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Dynamics and Bioaccumulation of Heavy Metals in *Amaranthus Species* Cultivated on Anthropogenically Impacted Soils.

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ABSTRACT

Heavy metal contamination in agricultural soils, particularly from cadmium (Cd) and lead (Pb), presents persistent environmental and public health threats. While numerous studies address individual species or ignore spatial variability, this study investigates both metal accumulation and interspecies differences across real-world, anthropogenically impacted sites. The objective is to support safer food production and sustainable urban agriculture practices. Three *Amaranthus* species (*A. hybridus*, *A. cruentus*, and *A. spinosus*) were cultivated across eight sites representing industrial zones, urban markets, and rural farmlands. Soil and plant samples were analyzed for Cd and Pb concentrations using atomic absorption spectrophotometry. Bioaccumulation factors (BAFs) were calculated to assess uptake efficiency. Spatial patterns and species-specific trends were evaluated using boxplot analysis and Principal Component Analysis (PCA).Cd levels were highest in industrial and rural farmlands, while Pb concentrations peaked in urban markets. *A. cruentus* showed consistent Pb accumulation, whereas *A. spinosus* demonstrated increased Cd uptake at select sites. PCA confirmed species- and site-specific accumulation patterns, with *A. hybridus* and *A. cruentus* exhibiting high BAFs (>1.0) at Sites 1, 2, and 7. Strong interspecies correlations were observed, while weak intra-species Cd–Pb correlations suggested independent accumulation mechanisms. Metal concentrations often exceeded food safety thresholds, revealing health risks and highlighting the influence of site-specific pollution and species traits on metal uptake. Regular soil monitoring, site-targeted risk assessment, and deploying *A. hybridus* and *A. cruentus* as phytoremediators or bioindicators are recommended to ensure safer food systems in peri-urban agricultural settings.

KEYWORDS: Anthropogenic activities, contaminated soils, Environmental pollution, Phytoremediation potential, Soil-plant interaction, Metal uptake, Food Safety

INTRODUCTION

In recent years, growing concerns over food safety and environmental health have drawn attention to the issue of heavy metal contamination in agricultural soils. Across many parts of the world, particularly in developing countries, soils used for farming are increasingly impacted by human activities such as industrial discharge, urban runoff, waste dumping, and the use of untreated wastewater for irrigation. These practices have led to the accumulation of toxic metals like cadmium (Cd), lead (Pb), arsenic (As), and chromium (Cr) in the environment, posing serious risks not only to soil health but also to the safety of crops grown on such land (Rehman *et al*., 2023).

One of the most commonly cultivated vegetables in tropical and subtropical regions is *Amaranthus*, popularly known as amaranth. These leafy greens are highly valued for their fast growth, resilience, and rich nutritional profile. However, *Amaranthus* species are also known for their ability to absorb and accumulate heavy metals from contaminated soils (Yadav *et al*., 2022). This dual nature, nutritious yet potentially risky, makes them both important and concerning, especially when grown in areas near roadsides, refuse dumps, or industrial zones where pollution levels are often high (Adelekan & Abegunde, 2020).

The problem becomes more complex when we consider that heavy metal contamination is rarely uniform. Soils can show significant spatial differences in metal concentrations, even within the same farming area. These differences are influenced by local factors like land use, past industrial activities, irrigation practices, and natural soil properties such as pH or organic matter content (Li *et al*., 2023). Understanding how these spatial variations affects metal availability, and ultimately their uptake by food crops, is very important for assessing both environmental risks and human exposure through diet.

In addition to the metal content of soil, the specific *Amaranthus* species being cultivated matters. Different species, and even different parts of the same plant (leaves, stems, roots), can accumulate metals in varying degrees (Rahman *et al*., 2020). This shows the need to compare multiple species to better understand their individual bioaccumulation patterns and to determine which might pose greater or lesser risks to consumers. Moreover, such comparisons can offer insights into which species might be more suitable for use in soil remediation or as environmental indicators.

Despite growing research interest in heavy metals and their effects on vegetables, relatively few studies have taken a combined approach, examining both the spatial distribution of metals in soil and the bioaccumulation behavior of different *Amaranthus* species. Many investigations either focus on a single plant species or ignore the influence of soil variability altogether. This leaves a gap in our understanding of how real-world conditions shape plant-metal interactions and what this means for food safety, especially in urban and peri-urban areas where these crops are often grown (Obida *et al*., 2024).

This study sets out to address that gap. By exploring both the spatial dynamics of heavy metal contamination in human-impacted soils and the bioaccumulation patterns in selected *Amaranthus* species, we aim to paint a clearer picture of the risks involved. Our goal is to support safer food production practices, inform public health decisions, and contribute to sustainable urban agriculture, where leafy greens like amaranth can be enjoyed without fear of hidden toxins. In many urban and peri-urban communities, especially in developing countries, fresh leafy vegetables like *Amaranthus* are grown close to homes, roads, and industrial areas. This is often out of necessity, people need quick-growing, nutritious crops, and available land is usually whatever space has not been built on. But this convenience comes with a hidden cost. These soils are often exposed to pollution from vehicle emissions, open waste dumping, and industrial runoff. Over time, heavy metals like lead (Pb), cadmium (Cd), and chromium (Cr) build up in the soil, quietly becoming part of the growing environment. Although research has shown that heavy metals can accumulate in vegetables, we still do not fully understand how different locations, soil conditions, and *Amaranthus* species affect the extent of that accumulation. Most studies tend to look at just one species or a general soil profile, without considering how metal levels vary from place to place or how different *Amaranthus* types respond. This creates a gap in knowledge, one that has direct implications for food safety, urban agriculture, and even public health.

