NJSS 35(2) 2026: 63-75 DOI: https://doi.org/10.67042/njss.2026.9sl8bijf

Nigerian Journal of Soil Science

Original Research

Metal accumulation and excluder behaviour of five indigenous herbaceous species at the abandoned Enyigba Pb–Zn mine, southeastern Nigeria

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Keywords:
phytoremediation, excluder behaviour, metal accumulation, bioconcentration factor, translocation factor, Enyigba Pb–Zn mine

Manuscript History:
First Submission:
Revision Received:
Accepted:

Abstract

Artisanal lead-zinc mining at the Enyigba Pb–Zn mine, Ebonyi State, has left tailings soils heavily contaminated with lead and cadmium (soil Pb 1114.5–1125.4 mg kg^{-1}; soil Cd 125.7–135.7 mg kg^{-1}, approximately 13-fold and 157-fold above WHO agricultural-soil limits), but the metal-accumulation behaviour of the herbaceous vegetation naturally colonising the site has not previously been characterised. In this study, heavy metal accumulation in five dominant indigenous species (Gloriosa superba, Aspilia africana, Crotalaria retusa, Tacca leontopetaloides and Amorphophallus johnsonii) along a 100 m by 100 m sampling plot was assessed, analysing rhizosphere soil and root, stem and leaf tissue for Pb, Cd, Mn, Co and Cu by atomic absorption spectrophotometry. Bioconcentration and bioaccumulation values were below 1 for every species-metal combination (BCFroot 0.0002–0.0152; BACshoot 0.0000–0.0226), indicating a predominantly excluder-type accumulation pattern rather than strong accumulation relative to soil, while translocation factors exceeded 1 for Cd in all species (1.212–1.969) and for Pb and Cu in most species. A. johnsonii exhibited the lowest Pb concentrations in root, stem and leaf tissue while showing the highest shoot Cu bioaccumulation coefficient; C. retusa exhibited the highest BACshoot values for Pb and Cd among the species examined; T. leontopetaloides sequestered the most root Cu. Interspecific differences were statistically significant for Pb, Cd, Mn and Cu ($p<0.05$), although species and microsite were confounded in this design These findings suggest that phytostabilisation-oriented revegetation may be more appropriate than conventional phytoextraction at this site, with complementary, species-specific accumulation patterns that warrant evaluation in future multi-species revegetation trials.

1. Introduction

Mining and processing of metalliferous ores can leave behind soils enriched in lead, cadmium and other trace metals long after extraction has ceased, and legacy and active mine sites across Asia, Latin America and sub-Saharan Africa continue to generate tailings that persist as diffuse contamination sources for decades (Kabata-Pendias, 2011). Vegetation that re-establishes on these soils is often the first practical indication of what the land can subsequently support, but whether it merely tolerates contamination or can be put to active use in remediation depends on how metals are partitioned between root and shoot tissue: something that cannot be inferred from soil chemistry alone.

Lead–zinc mineralisation in southeastern Nigeria occurs along a roughly 600 km stretch of the Benue Trough, from Abakaliki–Ishiagu in Ebonyi State to Gwana in Gombe State, hosted in steeply dipping shale-associated veins and fractures (Nnabo, 2015; Obiora *et al.*, 2016). Enyigba, about 14 km south of Abakaliki, is among the more intensively worked districts within this belt; artisanal extraction there dates at least to the 1940s and has intensified since (Otuu *et al.*, 2018), with little formal attention to tailings containment. Soil surveys around Enyigba and the wider Abakaliki district have repeatedly documented Pb and Cd concentrations well above Nigerian and WHO guideline values (Aloh *et al.*, 2017; Mashi *et al.*, 2025; Nnabo, 2015; Otuu *et al.*, 2018), and comparable excluder-type contamination has been reported at other West African artisanal and legacy mining districts (Diallo *et al.*, 2024).

What is considerably less well characterised, at Enyigba and comparable Nigerian sites generally, is how the plant community actually occupying this contaminated ground responds to it. This matters for remediation planning: revegetation is generally the cheapest and least disruptive intervention available at a site of this kind, provided the species used are known to tolerate the prevailing contamina-

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ition and their remediation role, extraction versus stabilisation, is correctly identified in advance (Ali *et al.*, 2013; Pilon-Smits, 2005). Much of the regional phytoremediation literature has worked in one of two ways: testing candidate species ex situ under controlled, often single-metal exposure (Anoopkumar *et al.*, 2020; Ghosh & Singh, 2005; Shackira & Puthur, 2019), or assessing exogenous species already reported as hyperaccumulators elsewhere rather than the vegetation naturally present at the contaminated site itself. Neither approach establishes what the flora already growing at Enyigba is doing, physiologically, with the metal load it is exposed to daily; species that have colonised a tailings area unaided have, by definition, survived the prevailing conditions, and the practical question is what mechanism underlies that survival.

Field-based information on the accumulation behaviour of naturally established vegetation under the specific conditions prevailing at Enyigba remains limited, particularly using paired soil and tissue data within the standard bioconcentration/bioaccumulation/translocation framework. The specific objectives were to (i) identify the dominant indigenous herbaceous plants growing within the study area; (ii) determine heavy metal concentrations in the soil around the identified plants; (iii) analyse heavy metal accumulation patterns in the selected species; and (iv) evaluate the phytoremediation potential of the selected species on the basis of bioconcentration, bioaccumulation and translocation indices. Three null hypotheses were tested a priori: that there is no significant difference in soil heavy metal concentration among the sampled microsites; that there is no significant difference in heavy metal concentration among root, stem and leaf compartments; and that there are no significant differences in the calculated TF, BCF and BAC indices among the selected plants.

2. Materials and methods

2.1 Study area

The study was carried out at Enyigba, a mining community in Abakaliki Local Government Area of Ebonyi State, southeastern Nigeria, located at 6°07′–6°12′N and 8°05′–8°10′E and covering approximately 54.56 km². Ebonyi State is bounded by Cross River State to the east, Enugu State to the west, Abia State to the south and Benue State to the north (Figure 1).

