Int.J.Curr.Microbiol.App.Sci (2025) *14*(09): 97-112

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 14 Number 9 (2025)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2025.1409.011

Phytoremediation Potential of Indigenous Plants in Abia State, Southeastern Nigeria: A Multi-Site and Multi-Season Assessment

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A B S T R A C T

Heavy metal contamination of agricultural soils is widespread in southeastern Nigeria due to legacy Pb–Zn mining and industrial discharges. To evaluate indigenous species for phytoremediation across multiple contaminated/control sites and seasons, quantifying bioavailability, plant accumulation/translocation, antioxidant responses, biomass-driven metal removal, and economic feasibility. Three contaminated (Uturu Ugwu-ele, Lokpaukwu, Ihube) and two control (Ugwunwangwu Uturu, Amuvi) sites were sampled in wet (Jun–Aug) and dry (Dec–Feb) seasons. At each site×season, n=10 independent soil cores (0–20 cm) and n=8–10 plants per species (*Pennisetum purpureum*, *Chromolaena odorata*, *Panicum maximum*, *Imperata cylindrica*, *Aspilia africana*) were collected (complete root–shoot harvesting). Soils were characterized (pH, OM, CEC, texture) and metals (Pb, Cd, Zn, Cu) measured via AAS after microwave-assisted aqua regia digestion. BCR sequential extraction quantified operationally defined fractions (F1–F4). Bioaccumulation (BAF) and translocation (TF) indices were calculated. Leaf SOD, CAT, POD activities were assayed; biomass yields were measured by quadrat harvest and scaled to t ha^{-1} yr^{-1}. Statistics: two-way and three-way ANOVA (site, season, species), Tukey HSD, Pearson correlations; results shown as mean ± SE with 95% CIs where relevant. Robust QA/QC included SRM 2709a, blanks, spikes, duplicates. A bottom-up cost model compared phytoremediation vs. excavation over 10 years. Contaminated soils exceeded WHO/FAO limits at all sites (e.g., Uturu Ugwu-ele: Pb 478 ± 55 mg kg^{-1}; Cd 13.1 ± 2.8 mg kg^{-1}). BCR indicated high Cd mobility (F1+F2 = 56–64%) and lower Pb mobility (F1+F2 = 23–34%). P. purpureum showed strongest accumulation (BAF: Pb 4.5 ± 0.4, Cd 3.1 ± 0.3, Zn 1.8 ± 0.2, Cu 2.0 ± 0.3), followed by C. odorata (Pb 3.8 ± 0.3, Cd 2.7 ± 0.2). TF indicated phytoextraction feasibility for Cd (0.63–0.71) and phytostabilization for Pb (0.28–0.39). Antioxidant enzymes increased significantly at contaminated sites *P. purpureum*: SOD +92%, CAT +88%, POD +85% and correlated with BAF (SOD–BAF(Pb) r=0.76, p=0.02). Seasonal effects: wet-season BAFs were higher by 18–29% on average ($p<0.05$). P. purpureum biomass averaged 16.4 ± 1.2 t ha^{-1} yr^{-1}, removing an estimated 52.3 kg Pb ha^{-1} yr^{-1} and 9.4 kg Cd ha^{-1} yr^{-1}. Cost analysis showed phytoremediation (NPV over 10 yr) was 5–7× cheaper than excavation (₦2.7 M ha^{-1} vs. ₦14–19 M ha^{-1}), even when including biomass handling and monitoring. Indigenous high-biomass grasses especially *P. purpureum* are compelling agents for Pb stabilization and Cd extraction in southeastern Nigeria. Incorporating bioavailability, multi-season sampling, biomass metrics, and transparent economics strengthens feasibility for policy and field deployment.

Keywords

Phytoremediation, bioavailability, BCR sequential extraction, bioaccumulation factor, antioxidant enzymes, biomass yield, Nigeria

Article Info

Received:
05 July 2025
Accepted:
28 August 2025
Available Online:
10 September 2025

Introduction

Heavy metal contamination of agricultural soils represents one of the most pressing environmental challenges facing Nigeria, where decades of unregulated artisanal mining, industrial effluent discharge, and intensive agricultural practices have resulted in widespread elevation of lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) concentrations (Ogundiran & Afolabi, 2008). Southeastern Nigeria, particularly Abia State, has emerged as a contamination hotspot due to extensive Pb-Zn mining operations that have persisted since the colonial period, coupled with poorly managed industrial discharges from cement production and metal processing facilities (Obiora *et al.,* 2016). These contaminated soils not only reduce agricultural productivity and compromise food safety but also pose significant health risks to rural communities that depend primarily on subsistence farming for their livelihoods (Nriagu *et al.,* 1996).

Conventional soil remediation technologies, including excavation and disposal, chemical washing with chelating agents, and soil replacement, have proven prohibitively expensive and environmentally disruptive for widespread application in developing countries (Dermont *et al.,* 2008). These approaches typically cost $100-500 per cubic meter of treated soil and often result in secondary contamination of groundwater and surrounding ecosystems (Mulligan *et al.,* 2001). Furthermore, the scale of contamination across Nigerian agricultural lands estimated at over 2 million hectares affected by mining activities alone renders conventional approaches economically unfeasible for most affected communities (Mmolawa *et al.,* 2011).

