contaminated soils (Li *et al.*, 2023; Chen *et al.*, 2024). Interestingly, *L. sativa* demonstrated preferential Hg accumulation in roots (7.30 mg/kg), surpassing safety limits by 146-fold, yet showed limited translocation to edible parts. This pattern suggests *L. sativa* may employ root sequestration as a primary detoxification mechanism, consistent with observations by Singh *et al.* (2023) in other leafy vegetables. The Zn accumulation patterns in both species, particularly the high root concentrations in *L. sativa* (24.70 mg/kg), support recent findings on essential metal homeostasis under stress conditions (White *et al.*, 2023).

The bioconcentration and translocation factors (Table 3) provide critical insights into metal management strategies. *A. viridis* exhibited TF>1 for Cd and Hg under contamination, indicating its potential for phytoextraction applications. This supports emerging evidence that certain *Amaranthus* cultivars can effectively mobilize and translocate these metals (Goswami *et al.*, 2023). The exceptionally high Pb BCF (3.50) in *A. viridis* roots suggests this species could be particularly valuable for Pb Phyto stabilization, complementing recent field studies by Rahman *et al.* (2024). For *L. sativa*, the unexpected Pb translocation factor (TF=3.81) despite low root accumulation presents an interesting contrast to most published data (Abdullahi *et al.*, 2023), possibly indicating novel transport mechanisms requiring further investigation.

From an environmental management perspective, these findings have important implications. The heavy metal concentrations in edible plant parts, particularly the Pb levels in *A. viridis* leaves (4.50 mg/kg), far exceed safety limits and corroborate growing concerns about food chain contamination (FAO/WHO, 2023). However, the relatively low metal accumulation in *L. sativa* leaves suggests this species may be safer for cultivation in marginally contaminated soils, supporting recommendations by the Food Safety and Standards Authority (FSSAI, 2023). The differential metal partitioning patterns between species reinforce the need for plant-specific remediation strategies (Pandey *et al.*, 2023) and highlight the importance of considering both BCF and TF values when selecting species for phytoremediation (Yan *et al.*, 2024).

The contrasting physiological responses observed between *A. viridis* and *L. sativa* under metal stress contribute to ongoing discussions about plant adaptation strategies. The relatively stable biomass production in *A. viridis* despite metal accumulation suggests efficient detoxification mechanisms, possibly involving vacuolar sequestration as described by Sharma *et al.* (2023). In contrast, the significant biomass reduction in *L. sativa*, particularly in dry weight (57% decrease under contamination), indicates greater sensitivity to metal toxicity, consistent with findings for other sensitive crops (Gupta *et al.*, 2024). These differences underscore the importance of species selection for specific remediation or agricultural applications.

CONCLUSION

This study demonstrates that *Amaranthus viridis* and *Lactuca sativa* exhibit fundamentally different strategies for coping with heavy metal (Cd, Pb, Hg, and Zn) contamination. *A. viridis* showed superior tolerance to metal stress, maintaining higher growth metrics and biomass production under contamination compared to *L. sativa*. The species effectively accumulated Pb in roots (35.00 mg/kg) while demonstrating significant translocation of Cd and Hg to shoots (TF>1), highlighting its potential for Phyto extraction applications. In contrast, *L. sativa* displayed preferential root sequestration of Hg (7.30 mg/kg) but unexpectedly high Pb mobility (TF=3.81), raising concerns about its safety as a food crop in Pb-contaminated soils. Both species exceeded WHO/FAO safety

limits for Pb and Hg in edible tissues, though Zn accumulation remained within permissible thresholds. These findings underscore the importance of species-specific approaches in managing metal-polluted agro ecosystems, *A. viridis* for remediation and *L. sativa* for cautious cultivation with rigorous Pb monitoring.

RECOMMENDATIONS

Phytoremediation Applications

Deploy *A. viridis* in Pb- and Cd-contaminated sites due to its high root accumulation (BCF=3.50 for Pb) and shoot translocation capacity. Field trials should validate its performance under real-world conditions.

Investigate *A. viridis*'s Hg phytoextraction potential further, given its high TF (3.13), possibly through chelate-assisted phytoremediation to enhance efficiency.

Food Safety Measures

Restrict *L. sativa* cultivation in soils with >1.0 mg/kg Pb due to its anomalous Pb translocation. Instead, prioritize its use in Zn-contaminated soils (BCF=1.24) where risks are lower.

Implement routine screening of leafy vegetables for Pb and Hg in the Sudan Savannah region, where irrigation with contaminated water is prevalent.

Policy and Farmer Engagement

Develop extension programs to educate farmers on species selection based on soil metal profiles, promoting *A. viridis* for phytoremediation and *L. sativa* only in low-Pb soils.

Advocate for stricter enforcement of WHO/FAO heavy metal limits in agricultural produce, particularly for Pb and Hg.

Future Research

Elucidate the molecular mechanisms behind *L. sativa*'s Pb translocation using transcriptomics or X-ray fluorescence imaging to identify transport pathways.

Explore genetic engineering or breeding to enhance *A. viridis*'s metal specificity and *L. sativa*'s exclusion capabilities.

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