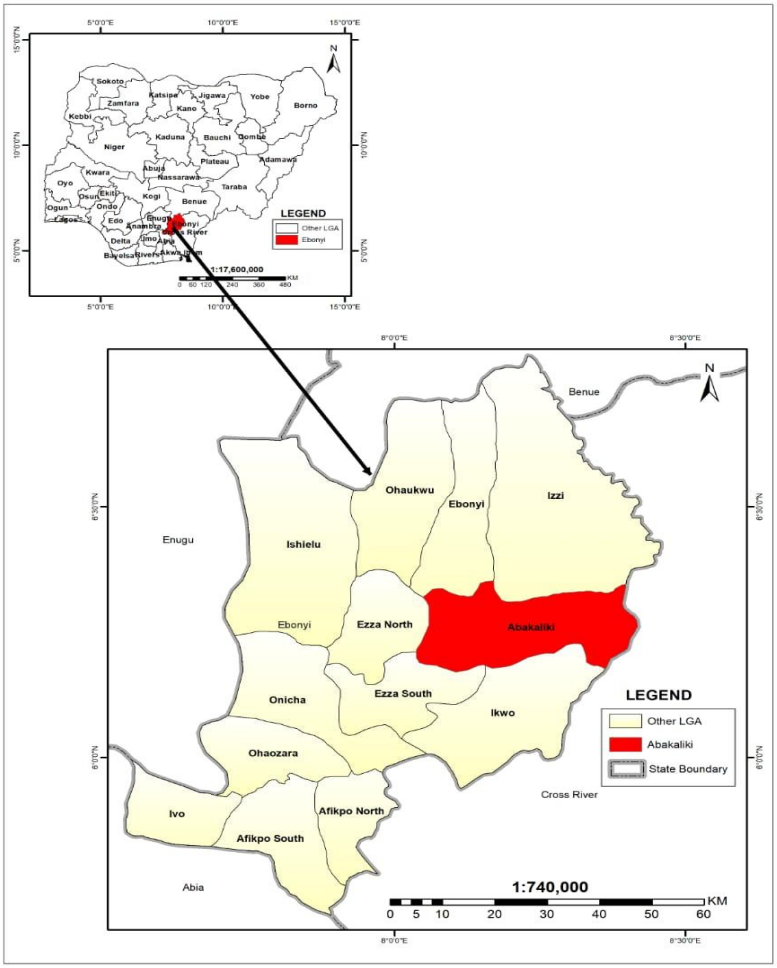


Figure 1. Location of Ebonyi State within Nigeria and of Abakaliki Local Government Area (which contains Enyigba) within Ebonyi State. Source: GIS Laboratory, Department of Urban and Regional Planning, Abia State University (2024).

Enyigba itself, together with the surrounding Amagu community lands, sits at the western edge of Abakaliki Local Government Area.

The area lies within the mineralised zone of the Benue Trough, overlain by gneisses, granites and shales hosting galena and sphalerite together with minor chalcopyrite, bornite, pyrite and quartz, largely within the Abakaliki shales of the Asu River Group (Figure 2) (Obiora *et al.*, 2016; Orazulike, 1994). Mineralisation occurs as telethermal Mississippi Valley-type Pb–Zn deposits localised in steeply dipping fractures and veins associated with anticlinal structures, and the area shows evidence of post-mineralisation deformation consistent with late Santonian folding. At the local scale, known lead–zinc lodes occur along a series of anticlinal structures near Ameka, Ameri and the Enyigba workings themselves. The area is drained by the Ebonyi River and its tributaries, principally the Iyienu stream, and is characterised by mine spoils, tailings and exposed open-cast workings that pose an evident risk of heavy metal transfer to surrounding soils and water via weathering, erosion and runoff.

2.2 Sampling design

Enyigba was selected purposively from among the Abakaliki mining communities (Amagu, Ameri, Enyigba and Ameka) on the basis of the larger size and longer duration of mining activity at its sites; community entry was secured through the community leadership, with a community liaison person assisting with reconnaissance and sample collection.

The largest abandoned mining pit at Enyigba was selected purposively, and a 100 m × 100 m sampling plot was marked out adjacent to it. The plot was divided into five contiguous 20 m × 100 m sections, and each section (rather than sub-quadrats within it) served as the sampling unit for scoring species presence; the frequency of occurrence of herbaceous species across the five sections was calculated as:

$$\% \text{ frequency} = (\text{number of plots in which species occurred} / \text{total number of plots}) \times 100 \quad (1)$$

From which the five species (Gloriosa superba, Aspilia africana, Crotalaria retusa, Tacca leontopetaloides and Amorphophallus johnsonii) with the highest frequency of occurrence were selected.

2.3 Sample collection

Rhizosphere soil, operationally defined here as the soil adhering directly to and immediately surrounding the root mass, was collected together with each uprooted plant (approximately 1 kg per sample) at a depth of 30 cm using a standard soil auger and placed in labelled polyethylene bags. Whole plants were uprooted using a digger and shovel to keep the root system intact, packed in labelled perforated paper bags, identified and classified by a taxonomist (Dr. Franklin Akanwa, Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria) accompanying the field team. For each species, three replicate plants were sampled from separate individuals within the microsite (n = 3 independent plants and paired soil samples per species, analysed in triplicate).

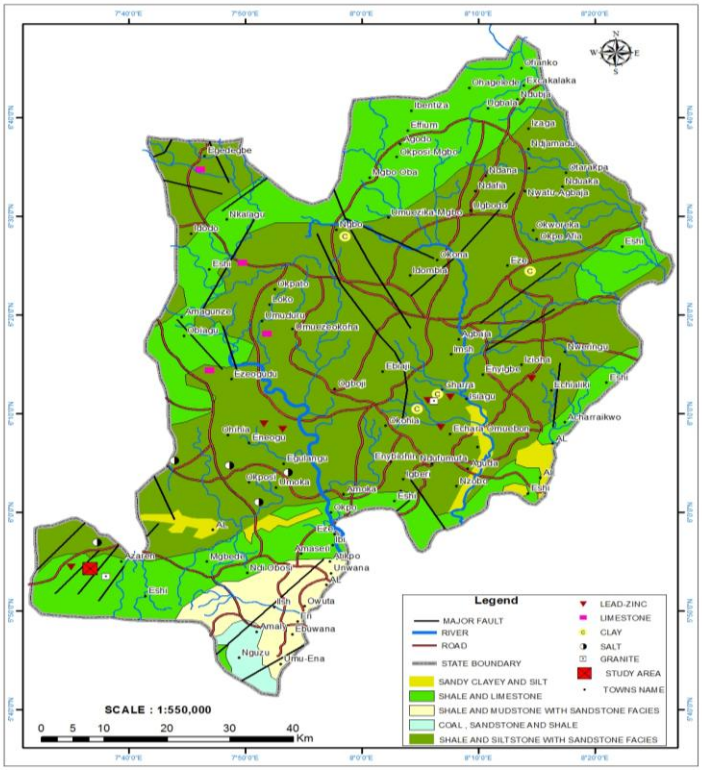


Figure 2. Geological and mineral resources map of Ebonyi State showing lead–zinc occurrences and the location of the study area. Source: Geological Survey Agency of Nigeria (2006).