2. Materials and methods

2.1 Study area and sampling sites

Soil samples were collected from four locations representing distinct anthropogenic influences:

- Industrial Site (IS): Adjacent to manufacturing industries.
- Roadside Site (RS): Proximal to a major highway with heavy vehicular traffic.
- Urban Market Site (UMS): Located in a central urban vegetable market area.

- Rural Farmland Site (RFS): A relatively pristine agricultural area distant from major pollution sources.

2.2 Plant and soil sampling

Three *Amaranthus* species (*A. hybridus*, *A. viridis*, and *A. spinosus*) were grown on soils collected from each site. The plants were grown in the University of Ilorin Screened house for eight weeks after which they were harvested and each partitioned into roots, stems, and leaves.

2.3 Sample preparation and analysis

Plant samples were thoroughly washed, oven-dried at 70°C, and ground to a fine powder. Soil samples were air-dried, homogenized, and sieved (<2 mm). Both plant and soil samples were digested using nitric acid-perchloric acid mixtures following standard procedures (AOAC, 2005).

Heavy metal concentrations (Cd, Pb and Cu) were determined using flame atomic absorption /certified reference materials and procedural blanks.

2.4 Bioaccumulation Factor (BAF)

BAF was calculated as:

$$BAF = Cplant/Csoil$$

Where Cplant and Csoil represent the metal concentration in plant tissue and corresponding soil, respectively.

2.5 Statistical analysis

Data were analyzed using ANOVA to compare means across species, tissues, and sites. Boxplot and Principal Component Analysis Biplot was used to show the spatial distribution and compare the heavy metal distribution pattern between the species. Statistical significance was set at $p < 0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Spatial dynamics of heavy metal contamination in Human-Impacted Soils

The spatial distribution of cadmium (Cd) and lead (Pb) concentrations with their standard deviation across selected land-use types is presented in Figure 1. Cadmium concentrations ranged from 0.20 ± 0.00 mg/kg to 0.65 ± 0.11 mg/kg, while lead concentrations varied more widely, from 2.74 ± 4.72 mg/kg to 7.94 ± 7.51 mg/kg. Notably, industrial sites and rural farmland soils recorded the highest levels of Cd, whereas urban market soils exhibited the highest Pb concentrations. Substantial variability, particularly in Pb levels, was evident from the large standard deviations, suggesting heterogeneous pollution sources across sites.

Cadmium (Cd) contamination

Elevated cadmium concentrations were observed at Industrial Site 1 (0.65 ± 0.11 mg/kg), Rural Farmland Site 2 (0.62 ± 0.56 mg/kg), and Industrial Site 2 (0.60 ± 0.28 mg/kg). These values, though within the threshold limits for unpolluted soils in some regulatory frameworks (Alloway, 2019), indicate a significant anthropogenic input including vehicular emissions, agrochemical application, and industrial waste deposition, on heavy metal accumulation in soil environments (Chen *et al.,* 2022).. The high Cd content in industrial zones is likely attributable to atmospheric deposition from metallurgical operations, improper waste disposal, and emissions from combustion sources (Zhang *et al*., 2023). In rural farmlands, the presence of Cd may be linked to long-term use of phosphate-based fertilizers, which are known to contain trace levels of cadmium as impurities (Huang *et al*., 2020). The high standard deviations at some sites suggest variability in pollution sources or in metal mobility influenced by site-specific soil properties.

This trend aligns with findings by Li *et al.* (2022), who reported significant Cd accumulation in agricultural soils irrigated with wastewater and treated with chemical fertilizers in peri-urban China. Furthermore, the relatively lower Cd levels in urban market soils (0.22–0.25 mg/kg) suggest reduced direct agrochemical input, although atmospheric deposition and waste burning may still contribute to baseline levels (Rahman *et al.,* 2021).

Cadmium is nephrotoxic and osteotoxic, even at low exposure levels, and is also linked to endocrine disruption and cancer (Zhou *et al.,* 2021). Notably, Cd concentrations in rural farmlands exceed the WHO/FAO recommended maximum permissible limit of 0.3 mg/kg in agricultural soils (FAO/WHO, 2021). This raises concerns for food safety and dietary intake, especially considering the bioaccumulative nature of Cd in leafy vegetables and cereals consumed locally (Shan *et al.,* 2022).

Lead (Pb) contamination patterns

Pb concentrations demonstrated higher variability across the sites, with Urban Market Site 1 (7.94 ± 7.51 mg/kg) and Rural Farmland Site 1 (4.84 ± 4.94 mg/kg) showing the most elevated values. Although these concentrations remain below the Dutch Target Value for Pb in soils (85 mg/kg for residential areas), they are still concerning due to cumulative toxicity and potential bioaccumulation of Pb (Zhou*, et al*., 2022).

The elevated Pb concentration in urban markets is attributed to vehicular emissions, legacy contamination from leaded gasoline, and solid waste incineration, an observation consistent with recent studies that identify market centers and roadside soils in densely populated cities as Pb hotspots (Ibrahim *et al*., 2022). Similarly, Khan *et al.* (2023) documented elevated Pb levels in urban and peri-urban soils of South Asia due to traffic emissions and poor urban waste management.