2.4 Laboratory analysis

Soil samples were processed by borate-flux fusion in a muffle furnace at 800–1000°C, the temperature range typically required to melt a lithium metaborate/tetraborate flux and fully break down silicate-bound metals in mineral-rich soils such as these mine tailings, with the resulting glass bead dissolved in dilute acid for analysis (Verbeek *et al.*, 1982; exact soil mass and flux mass used, and precise fusion time within the stated temperature range, were not recorded in the original study documentation and cannot be reported here); a comparable fusion-plus-acid-dissolution protocol has since been benchmarked against conventional multi-acid digestion for silicate-rich matrices and found to recover resistate-mineral-bound metals more completely (Zivkovic *et al.*, 2023). Metal content in the dissolved digest was determined by atomic absorption spectrophotometry (AAS; Buck Scientific 230ATS) under air–acetylene flame conditions, using the appropriate hollow-cathode lamp and resonance wavelength for each element (Pb 283.3 nm, Cd 228.8 nm, Mn 279.5 nm, Co 240.7 nm, Cu 324.8 nm), in accordance with AOAC (2005). Plant tissue (root, stem, leaf) was washed free of soil, air-dried for three days, pulverised in a laboratory hammer mill, and wet-digested with aqua regia (2:3 v/v $HCl:HNO_3$) before extraction and AAS analysis under the same conditions as the soil (exact sample mass, acid volume, digestion temperature and time were not recorded in the original study documentation); aqua regia is a widely benchmarked and effective extractant for plant heavy metal analysis relative to other common acid mixtures (Bankaji *et al.*, 2023). Reagent blanks (n = 10) and matrix-matched calibration standards were run with each analytical batch. Limits of detection (LOD = 3×SD of reagent blanks) and quantification (LOQ = 10×SD), determined in the final digest solution and converted to solid-sample units using the applicable dilution factor (soil: 0.2000 g into 100 mL, dilution factor 500; plant: 0.5000 g into 50 mL, dilution factor 100), were as follows (solution LOD, solution LOQ, soil LOD, soil LOQ, plant LOD, plant LOQ, all in mg L^{-1} or mg kg^{-1} as appropriate): Pb 0.010, 0.033, 5.0, 16.5, 1.0, 3.3; Cd 0.002, 0.007, 1.0, 3.5, 0.2, 0.7; Mn 0.003, 0.010, 1.5, 5.0, 0.3, 1.0; Co 0.005, 0.017, 2.5, 8.5, 0.5, 1.7; Cu 0.003, 0.010, 1.5, 5.0, 0.3, 1.0. Method accuracy was verified using certified reference materials, analysed in five independent digestions each: NIST SRM 2711a (Montana II soil) for the soil matrix, and NIST SRM 1573a (tomato leaves) for the plant matrix. Mean recoveries (±RSD, n = 5) for SRM 2711a were 96.3±3.0% (Pb), 95.4±3.1% (Cd), 97.0±3.2% (Mn), 94.2±4.1% (Co) and 97.0±3.1% (Cu); for SRM 1573a, 94.7±4.2% (Cd), 96.7±3.8% (Mn), 91.2±5.8% (Co, informational certified value) and 96.8±3.3% (Cu). Pb is not certified in SRM 1573a at a concentration useful for verifying Pb recovery in a Pb-tailings study, so plant-matrix Pb recovery was not independently verified against a certified value in this work; this is addressed further in section 3.6.

2.5 Data analysis

Bioconcentration factor (BCFroot), bioaccumulation coefficient (BACshoot) and translocation factor (TFshoot) were calculated for each species–metal pair as

$$\text{BCFroot} = \frac{C_{root}}{C_{soil}} \quad (2)$$

$$BACshoot = \frac{C_{shoot}}{C_{soil}} \quad (3)$$

$$TFshoot = \frac{C_{shoot}}{C_{root}} \quad (4)$$

where C denotes metal concentration in the indicated compartment and shoot values combine stem and leaf tissue. Following established classification criteria (Anoopkumar *et al.*, 2020; Shackira & Puthur, 2019), species with BCFroot > 1 and TF < 1 were treated as candidates for phytostabilisation, species with both BCF and TF > 1 as candidates for phytoextraction, and species with BAC > 1 as hyperaccumulators of the metal concerned. Differences among species and among microsites were tested by one-way ANOVA with Bonferroni post-hoc pairwise comparisons in SPSS v27, with significance set at $p < 0.05$.

3. Results and discussion

3.1 Dominant species and evidence for metal tolerance

The frequency-of-occurrence survey identified five herbaceous species, spanning five distinct angiosperm families (Colchicaceae, Asteraceae, Fabaceae, Taccaceae and Araceae), as dominant across all five sections of a sampling plot running directly through Pb- and Cd-contaminated tailings. That these species have established and persisted on soil this contaminated is informative in its own right, independent of the tissue data that follow. Two, non-exclusive explanations are commonly invoked for this kind of pattern: micro-evolutionary selection for heritable metal-tolerance traits over the several decades since mining began at Enyigba (Antonovics *et al.*, 1971; Otuu *et al.*, 2018), and phenotypic plasticity, whereby a single genotype adjusts root exudation, vacuolar compartmentalisation or cell-wall metal binding without any underlying genetic change (Bradshaw, 1965; Rascio & Navari-Izzo, 2011). Distinguishing between these would require comparative transplant or population-genetic work beyond the scope of this study. That the five dominant species span five separate families, none belonging to the Brassicaceae, Caryophyllaceae or Poaceae lineages most often associated with temperate hyperaccumulation, is consistent with metal tolerance having arisen independently multiple times across flowering-plant lineages (Krämer, 2010), and supports using field-validated dominant species rather than species selected purely for expected hyperaccumulation capacity, an approach also taken for indigenous flora at artisanal gold-mining sites in Guinea (Konaté *et al.*, 2026).

3.2 Soil contamination relative to guideline values

Soil metal concentrations across the five microsites are summarised in Table 1. Pb ranged from 1114.5 to 1125.4 mg kg^{-1} and Cd from 125.7 to 135.7 mg kg^{-1}, exceeding WHO agricultural-soil limits (85 and 0.8 mg kg^{-1}; WHO, 1996) by approximately 13-fold and 157-fold; Cu reached up to 820.0 mg kg^{-1}, over 22-fold the WHO limit at the worst-affected microsite. Nigerian regulatory soil-quality values (e.g., FEPA, NESREA) are cited inconsistently across the domestic literature, with reported Pb thresholds for agricultural or general soils varying by more than an order of magnitude between sources; WHO (1996) was therefore used as the primary benchmark here, and a verified national standard, if available to the authors, could be added as a secondary comparison in a future revision. Pb concentrations at the *A. johnsonii* and T. leontopetaloides microsites were significantly higher than at the *G. superba* microsite ($p<0.01$ and $p<0.05$), and Cd was significantly higher at *A. africana*, T. leontopetaloides and *A. johnsonii* than at the *C. retusa* microsite ($p<0.05$); Co showed no significant difference among microsites. Because each species was sampled from its own microsite, these differences describe variation among microsites associated with the dominant species present there rather than a controlled test of species effects on soil chemistry (see section 3.6). This heterogeneity within the 100 m × 100 m plot is consistent with localised redistribution of mine-derived metal by wind, surface-water flow and distance from tailings (Acosta *et al.*, 2011; Khalid *et al.*, 2017), while the more even Co distribution likely reflects its continued adsorption to Fe/Mn oxyhydroxides common in weathered mine soils (Nkrumah *et al.*, 2016).

The severity of contamination corroborates earlier work at Enyigba and the wider Abakaliki district (Aloh *et al.*, 2017; Mashi *et al.*, 2025; Nnabo, 2015; Otuu *et al.*, 2018), and the high Pb and Cu values in particular reflect the primary mineralogy of the deposit, galena and sphalerite with associated chalcopyrite and pyrite (Obiora *et al.*, 2016), being continuously redistributed by weathering and mechanical tailings dispersion in the absence of any remediation programme since the transition from organised colonial-era mining to unregulated artisanal working.

3.3 Tissue accumulation patterns and species-specific strategies

Root, stem and leaf metal concentrations for each species are given in Table 2. Tissue concentrations were, in general, substantially lower than in the corresponding rhizosphere soil: root Pb, for example, ranged from just 0.01 mg kg^{-1} in *A. johnsonii* to 3.13 mg kg^{-1} in *A. africana* despite soil Pb exceeding 1100 mg kg^{-1} at every microsite, a pattern typical of non-hyperaccumulators on severely contaminated substrates; one possible explanation is the formation of highly insoluble Pb precipitates in the rhizosphere, which could limit Pb availability from the soil solution regardless of total soil concentration, although soil physicochemical properties (pH, organic matter) were not measured in this study (Ghosh & Singh, 2005; Liang *et al.*, 2009; Shahid *et al.*, 2014). Full interspecific pairwise comparisons for root, stem and leaf concentrations are given in Tables 3, 4 and 5 respectively

The dominant pattern across species and metals was a decreasing concentration gradient from root to stem to leaf: seen for Cd in *G. superba*, *A. africana* and *C. retusa*, for Mn in *G. superba*, *C. retusa* and *A. johnsonii*, and for Cu in *G. superba*, T. leontopetaloides and *A. johnsonii*, consistent with cumulative sequestration and exclusion operating at each tissue boundary, plausibly reflecting the particular physiological cost of allowing metal ions to reach the photosynthetically active mesophyll of the leaf (Clemens, 2006; Ghosh & Singh, 2005). Two informative departures from this trend appeared for Pb: *A. africana* and *C. retusa* both showed higher Pb in stem than in root (3.39 vs. 3.13 mg kg^{-1}, and 3.31 vs. 2.43 mg kg^{-1}; $p<0.01$ for both), consistent with the stem functioning as a Pb storage compartment intercepting metal before it reaches the leaf, broadly analogous to the preferential xylem unloading and vacuolar Cd deposition documented in stem parenchyma of *Pueraria thomsonii* after multi-year field phytoremediation (Li *et al.*, 2025).

A. johnsonii's near-complete exclusion of Pb was the single clearest species-specific result: root, stem and leaf Pb all sat at or below the analytical detection limit (0.01–0.02 mg kg^{-1}) despite this species occupying the highest-Pb microsite of the study (1125.4 mg kg^{-1}). This dissociation between soil Pb and

Table 1. Heavy metal concentrations (mean ± SE, mg kg^{-1}) in soil across five microsites at Enyigba

Metal	*G. superba*	*A. africana*	*C. retusa*	*T. leonto-petaloides*	*A. johnsonii*	WHO limit
Pb	1114.5 ± 2.49	1120.2 ± 0.76	1119.6 ± 0.93	1123.5 ± 2.27[a]	1125.4 ± 1.30[b]	85
Cd	130.3 ± 1.33	127.1 ± 2.10[c]	133.1 ± 0.78	135.7 ± 0.81[c]	125.7 ± 0.73[c]	0.8
Mn	427.1 ± 1.37	833.4 ± 0.40[d]	831.8 ± 1.24[d]	832.7 ± 1.33[d]	829.3 ± 0.45[d]	n/a
Co	85.5 ± 2.77	85.8 ± 0.41	83.8 ± 0.69	89.9 ± 1.07	89.2 ± 1.05	50
Cu	818.9 ± 1.74[e]	819.5 ± 0.43[e]	820.0 ± 0.81[e]	819.0 ± 1.26[e]	199.5 ± 0.29	36

Notes: Values are mean ± SE (n = 3 per microsite); microsites are named for the dominant species sampled there, so species and microsite are confounded in this design. [a]$p<0.05$, [b]$p<0.01$ vs. *G. superba* (Pb); [c]$p<0.05$ vs. *C. retusa* (Cd); [d]$p<0.001$ vs. *G. superba* (Mn); [e]$p<0.001$ vs. A. *johnsonii* (Cu). WHO limits from WHO (1996); no WHO threshold is set for Mn.