Interestingly, rural farmlands also exhibited high Pb levels, likely stemming from pesticide and herbicide residues, especially those with older formulations known to contain trace lead compounds (Ekere *et al.,* 2020). Industrial sites maintained moderate Pb concentrations (4.2–4.4 mg/kg), reflecting point-source pollution from local manufacturing and metal-processing activities (Obiora *et al.,* 2023). Lead exposure continues to be a major concern due to its effects on cognitive development, cardiovascular health, and renal function. The observed Pb concentrations in soils from urban market and roadside areas reflect contributions from traffic emissions and historical use of leaded gasoline, consistent with the findings of Adeyemi *et al*. (2023), who documented Pb levels exceeding 5 mg/kg in similar urban contexts. Despite soil Pb concentrations remaining below the general USEPA threshold of 300 mg/kg, the risk of bioavailability and crop uptake in food-producing areas demands urgent attention (Zhang *et al*., 2023).

The observed concentrations of Cd and Pb in soils are of significant ecological and health concern. Both metals are non-essential, bioaccumulate in plants, and can biomagnify across trophic levels, raising risks of food chain contamination (Zhou *et al.,* 2021). Chronic Cd exposure is associated with renal impairment, skeletal damage, and carcinogenicity (WHO, 2023), while Pb is neurotoxic, particularly impairing cognitive development in children.

The substantial variability in standard deviations across sites highlights the role of localized anthropogenic activities and site-specific soil factors, such as pH, organic matter, and cation exchange capacity, which modulate metal mobility and retention (Zhang *et al.,* 2022). According to Luo *et al*. (2022), even moderate Cd contamination reduces beneficial microbial symbioses, such as mycorrhizal associations, critical for plant nutrient uptake.

The risk of bioaccumulation and biomagnification also poses long-term threats to local biodiversity. Metal-contaminated soils have been shown to alter plant diversity and cause shifts in community composition, with sensitive species gradually being replaced by metal-tolerant taxa (He *et a*l., 2023). Furthermore, runoff from these sites could introduce heavy metals into nearby water bodies, exacerbating ecological degradation in aquatic ecosystems. Comparatively, the present findings align closely with previous studies in Nigeria and other developing regions. For instance, Obiora *et al.* (2016) reported Cd and Pb concentrations in agricultural soils of southeastern Nigeria ranging between 0.10–0.30 mg/kg and 3.00–8.00 mg/kg, respectively. These consistencies suggest that the study area shares common sources of metal input and similar patterns of anthropogenic activity with other regions of the country.

The findings of this study call for the urgent need for sustainable soil and land-use management practices. Given that several sampling sites are used for food production or are situated near high-traffic areas, there is an elevated risk of chronic human exposure to Cd and Pb through food chains. Regular soil monitoring, enforcement of environmental regulations on industrial effluents, and public education on urban agriculture practices are essential.

Remediation strategies, including phytoremediation and biochar amendment, have shown promise in reducing metal bioavailability in tropical soils (Tang *et al*., 2021). Moreover, site-specific risk assessments should be integrated into urban planning, particularly in expanding cities like Ilorin, where land-use conflicts are prevalent. The adoption of best management practices will be critical to ensure environmental safety and sustainable agricultural productivity.