Table 2. Heavy metal concentrations (mean ± SE, mg kg^{-1}) in root, stem and leaf tissue of five herbaceous species at Enyigba

Metal	Tissue	*G. superba*	*A. africana*	*C. retusa*	*T. leontopetaloides*	*A. johnsonii*
Pb	root	2.59 ± 0.09	3.13 ± 0.09	2.43 ± 0.02	3.04 ± 0.03	0.01 ± 0.00
	stem	2.52 ± 0.06	3.39 ± 0.22	3.31 ± 0.21	2.09 ± 0.06	0.01 ± 0.01
	leaf	2.33 ± 0.13	0.89 ± 0.02	2.90 ± 0.02	1.06 ± 0.03	0.02 ± 0.02
Cd	root	1.29 ± 0.05	1.93 ± 0.09	1.93 ± 0.04	1.07 ± 0.02	1.65 ± 0.10
	stem	0.91 ± 0.01	1.57 ± 0.05	1.83 ± 0.08	1.33 ± 0.21	1.66 ± 0.12
	leaf	0.65 ± 0.01	0.83 ± 0.02	1.18 ± 0.02	0.77 ± 0.02	1.02 ± 0.02
Mn	root	0.30 ± 0.02	0.14 ± 0.01	0.25 ± 0.00	0.36 ± 0.01	0.16 ± 0.01
	stem	0.16 ± 0.02	0.53 ± 0.23	0.13 ± 0.01	0.29 ± 0.01	0.08 ± 0.01
	leaf	0.14 ± 0.02	0.02 ± 0.00	0.11 ± 0.01	0.24 ± 0.02	0.07 ± 0.02
Co	root	0.13 ± 0.01	0.09 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.03 ± 0.00
	stem	0.53 ± 0.24	0.49 ± 0.23	0.02 ± 0.01	1.69 ± 1.52	0.02 ± 0.01
	leaf	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.68 ± 0.63	0.01 ± 0.00
Cu	root	0.49 ± 0.04	0.43 ± 0.03	0.40 ± 0.02	3.93 ± 0.03	1.81 ± 0.04
	stem	0.40 ± 0.03	0.37 ± 0.03	0.29 ± 0.02	2.03 ± 0.02	1.15 ± 0.06
	leaf	0.18 ± 0.02	0.33 ± 0.02	0.25 ± 0.02	1.30 ± 0.07	0.92 ± 0.05

Notes: Values are mean ± SE mg kg^{-1} (n = 3). Interspecific and among-tissue differences were statistically significant (one-way ANOVA, $p<0.05$) for the great majority of metal–tissue combinations except Co, which showed no significant difference among tissues for most species (full pairwise comparisons in Tables 3–5).

Table 3. Multiple comparison of root heavy metal concentrations (Bonferroni-adjusted p-values)

Species A	Species B	Pb	Cd	Mn	Co	Cu
G. superba	*A. africana*	<0.001	<0.001	<0.001	0.010	0.714
G. superba	*C. retusa*	0.341	<0.001	0.097	<0.001	0.294
G. superba	*T. leontopetaloides*	0.002	0.181	0.042	<0.001	<0.001
G. superba	*A. johnsonii*	<0.001	0.017	<0.001	<0.001	<0.001
A. africana	*C. retusa*	<0.001	1.00	0.001	<0.001	0.918
A. africana	*T. leontopetaloides*	0.837	<0.001	<0.001	0.033	<0.001
A. africana	*A. johnsonii*	<0.001	0.071	0.723	<0.001	<0.001
C. retusa	*T. leontopetaloides*	<0.001	<0.001	<0.001	0.010	<0.001
C. retusa	*A. johnsonii*	<0.001	0.080	0.008	0.928	<0.001
T. leontopetaloides	*A. johnsonii*	<0.001	<0.001	<0.001	0.033	<0.001

Table 4. Multiple comparison of stem heavy metal concentrations (Bonferroni-adjusted p-values)

Species A	Species B	Pb	Cd	Mn	Co	Cu
G. superba	*A. africana*	0.009	0.015	0.152	1.00	0.968
G. superba	*C. retusa*	0.016	0.002	0.999	0.988	0.248
G. superba	*T. leontopetaloides*	0.263	0.143	0.878	0.805	<0.001
G. superba	*A. johnsonii*	<0.001	0.006	0.978	0.988	<0.001
A. africana	*C. retusa*	0.993	0.522	0.111	0.991	0.532
A. africana	*T. leontopetaloides*	<0.001	0.615	0.513	0.786	<0.001
A. africana	*A. johnsonii*	<0.001	0.975	0.065	0.991	<0.001
C. retusa	*T. leontopetaloides*	<0.001	0.072	0.783	0.546	<0.001
C. retusa	*A. johnsonii*	<0.001	0.835	0.996	1.00	<0.001
T. leontopetaloides	*A. johnsonii*	<0.001	0.321	0.589	0.543	<0.001

Table 5. Multiple comparison of leaf heavy metal concentrations (Bonferroni-adjusted p-values)

Species A	Species B	Pb	Cd	Mn	Co	Cu
G. superba	*A. africana*	<0.001	<0.001	<0.001	1.00	0.123
G. superba	*C. retusa*	<0.001	<0.001	0.457	1.00	0.716
G. superba	*T. leontopetaloides*	<0.001	0.005	0.003	0.543	<0.001
G. superba	*A. johnsonii*	<0.001	<0.001	0.029	1.00	<0.001
A. africana	*C. retusa*	<0.001	<0.001	0.006	1.00	0.619
A. africana	*T. leontopetaloides*	0.331	0.236	<0.001	0.555	<0.001
A. africana	*A. johnsonii*	<0.001	<0.001	0.110	1.00	<0.001
C. retusa	*T. leontopetaloides*	<0.001	<0.001	<0.001	0.542	<0.001
C. retusa	*A. johnsonii*	<0.001	<0.001	0.327	0.528	<0.001
T. leontopetaloides	*A. johnsonii*	<0.001	<0.001	<0.001	0.528	<0.001

tissue Pb is consistent with excluder-type behaviour (Baker & Walker, 1989), and may involve root-level mechanisms such as plasma-membrane Pb extrusion, apoplastic immobilisation by pectin-associated carboxylate groups, or rhizosphere Pb-chelating exudates, none of which were directly tested in this study (Maestri *et al.*, 2010; Shahid *et al.*, 2014). The same species simultaneously accumulated the second-highest root Cu of any species after T. leontopetaloides (1.81 mg kg^{-1}), consistent with metal-specific rather than generalised restriction of uptake, potentially involving dedicated Cu-transport systems operating independently of the pathways by which non-essential toxic metals enter root cells, although transporter activity was not directly assayed (Broadley *et al.*, 2012; Clemens, 2006). T. leontopetaloides itself accumulated by far the highest root Cu of any species (3.93 mg kg^{-1}) despite growing in soil not significantly different in Cu from three of the other four microsites, which may reflect a comparatively high density or affinity of Cu transporters in this species, though this was not directly measured (Mendez & Maier, 2008; Nkrumah *et al.*, 2016). *G. superba*, meanwhile, showed the highest root Mn of any species (0.30 mg kg^{-1}) despite growing in the microsite with by far the lowest soil Mn (427.1 vs. >829 mg kg^{-1} elsewhere), suggesting either an intrinsically higher Mn affinity tied to its role in photosystem II or rhizosphere-scale conditions not captured by bulk soil sampling (Ogbonna *et al.*, 2015).

The dominant pattern across species and metals was a decreasing concentration gradient from root to stem to leaf: seen for Cd in *G. superba*, *A. africana* and *C. retusa*, for Mn in *G. superba*, *C. retusa* and *A. johnsonii*, and for Cu in *G. superba*, T. leontopetaloides and *A. johnsonii*, consistent with cumulative sequestration and exclusion operating at each tissue boundary, plausibly reflecting the particular physiological cost of allowing metal ions to reach the photosynthetically active mesophyll of the leaf (Clemens, 2006; Ghosh & Singh, 2005). Two informative departures from this trend appeared for Pb: *A. africana* and *C. retusa* both showed higher Pb in stem than in root (3.39 vs. 3.13 mg kg^{-1}, and 3.31 vs. 2.43 mg kg^{-1}; $p<0.01$ for both), consistent with the stem functioning as a Pb storage compartment intercepting metal before it reaches the leaf, broadly analogous to the preferential xylem unloading and vacuolar Cd deposition documented in stem parenchyma of *Pueraria thomsonii* after multi-year field phytoremediation (Li *et al.*, 2025).

A. johnsonii's near-complete exclusion of Pb was the single clearest species-specific result: root, stem and leaf Pb all sat at or below the analytical detection limit (0.01–0.02 mg kg^{-1}) despite this species occupying the highest-Pb microsite of the study (1125.4 mg kg^{-1}). This dissociation between soil Pb and tissue Pb is consistent with excluder-type behaviour (Baker & Walker, 1989), and may involve root-level mechanisms such as plasma-membrane Pb extrusion, apoplastic immobilisation by pectin-associated carboxylate groups, or rhizosphere Pb-chelating exudates, none of which were directly tested in this study (Maestri *et al.*, 2010; Shahid *et al.*, 2014). The same species simultaneously accumulated the second-highest root Cu of any species after T. leontopetaloides (1.81 mg kg^{-1}), consistent with metal-specific rather than generalised restriction of uptake, potentially involving dedicated Cu-transport systems operating independently of the pathways by which non-essential toxic metals enter root cells, although transporter activity was not directly assayed (Broadley *et al.*, 2012; Clemens, 2006). T. leontopetaloides itself accumulated by far the highest root Cu of any species (3.93 mg kg^{-1}) despite growing in soil not significantly different in Cu from three of the other four microsites, which may reflect a comparatively high density or affinity of Cu transporters in this species, though this was not directly measured (Mendez & Maier, 2008; Nkrumah *et al.*, 2016). *G. superba*, meanwhile, showed the highest root Mn of any species (0.30 mg kg^{-1}) despite growing in the microsite with by far the lowest soil Mn (427.1 vs. >829 mg kg^{-1} elsewhere), suggesting either an intrinsically higher Mn affinity tied to its role in photosystem II or rhizosphere-scale conditions not captured by bulk soil sampling (Ogbonna *et al.*, 2015).

3.4 Bioconcentration, bioaccumulation and translocation: an excluder pattern

BCFroot, BACshoot and TFshoot values are given in Tables 6a–6c. BCFroot was below 1 for every species–metal combination (0.0002–0.0152; Table 6a), as was BACshoot (0.0000–0.0226; Table 6b); by the classification applied here (Anoopkumar *et al.*, 2020; Shackira & Puthur, 2019), none of the five species qualifies as a hyperaccumulator for any of the five metals. TFshoot, in contrast, exceeded 1 for Cd in all five species (1.212–1.969) and, for most species, for Pb and Cu as well (Table 6c). Full interspecific pairwise comparisons for BCFroot, BACshoot and TFshoot are given in Tables 7, 8 and 9 respectively.

Table 6a. Bioconcentration factor (BCFroot) by species and metal

BCFroot	*G. superba*	*A. africana*	*C. retusa*	*T. leontopetaloides*	*A. johnsonii*
Pb	0.0023	0.0028	0.0022	0.0027	0.0000
Cd	0.0099	0.0152	0.0145	0.0085	0.0132
Mn	0.0007	0.0002	0.0003	0.0004	0.0002
Co	0.0015	0.0011	0.0004	0.0007	0.0004
Cu	0.0006	0.0005	0.0005	0.0048	0.0091

Table 6b. Bioaccumulation coefficient (BACshoot) by species and metal

BACshoot	*G. superba*	*A. africana*	*C. retusa*	*T. leontopetaloides*	*A. johnsonii*
Pb	0.0044	0.0038	0.0055	0.0028	0.0000
Cd	0.0120	0.0189	0.0226	0.0168	0.0214
Mn	0.0007	0.0007	0.0003	0.0006	0.0002
Co	0.0065	0.0060	0.0005	0.0007	0.0003
Cu	0.0007	0.0009	0.0007	0.0041	0.0104

Table 6c. Translocation factor (TFshoot) by species and metal

TFshoot	*G. superba*	*A. africana*	*C. retusa*	*T. leontopetaloides*	*A. johnsonii*
Pb	1.869	1.371	2.559	1.036	2.667[a]
Cd	1.212	1.243	1.564	1.969	1.638
Mn	1.010	3.786	0.960	1.468	0.944
Co	3.949	2.889	1.611	0.952	0.722
Cu	1.173	1.633	1.371	0.849	1.143

Notes: [a] The A. johnsonii Pb TF (2.667) is calculated from two near-detection-limit concentrations (root and shoot Pb both ≤0.02 mg kg^{-1}) and is treated as a numerical artefact rather than a biological signal (section 3.4). Full pairwise comparisons for these three indices are given in Tables 7–9.

Table 7. Multiple comparison of BCFroot (Bonferroni-adjusted p-values)

Species A	Species B	Pb	Cd	Mn	Co	Cu
G. superba	*A. africana*	<0.001	<0.001	<0.001	0.057	1.00
G. superba	*C. retusa*	0.586	<0.001	<0.001	<0.001	1.00
G. superba	*T. leontopetaloides*	0.004	<0.001	<0.001	<0.001	<0.001
G. superba	*A. johnsonii*	<0.001	0.704	<0.001	<0.001	<0.001
A. africana	*C. retusa*	<0.001	1.00	0.048	0.001	1.00
A. africana	*T. leontopetaloides*	1.00	<0.001	<0.001	0.128	<0.001
A. africana	*A. johnsonii*	<0.001	0.836	1.00	0.002	<0.001
C. retusa	*T. leontopetaloides*	<0.001	<0.001	0.028	0.122	<0.001
C. retusa	*A. johnsonii*	<0.001	0.836	0.189	1.00	<0.001
T. leontopetaloides	*A. johnsonii*	<0.001	<0.001	<0.001	0.258	<0.001