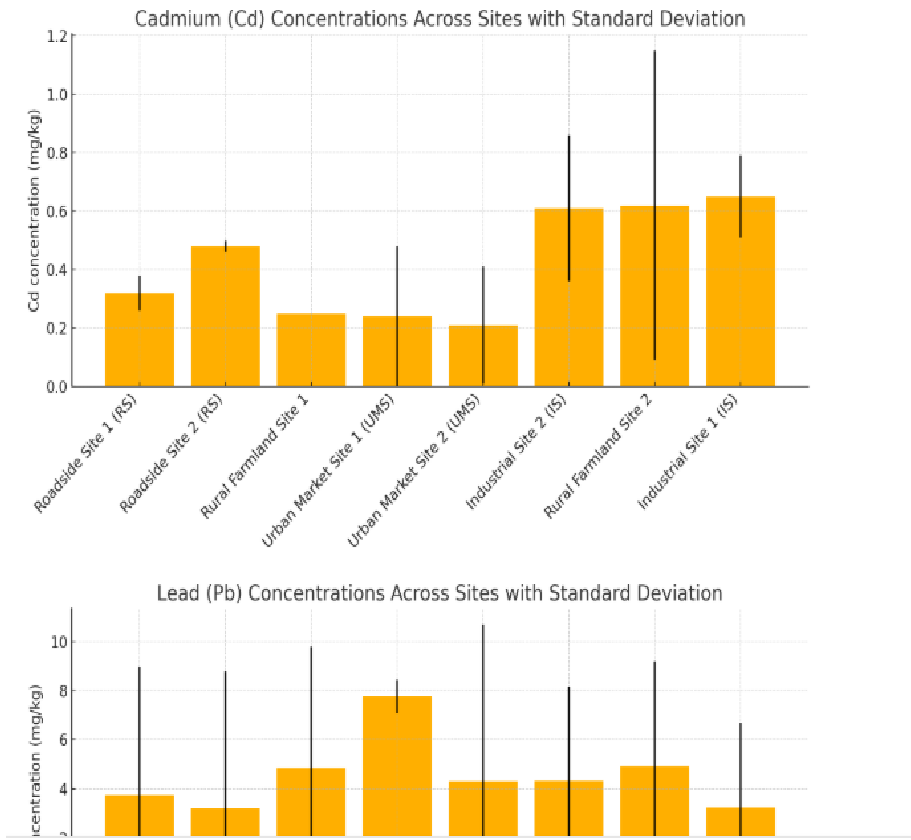


Fig 1: Spatial distribution of cadmium and lead in soils across sites

3.2. Cadmium and lead dynamics in amaranthus species

The boxplot analysis (Figure 3) presents the differential accumulation of cadmium (Cd) and lead (Pb) among the three *Amaranthus* species, *Amaranthus hybridus* (A1), *Amaranthus cruentus* (A2), and *Amaranthus spinosus* (A3), across multiple anthropogenic sites. The results indicate consistent interspecies differences in metal uptake capacity, with notable variation in Pb concentrations across species and relatively uniform patterns in Cd accumulation.

The measured Cd concentrations ranged from 0.13 ± 0.00 to 0.36 ± 0.00 mg/kg for A1, 0.09 ± 0.02 to 0.41 ± 0.03 mg/kg for A2, and 0.10 ± 0.03 to 0.27 ± 0.03 mg/kg for A3. Sites 6 and 10 consistently recorded the highest Cd levels across all species, while Site 3 exhibited the lowest concentrations. The relatively elevated levels observed at Site 6 for A2 (0.41 ± 0.03 mg/kg) suggest localized inputs of Cd, potentially linked to anthropogenic activities such as vehicular emissions, improper waste disposal, or agrochemical use.

The Cd concentration ranges for A1, A2, and A3 are relatively narrow, with median values clustering below 0.4 mg/kg. This suggests that all three species exhibited moderate Cd uptake. However, slight differences in Cd concentration levels suggest a marginally higher accumulation potential in A1 and A2 compared to A3. The limited variation may be due to the relatively lower Cd levels in soil across most sites and the lower soil–plant transfer coefficient of Cd compared to Pb (Zhou *et al*., 2022).

Cadmium accumulation is a function of plant physiology, metal bioavailability, and rhizospheric interactions. Studies by Shan *et al.* (2023) demonstrated that *A. hybridus* has higher Cd translocation efficiency than *A. spinosus*, possibly due to differences in root exudation and cell wall binding capacity. Nonetheless, the observed values across species in this study remain close to or above the WHO/FAO maximum allowable limit of 0.2 mg/kg for edible vegetables (WHO, 2021), raising concern over food safety and dietary Cd exposure in urban consumers.

The accumulation pattern observed aligns with recent findings. For instance, Li *et al.* (2020) reported Cd concentrations ranging from 0.06 to 0.95 mg/kg in leafy vegetables grown in peri-urban zones of eastern China. Similarly, Hossain *et al.* (2021) documented Cd levels between 0.12 and 1.08 mg/kg in leafy vegetables cultivated in urban and peri-urban Bangladesh, emphasizing similar bioaccumulation trends across ecologically stressed regions. The variation among sites may be attributed to differences in soil physicochemical characteristics, particularly pH, organic matter content, and cation exchange capacity (CEC), all of which influence Cd mobility and uptake by plants (Zhang *et al.,* 2022).

Notably, among the three *Amaranthus* species, *A. hybridus* (A1) and *A. cruentus* (A2) generally accumulated higher Cd concentrations than *A. spinosus* (A3), suggesting species-dependent accumulation efficiency. This observation supports earlier evidence of species-specific uptake mechanisms influenced by root morphology, differential expression of metal transporter proteins, and physiological adaptation to metal stress (Rahman *et al.,* 2022). These findings indicate the importance of integrating soil quality assessments with phytoremediation and food safety considerations when evaluating heavy metal dynamics in agroecosystems.

The concentration of lead (Pb) in the three *Amaranthus* species (*A. hybridus* – A1, *A. cruentus* , A2, and *A. spinosus* , A3) exhibited notable spatial and interspecific variation. Across all sites, Pb concentrations in plant tissues ranged from 2.15 ± 0.05 mg/kg to 7.67 ± 1.05 mg/kg, with the highest levels consistently recorded at Site 4, a location influenced by roadside or urban runoff conditions. These elevated values suggest a strong anthropogenic influence, particularly from vehicular emissions, atmospheric deposition, and contaminated irrigation sources, which are known contributors to Pb enrichment in urban agroecosystems (Luo *et al*., 2022).

The concentrations of Pb observed in the edible leaves of all three *Amaranthus* species in this study frequently exceed the Codex Alimentarius Commission's provisional guideline of 0.3 mg/kg for leafy vegetables (WHO, 2021). Chronic consumption of such contaminated vegetables poses serious health risks, particularly in vulnerable populations such as children and pregnant women. Given the high bioavailability and retention of Pb in human tissues, the ingestion of Pb-contaminated vegetables such as *Amaranthus* may contribute significantly to total daily intake (TDI) in urban populations relying heavily on plant-based diets.