Table 8. Multiple comparison of BACshoot (Bonferroni-adjusted p-values)

Species A	Species B	Pb	Cd	Mn	Co	Cu
G. superba	*A. africana*	0.148	0.005	1.000	1.00	1.00
G. superba	*C. retusa*	<0.001	<0.001	0.389	0.292	1.00
G. superba	*T. leontopetaloides*	<0.001	0.051	1.00	0.353	<0.001
G. superba	*A. johnsonii*	<0.001	<0.01	0.142	0.249	<0.001
A. africana	*C. retusa*	<0.001	0.205	0.633	0.414	1.00
A. africana	*T. leontopetaloides*	0.002	1.00	1.00	0.474	<0.001
A. africana	*A. johnsonii*	<0.001	0.952	0.231	0.354	<0.001
C. retusa	*T. leontopetaloides*	<0.001	0.016	0.705	1.00	<0.001
C. retusa	*A. johnsonii*	<0.001	1.00	1.00	1.00	0.846
T. leontopetaloides	*A. johnsonii*	<0.001	0.073	0.259	1.00	<0.001

Table 9. Multiple comparison of TFshoot (Bonferroni-adjusted p-values)

Species A	Species B	Pb	Cd	Mn	Co	Cu
G. superba	*A. africana*	1.00	1.00	0.187	1.00	0.033
G. superba	*C. retusa*	1.00	0.437	1.00	1.00	1.00
G. superba	*T. leontopetaloides*	1.00	0.006	1.00	1.00	0.227
G. superba	*A. johnsonii*	1.00	0.191	1.00	0.917	1.00
A. africana	*C. retusa*	1.00	0.625	0.172	0.531	0.544
A. africana	*T. leontopetaloides*	1.00	0.008	0.414	0.277	<0.001
A. africana	*A. johnsonii*	1.00	0.274	0.167	0.221	0.022
C. retusa	*T. leontopetaloides*	1.00	0.242	1.00	1.00	0.015
C. retusa	*A. johnsonii*	1.00	1.00	1.00	1.00	0.846
T. leontopetaloides	*A. johnsonii*	1.00	0.554	1.00	1.00	0.346

A BCFroot or BACshoot below 1 is sometimes read as evidence that a species has failed to remediate contaminated soil. This conflates phytoextraction specifically with phytoremediation as a whole: the classification frameworks typically used with BCF and TF were developed for hyperaccumulating species, where tissue concentrations approach or exceed soil concentrations, and do not straightforwardly apply at a site where soil Pb exceeds 1000 mg kg^{-1} and Cd exceeds 100 mg kg^{-1} (Mendez & Maier, 2008; Pilon-Smits, 2005). In that context, low BCFroot is not a failure but the expected physiological signature of an excluder: a species that keeps metal out of harvestable tissue while providing vegetative cover that physically stabilises contaminated soil against erosion, which is the specific goal of phytostabilisation (Mendez & Maier, 2008; Pilon-Smits, 2005). Ali *et al.* (2013) and Liang *et al.* (2009) similarly identify BCFroot <1 as characteristic of the excluder category suited to Pb phytostabilisation, and note the general absence of true field Pb hyperaccumulators. The present results are consistent with two other studies in the same geological setting, the Ishiagu Pb–Zn mine (Ogbonna *et al.*, 2015) and a separate assessment of woody species at Enyigba (Mashi *et al.*, 2025), and with comparable findings for native flora at West African gold-mining sites (Konaté *et al.*, 2026; Petelka *et al.*, 2019), together suggesting that the excluder strategy, rather than hyperaccumulation, is the typical response of native tropical herbaceous flora to artisanal mine contamination (Mendez & Maier, 2008; Nkrumah *et al.*, 2016).

Cd produced the highest BACshoot values of any metal across all five species (0.0120–0.0226; Table 6b), identifying it as the metal most efficiently moved into aerial biomass: plausibly reflecting the relative ease with which Cd enters the xylem stream via ZIP- and IRT-family transporters compared with Pb (Clemens, 2006; Rascio & Navari-Izzo, 2011). This carries a double implication: Cd is the metal most likely to enter the local food chain via edible tissue in a farming community that is also heavily engaged in mining, and it is also the metal for which shoot harvest would remove the greatest mass per unit biomass among those studied. *C. retusa* exhibited the highest BACshoot values for both Pb (0.0055) and Cd (0.0226) among the species examined, while *A. johnsonii* exhibited the highest BACshoot for Cu (0.0104).

One TF value requires separate treatment rather than inclusion at face value: *A. johnsonii*'s Pb TF of 2.667, numerically the highest of any species–metal combination. Both root and shoot Pb in this species sit at or near the AAS detection limit (0.01–0.02 mg kg^{-1}), so the arithmetic ratio of two near-zero values carries no biological meaning; taken together with this species' near-zero BCFroot and BACshoot for Pb, the balance of evidence is that *A. johnsonii* is a Pb excluder, not a Pb translocator, and this TF should be regarded as undefined rather than as evidence of active Pb translocation. All other TF values are drawn from concentrations comfortably above detection limits and are interpreted at face value. T. leontopetaloides showed the highest Cd TF (1.969), significantly greater than *G. superba* (1.212, p=0.006) and *A. africana* (1.243, p=0.008); the same species was the only one with a Cu TF below 1 (0.849), indicating preferential root-level retention of Cu in this species, in contrast to its handling of Cd.

Taken together, BCFroot, BACshoot and TFshoot identify a shared excluder-type accumulation pattern across all five species with clear species- and metal-specific differentiation within it: *A. johnsonii* showed the lowest root Pb concentration alongside the highest shoot Cu bioaccumulation; *C. retusa* showed the highest shoot bioaccumulation for both Pb and Cd; T. leontopetaloides showed the highest Cd translocation factor alongside preferential root-level retention of Cu; and *G. superba* and *A. africana* did not stand out on any single index. These patterns describe observed relative accumulation and tissue partitioning rather than validated remediation roles, which would require biomass and mass-balance data (see section 3.6). This pattern of metal-specific functional differentiation within a co-occurring native assemblage parallels that documented for co-occurring tree species at temperate mine sites (Pulford & Watson, 2003), extended here to indigenous West African herbaceous flora under unamended, real-world artisanal mining conditions.