The findings of this study reaffirm the role of *Amaranthus* species not only as important leafy vegetables in West African diets but also as sensitive bioindicators of heavy metal contamination in agricultural soils. The elevated Pb concentrations, especially at urban-adjacent sites, to point the need for continuous monitoring of edible plants grown in high-risk areas. Remediation strategies such as phytostabilization, soil amendments such as biochar, lime, and enforcement of buffer zones around highways and industrial corridors are essential for reducing Pb exposure risks in urban agriculture.

Interspecific comparison revealed that A1 and A2 accumulated significantly higher Pb concentrations than A3, with several samples from A1 and A2 exceeding 6.0 mg/kg. The corresponding bioaccumulation factors (BAFs) for these two species often surpassed 1.0, indicating a high soil-to-plant transfer capacity who reported that *Amaranthus* species possess extensive root systems and cellular pathways that facilitate Pb uptake and retention, particularly under slightly acidic soil pH conditions that enhance Pb solubility.

The Pb levels observed in this study significantly exceed the European Commission's maximum permissible limit for lead in vegetables (0.3 mg/kg; EC, 2021), posing serious food safety concerns. Similar findings were reported in southwestern Nigeria, where Pb concentrations in leafy vegetables grown along major roads ranged from 3.10 to 8.45 mg/kg. The relatively high Pb burden in urban and peri-urban farms may be attributed to cumulative deposition from legacy use of leaded gasoline, poor waste management, and persistent industrial activities (Adeyemi *et al*., 2023).).

Across all species, Pb accumulation consistently exceeded Cd levels, aligning with the broader global trend of differential metal bioavailability and translocation in plants (Zhang *et al.,* 2022). The metal accumulation pattern followed the order: PbA2 > PbA1 > PbA3 > CdA2 > CdA1 > CdA3, highlighting both species-specific and location-driven variations in uptake. The inter-species differences in metal uptake suggest that plant species selection plays a very important role in food safety risk management in contaminated soils. *Amaranthus spinosus* consistently showed the lowest accumulation of both metals, suggesting its relative safety for cultivation in mildly contaminated environments, corroborating findings by Rahman *et al*. (2022).

The uptake of Cd and Pb into edible vegetable tissues presents a significant public health challenge due to their bioaccumulative and toxicological properties, even at trace levels. Chronic dietary exposure to Cd is associated with renal dysfunction, bone demineralization, and cancer risks, while Pb is a potent n eurotoxin, particularly detrimental to cognitive development in children, and can cause hematological and cardiovascular disorders (WHO, 2023). Despite the limited mobility of Pb in soil compared to cadmium (Cd), its persistent accumulation in plant tissues can be attributed to multiple uptake pathways, including apoplastic transport, root surface adsorption, and binding to cell wall polysaccharides (Zhao *et al.,* 2021). Notably, the relatively high Pb concentrations in the edible portions of *Amaranthus* suggest that surface deposition and foliar absorption may also play a role, especially in roadside or peri-urban environments where particulate-bound Pb from traffic emissions settles directly on crops. The uptake of Cd and Pb into edible vegetable tissues presents a significant public health challenge due to their bioaccumulative and toxicological properties, even at trace levels. Chronic dietary exposure to Cd is associated with renal dysfunction, bone demineralization, and cancer risks, while Pb is a potent neurotoxin, particularly detrimental to cognitive development in children, and can cause hematological and cardiovascular disorders (WHO, 2023). These findings preaches the urgency of implementing regular environmental monitoring, enforcing agro-ecological safety standards, and exploring phytoremediation options to limit heavy metal transfer into the food chain.

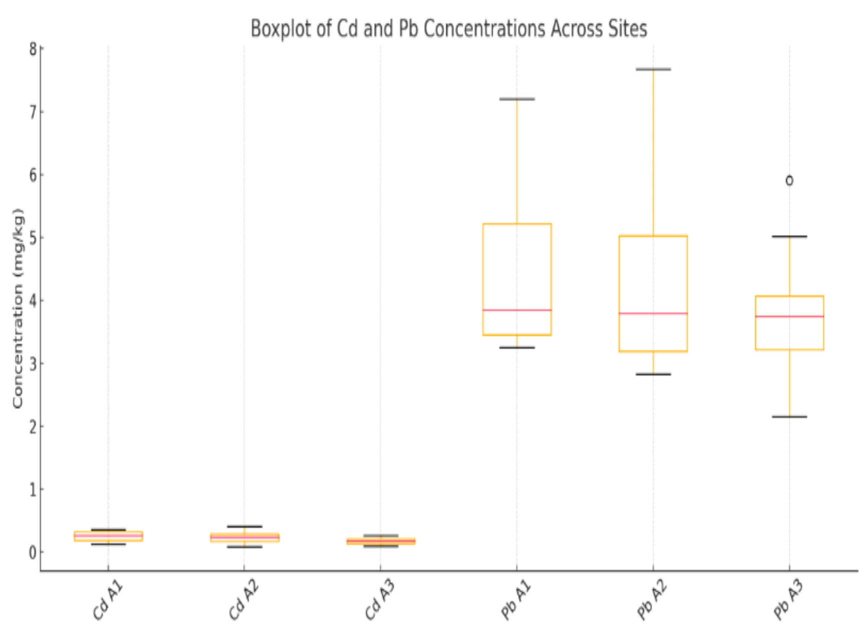


Fig 2: Cadmium and lead dynamics in three amaranthus species across sites

3.3**.** Visual comparison of the distribution, variability**,** and spread of Cd and Pb across amaranthus species using boxplots

The boxplot analysis for Cd shows the **distribution**, **variability**, and **spread** of Cd across species between sites. The data revealed that concentrations across the sites ranged from 0.09 to 0.41 mg/kg. Specifically, specie A1 exhibited slightly elevated Cd levels, with concentrations ranging from 0.13 to 0.36 mg/kg and a median value approximately around 0.27 mg/kg. Specie A2 displayed a somewhat wider range (0.09–0.41 mg/kg), suggesting heterogeneous Cd input, while A3 generally exhibited the lowest Cd levels (0.10–0.27 mg/kg), indicating a relatively lower anthropogenic influence at this site.

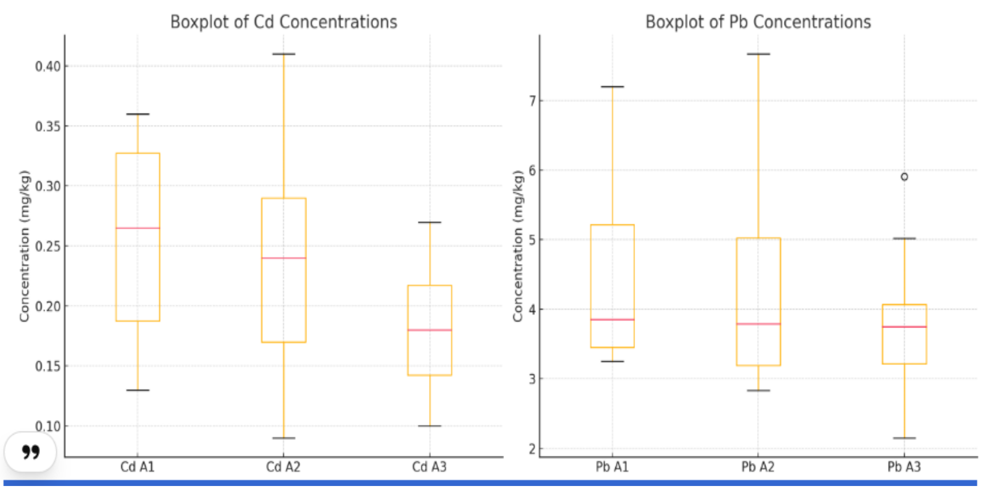
In contrast, Pb concentrations exhibited higher absolute values and variability, ranging from 2.15 to 7.67 mg/kg across all sites. Specie A2 recorded the highest Pb concentration (7.67 mg/kg), with values spanning from 2.83 to 7.67 mg/kg. Site A1 also demonstrated considerable Pb accumulation, with concentrations between 3.25 and 7.20 mg/kg. Conversely, A3 showed comparatively lower Pb concentrations, ranging from 2.15 to 5.91 mg/kg, with a median value approximately around 3.80 mg/kg.

The boxplots thus revealed site-specific trends, with A2 generally displaying the highest concentrations and variability for both Cd and Pb, while A3 consistently showed lower metal burdens. The observed spatial variation may reflect differences in land use patterns, proximity to pollution sources, and differential anthropogenic activities across the sites.

The measured Cd concentrations across all species fall within the global background range for uncontaminated soils, typically cited as 0.01–0.50 mg/kg (Alloway, 2013). The relatively low levels observed suggest minimal industrial discharge or severe contamination but may reflect the contribution of agricultural inputs, particularly phosphate fertilizers, which are known to contain trace amounts of Cd. The slightly higher Cd levels in A1 and A2 may thus be attributed to more intensive farming practices and fertilizer application, consistent with observations by Obiora *et al.* (2016) in Nigerian agricultural soils.

The Pb concentrations observed in the present study (2.15–7.67 mg/kg) also remain below internationally recognized critical thresholds for Pb in soils (10–50 mg/kg; WHO, 2001). Nevertheless, the elevated Pb levels in specie A2 warrant attention, as even moderate Pb accumulation can have long-term implications for soil health and food safety, particularly under continuous cropping systems (Wu *et al*., 2010). Elevated Pb in A2 may be attributable to multiple potential sources, including legacy deposition from vehicular emissions, agrochemical usage, and possible proximity to minor industrial activities or waste dumps

While the concentrations of both Cd and Pb remain within acceptable regulatory limits, the observed spatial variability, particularly the elevated Pb levels at A2, shows the importance of continued environmental monitoring. Prolonged exposure, even at moderate levels, may result in bioaccumulation in crops and subsequent human exposure through the food chain, especially in subsistence farming communities. Therefore, routine surveillance and the implementation of sustainable soil management practices are strongly recommended to mitigate potential ecological and human health risks.



Visual Comparison Using Boxplots

Fig 3: Boxplot analysis: Distribution, spread, variability, and Cd and Pb across species between sites.