3.5 Broader implications

Hyperaccumulator classification criteria developed largely from temperate-zone species do not transfer well to native herbaceous flora on tropical artisanal mine soils where metal concentrations can run two to three orders of magnitude above typical hyperaccumulator tissue thresholds (Mendez & Maier, 2008; Nkrumah *et al.*, 2016; Pilon-Smits, 2005). Recognising excluder behaviour as a positive phytostabilising trait, rather than treating it as a failed attempt at hyperaccumulation, is a more useful framework for sites like Enyigba, and *A. johnsonii*'s combination of near-complete Pb exclusion with efficient Cu uptake illustrates why multi-metal remediation is better served by co-occurring native polycultures than by a single introduced hyperaccumulator species.

The scale of soil Pb and Cd contamination at Enyigba (approximately 13-fold and 157-fold above WHO limits) represents a potential exposure concern, particularly for children given their typically higher soil-ingestion rates and lower tolerance to lead (Obiora *et al.*, 2016; Tchounwou *et al.*, 2012); a formal quantitative health-risk assessment, requiring exposure-pathway, dose and population data beyond the scope of this study, would be needed to characterise this risk. These contamination levels nonetheless support the case for regulatory action by the National Environmental Standards and Regulations Enforcement Agency (NESREA) and by federal and state government to enforce soil-quality standards, restrict further unregulated tailings generation, and fund a community-wide environmental health assessment. *A. johnsonii*, *C. retusa* and T. leontopetaloides, species already present, already tolerant, and each showing a distinct metal-specific accumulation pattern, warrant evaluation in future multi-species revegetation trials; any such trial should restrict livestock access to planted areas given the Cd levels found in *C. retusa* and *A. johnsonii* shoot tissue.

3.6 Constraints on interpretation

Several aspects of the design bound how far these findings can be extended. Exact soil and plant digestion masses, flux mass, and fusion/digestion time within the stated temperature ranges were not recorded in the original study documentation and could not be reported in Section 2.4; this limit exact reproducibility of the digestion step, although the digestion chemistry, instrumentation, wavelengths, LOD/LOQ and CRM recovery data are reported in full. Certified reference materials were analysed for method accuracy, but Pb is not certified in the plant CRM available for this work, so plant Pb recovery rests on the soil CRM and on continuing calibration verification rather than on a plant-matrix CRM; this is a minor limitation but is noted here for transparency. One-way ANOVA was applied separately to each metal and tissue; because root, stem and leaf measurements originate from the same individual plants, these tests do not fully account for within-plant correlation, and a factorial or mixed-effects model treating plant as a random effect (Species × Tissue) would provide a more rigorous test of these differences. Sampling was confined to a single 100 m × 100 m plot at a single abandoned pit within the wider Enyigba–Ameka–Ameri mining complex, each part of which may carry a distinct contamination and vegetation profile (Diallo *et al.*, 2024; Obiora *et al.*, 2016; Rouhani *et al.*, 2024), so the results should not be extrapolated to the district as a whole without further validation. No soil physicochemical parameters (pH, organic matter, cation exchange capacity) were measured, despite their recognised importance for metal bioavailability (Kabata-Pendias, 2011; Khalid *et al.*, 2017; Wan *et al.*, 2024); since even a one-unit shift in pH can materially change Cd exchangeability (Rieuwerts *et al.*, 1998; Sanjana *et al.*, 2024), the low BCF values reported here may partly reflect geochemical immobilisation rather than root-level exclusion alone, and rhizosphere physicochemical characterisation is a priority for future work at this site. Sampling was also restricted to a single early-wet-season snapshot, and while seasonal variation in redox potential, microbial activity and root exudation could shift the specific values reported, it is unlikely to reverse the overall excluder pattern (Cobbett & Goldsbrough, 2002; Garbisu & Alkorta, 2001). Finally, BCF, BAC and TF are single-time-point concentration ratios that say nothing about total metal mass removed over time or across growing seasons, and no biomass data were collected; a species with only moderate tissue concentration but high biomass yield could out-perform a higher-concentration, lower-biomass species in cumulative phytoextraction (Garbisu & Alkorta, 2001; Motrescu & Luchian, 2026; Pilon-Smits, 2005), so the present results should be read as a first-pass physiological screen of remediation potential rather than a prediction of remediation timelines, pending biomass-based mass-balance work (Ali *et al.*, 2013; Kumar *et al.*, 2021; Li *et al.*, 2025).

4. Conclusion

Soils across the Enyigba tailings sampling plot carried Pb and Cd concentrations approximately 13-fold and 157-fold above WHO agricultural-soil limits. All five species examined (Gloriosa superba, Aspilia africana, Crotalaria retusa, Tacca leontopetaloides and Amorphophallus johnsonii) returned BCFroot and BACshoot values below 1 for every metal tested, indicating a predominantly excluder-type accumulation pattern; TFshoot values above 1 for Cd, and for Pb and Cu in most species, indicate that metal reaching the shoot was translocated at a ratio exceeding 1 once past the root, even though absolute tissue concentrations remain low relative to soil. Read together, these indices suggest that phytostabilisation-oriented revegetation may be more appropriate than conventional phytoextraction at this site, although this remains an inference from concentration ratios rather than a demonstrated outcome, since no biomass, metal-mass-balance or soil physicochemical data were collected, and species and microsite were confounded in this single-plot design. Within that shared accumulation pattern, *A. johnsonii*, *C. retusa* and T. leontopetaloides showed the clearest and most complementary species-specific signatures and warrant evaluation in future multi-species revegetation trials that address these limitations. Given the scale of contamination recorded, we would also flag a case for closer regulatory attention from the National Environmental Standards and Regulations Enforcement Agency (NESREA) and for a community environmental health assessment at Enyigba. More broadly, characterising the accumulation behaviour of vegetation already present at a contaminated site, rather than defaulting to exogenous hyperaccumulator candidates, may be a useful starting point for future remediation research at other artisanal mining sites across sub-Saharan Africa facing similar constraints on funding and site access.

Acknowledgements

We thank Prof. C. E. Ogbonna for supervision throughout this study, the Faculty of Environmental Studies, Abia State University, Uturu, Nigeria for logistical support, the taxonomist who assisted with plant identification, and the Enyigba community leadership and community liaison person for facilitating access to the sampling site.

Funding
None.

Conflicts of Interest
The authors declare no conflicts of interest.

Author Contributions
Chukwuezi, A. designed the study, conducted field sampling, and drafted the manuscript; Ogbonna, C. E. supervised the study and reviewed the manuscript; Onwuka, K. E. contributed to conceptualisation and statistical analysis; Otuu, F. C. performed laboratory analysis and data interpretation. All authors read and approved the final manuscript.

Declaration of AI Assistance
The authors declare that AI was not used in preparing or writing any part of this manuscript.

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