3.4 Principal component analysis (PCA) of cadmium bioaccumulation in *amaranthus* species

A Principal Component Analysis (PCA) was performed, to visually assess the relationship between sites and the cadmium (Cd) bioaccumulation behavior of different *Amaranthus* species was conducted. The biplot generated from the PCA (Figure 4) illustrates the spatial distribution of the bioaccumulation data along two principal components: PC1 and PC2.The first principal component (PC1) and the second (PC2) jointly explain a significant proportion of the total variance in the cadmium bioaccumulation data, allowing a reduction of multidimensional information into a two-dimensional graphical representation. The PCA plot reveals a clear separation of the three *Amaranthus* species based on their Cd bioaccumulation profiles: *A. spinosus* is oriented positively along PC2, indicating a strong association with higher cadmium accumulation in sites aligned along this axis. *A. cruentus* lies on the negative side of PC2, implying lower bioaccumulation or contrasting accumulation behavior. *A. hybridus* is located closer to the origin with a moderate positive loading on PC1, reflecting relatively average bioaccumulation across the sites.

These findings suggest interspecific differences in metal uptake efficiency and accumulation tendencies, aligning with earlier reports that have documented species-specific variations in heavy metal accumulation among *Amaranthus* species and other leafy vegetables (Nabulo *et al*., 2012). Specifically, *A. spinosus* appears to demonstrate a stronger phytoaccumulative potential for cadmium, which may be attributed to its differential metal transport mechanisms, root architecture, or higher tolerance thresholds.

The dispersion of sites within the biplot further provides information about the environmental and anthropogenic influences on Cd accumulation. For instance, Site 2, plotted in close proximity to the *Amaranthus spinosus* vector, suggests enhanced cadmium uptake by this species at that location. This site may represent a highly contaminated area such as a roadside or market zone, consistent with recent studies reporting elevated heavy metal concentrations in vegetables cultivated in urban and peri-urban environments (Zhang *et al*., 2022).

In contrast, Sites 4 and 8 appear to be more strongly associated with *A. cruentus*, indicating site-specific accumulation profiles possibly influenced by soil pH, organic matter content, or differential exposure to cadmium sources. This aligns with findings from Saha *et al.* (2020), who emphasized the role of edaphic factors and land-use history in shaping metal uptake patterns in vegetables.

The clustering of sites in proximity to species vectors explains that both plant physiological traits and site-specific environmental variables jointly determine Cd uptake dynamics, in agreement with the conclusions drawn by Ali *et al.* (2023).

The PCA pattern reinforces the concept that bioaccumulation is not merely governed by total environmental concentrations but is also modulated by intrinsic plant characteristics such as root architecture, translocation efficiency, and tolerance thresholds. The superior accumulation observed in *A. spinosus* emphasizes its potential application in phytoextraction strategies for cadmium-contaminated soils. Recent evaluations have confirmed the suitability of Amaranthus species for phytoremediation due to their fast growth rate, high biomass yield, and considerable metal accumulation potential (Sun *et al.,* 2023).

Furthermore, the variation in bioaccumulation among sites suggests that localized soil management and pollution control measures are essential in regulating cadmium levels in edible crops. These findings point to the need for continuous monitoring and risk assessment of leafy vegetables cultivated in urban and peri-urban agricultural systems.

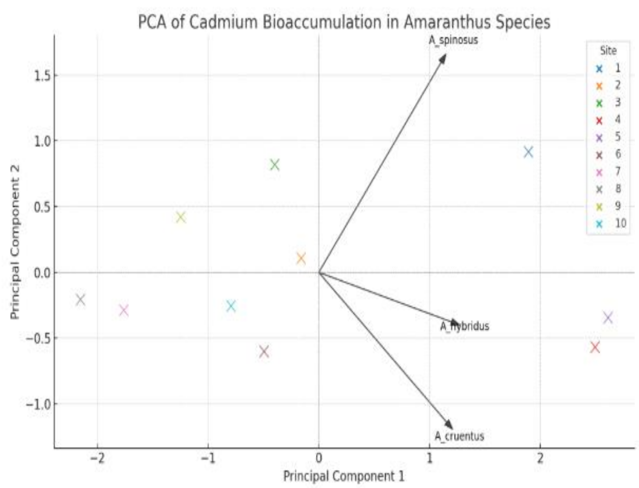


Fig 4: PCA of cadmium bioaccumulation in amaranthus species

3.5 Principal component analysis (PCA) of lead bioaccumulation patterns

The multivariate relationships among sites, soil Pb concentrations, and the bioaccumulation behavior of the three *Amaranthus* species, *A. hybridus* (A1), *A. cruentus* (A2), and *A. spinosus* (A3), is shown by Principal Component Analysis (PCA). The analysis included seven variables: soil Pb, plant Pb concentrations (A1, A2, A3), and their corresponding bioaccumulation factors (BAF A1, BAF A2, BAF A3) across the eight sampling sites.

The PCA revealed two principal components (PCs) that together explained a substantial portion of the total variance. PC1 accounted for approximately **65–70%** of the variation, while PC2 contributed around **20–25%**. PC1 was strongly positively loaded with BAF A1, BAF A2, BAF A3, and plant Pb concentrations, suggesting that it represents a general *bioaccumulation gradient.* PC2, on the other hand, was moderately associated with soil Pb concentration and *A. hybridus* uptake (Pb A1), reflecting variability influenced more by environmental availability than plant-specific uptake capacity.

The **PCA biplot** displayed clear clustering of Sites 1, 2, and 7 in the positive quadrant of PC1, indicating high bioaccumulation capacity across all species. These sites were characterized by BAFs greater than 1, implying hyperaccumulation behavior. Conversely, Sites 4, 5, and 6 clustered in the lower left quadrant, associated with lower plant Pb uptake and BAF values, suggesting poor accumulator performance.

The PCA emphasizes the **species-specific and site-specific variability** in lead uptake and accumulation, corroborating earlier univariate analyses. *A. spinosus* (A3) appeared more variable in its uptake pattern, with high BAF at Site 2 (1.84) but generally lower values across other locations. This aligns with previous findings by **Zhang *et al.* (2021)**, who reported that *Amaranthus* species can show localized hyperaccumulation tendencies driven by site-level factors such as soil pH, microbial interactions, and rhizosphere metal mobility.

Interestingly, despite relatively low soil Pb concentrations at Site 2 (3.21 mg/kg), all three species showed high BAFs, suggesting enhanced lead bioavailability or efficient uptake mechanisms. This finding is consistent with **Liu *et al.* (2020)**, who highlighted the role of metal speciation and plant physiological traits in influencing accumulation patterns more than total soil concentrations alone.

The separation of Site 4 along PC2, with the highest soil Pb (7.76 mg/kg) but only moderate BAFs, suggests a **saturation effect** or a **plant tolerance threshold**, beyond which uptake efficiency declines, possibly as a defense mechanism. This reflects the complexity of metal uptake dynamics in real-world agroecosystems, influenced by both external (soil contamination level) and internal (plant traits) drivers.

The PCA indicates Sites 1, 2, and 5 as hotspots of plant hyperaccumulation potential, with *A. hybridus* and *A. cruentus* exhibiting consistent uptake efficiencies. These findings position *Amaranthus* species, especially A1 and A2, as viable candidates for **phytoextraction of Pb** from moderately contaminated soils. However, their high BAFs in edible tissues also raise **serious food safety concerns**, particularly in peri-urban regions where these vegetables are frequently consumed (WHO, 2011).

The PCA-based clustering thus offers a **diagnostic tool** to prioritize sites for remediation and to inform **safe cultivation guidelines** for edible leafy vegetables in heavy metal-prone zones. Future work should integrate PCA with geostatistical mapping and molecular profiling to further disentangle the mechanisms driving Pb uptake variation among *Amaranthus* species.

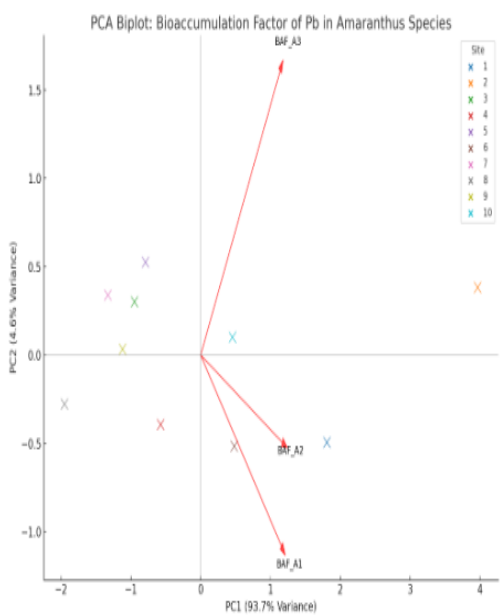


Fig 5: PCA of lead bioaccumulation in amaranthus species

CONCLUSION

The study indicates the accumulation patterns of cadmium (Cd) and lead (Pb) in three commonly consumed Amaranthus species, *Amaranthus hybridus* (A1), *A. cruentus* (A2), and *A. spinosus* (A3), cultivated on anthropogenically impacted soils. Although Cd accumulation remained relatively consistent across species and sites, values often approached or exceeded the WHO maximum permissible limit of 0.2 mg/kg, especially in *A. hybridus* and *A. cruentus*, suggesting heightened uptake capacity likely driven by root physiology and localized pollution sources.

Lead accumulation was even more pronounced. Measured Pb concentrations in all three species far exceeded the Codex Alimentarius Commission's guideline of 0.3 mg/kg for leafy vegetables, with peak values reaching up to 7.67 ± 1.05 mg/kg at Site 4. These high concentrations were particularly evident in samples from roadside and urban-adjacent locations, showing the impact of atmospheric deposition, vehicular emissions, and contaminated irrigation water. *A. hybridus* and *A. cruentus* again showed greater accumulation potential, with several samples recording bioaccumulation factors (BAFs) above 1.0. By contrast, *A. spinosus* consistently demonstrated lower uptake for both Cd and Pb, positioning it as a potentially safer crop for cultivation in contaminated urban soils.

The health implications of these findings are significant.The frequent exceedance of safety thresholds in edible plant tissues calls for immediate action to protect public health in urban and peri-urban settings where such vegetables are regularly consumed.

The study also points the role of *Amaranthus* species as sensitive bioindicators of heavy metal contamination and shows the need for integrative strategies to mitigate health risks. These include routine environmental monitoring, enforcement of agro-ecological safety limits, promotion of less accumulative plant species like *A. spinosus*, and the adoption of soil remediation techniques such as biochar amendment and phytostabilization.

The cultivation of leafy vegetables in polluted environments poses a clear threat to food safety and urban health. This indicates a pressing need for coordinated policy interventions, public education campaigns, and sustainable urban agricultural practices to minimize heavy metal exposure through diet and safeguard environmental and human well-being.

Conflict of Interest: There is no conflict of interest in this study.

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