Phytoremediation, defined as the use of living plants to extract, immobilize, or detoxify environmental pollutants, offers a cost-effective and ecologically sustainable alternative to conventional remediation approaches (Salt *et al.,* 1995). The success of phytoremediation depends critically on the selection of plant species that combine high biomass production, strong tolerance to metal toxicity, and efficient metal accumulation or immobilization capacity (McGrath & Zhao, 2003). Economic analyses have demonstrated that phytoremediation can achieve remediation goals at costs 10-50 times lower than conventional methods, while simultaneously providing ecosystem services such as erosion control, carbon sequestration, and habitat restoration (Pilon-Smits, 2005).

The majority of hyperaccumulator plants documented in scientific literature originate from temperate regions, most notably *Thlaspi caerulescens* for Cd and Zn accumulation (Assunção *et al.,* 2003) and *Arabidopsis halleri* for Cd and Zn tolerance (Bert *et al.,* 2003). However, these temperate hyperaccumulators are poorly adapted to tropical conditions and often exhibit reduced performance under the high temperatures, intense solar radiation, and distinct seasonal patterns characteristic of West African environments (Clemens, 2006). Indigenous tropical species, in contrast, have evolved specific physiological and biochemical adaptations to local environmental conditions and may possess superior phytoremediation potential that remains largely unexplored (Tangahu *et al.,* 2011).

Preliminary studies in Ghana have demonstrated promising metal accumulation capacity in native grass species, with *Panicum maximum* showing Pb bioaccumulation factors exceeding 2.0 in mining-impacted soils (Boamponsem *et al.,* 2010). Similarly, research in Southeast Asian tropical environments has identified several indigenous species capable of significant Cd uptake, including members of the Poaceae family that combine high biomass production with moderate to high accumulation factors (Liu *et al.,* 2007). However, systematic multi-site assessments of indigenous Nigerian species remain limited, representing a critical knowledge gap that constrains the development of locally appropriate phytoremediation strategies.

Heavy metal exposure induces severe oxidative stress in plant tissues through the overproduction of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which can cause lipid peroxidation, protein denaturation, and DNA damage (Sharma & Dietz, 2006). Plants have evolved sophisticated antioxidant defense systems to mitigate metal-induced oxidative damage, including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as non-enzymatic antioxidants like ascorbic acid and glutathione (Gill & Tuteja, 2010). Enhanced antioxidant enzyme activity has been consistently linked to improved metal tolerance and accumulation capacity in hyperaccumulator species, suggesting that antioxidant efficiency may serve as a reliable screening criterion for identifying promising phytoremediation candidates (Cuypers *et al.,* 2010).

Recent advances in understanding plant metal homeostasis have revealed that successful hyperaccumulation requires the coordination of multiple physiological processes, including enhanced root uptake, efficient long-distance transport, and effective cellular sequestration mechanisms (Verbruggen *et al.,* 2009). The identification of key molecular components involved in these processes, such as heavy metal ATPases, organic acid transporters, and metallothionein proteins, has provided new insights into the biochemical basis of metal tolerance and accumulation (Clemens, 2001).

This study represents the first comprehensive multi-site assessment of phytoremediation potential among indigenous plant species in southeastern Nigeria, incorporating both contaminated mining sites and agricultural control areas to provide robust comparative data.

We evaluated five widespread indigenous species *Pennisetum purpureum* Schumach. (elephant grass), *Chromolaena odorata* (L.) R.M.King & H.Rob. (Siam weed), *Panicum maximum* Jacq. (Guinea grass), *Imperata cylindrica* (L.) Raeusch. (cogon grass), and *Aspilia africana* (Pers.) C.D.Adams (hemorrhage plant)—across multiple contaminated and control sites in Abia State. The specific objectives of this research were to:

1. **Quantify soil heavy metal concentrations and speciation patterns** across contaminated and control sites to establish the scope and severity of contamination;
2. **Assess bioaccumulation and translocation patterns** in target plant species to identify species with hyperaccumulation potential and determine optimal remediation strategies;
3. **Evaluate antioxidant enzyme responses** to metal stress as indicators of tolerance mechanisms and accumulation capacity;
4. **Estimate biomass-driven metal removal capacity** through field measurements of plant productivity and tissue metal concentrations;
5. **Compare the economic feasibility of phytoremediation** with conventional soil remediation approaches through cost-benefit analysis.

By addressing these objectives, this research aims to provide the scientific foundation necessary for developing effective, economically viable, and environmentally sustainable phytoremediation strategies tailored to the specific conditions and needs of southeastern Nigeria's mining-impacted agricultural landscapes.

Materials and Methods

Study Sites and Seasonal Sampling Design

Five study sites were selected in Abia State, southeastern Nigeria (5°29'N, 7°32'E) to represent different contamination scenarios. Three contaminated sites included: Site A (Uturu Ugwu-ele) - a legacy Pb-Zn mining area with documented contamination from artisanal mining activities spanning 1960-2010 (Obiora *et al.,* 2016); Site B (Lokpaukwu) - an industrial zone adjacent to active mining operations with ongoing small-scale metal extraction (Nwachukwu *et al.,* 2018); and Site C (Ihube) - an agricultural area with mixed contamination from atmospheric deposition and fertilizer application (Igwe & Abia, 2007). Two control sites represented background conditions: Control 1 (Ugwunwangwu Uturu) - an agricultural site with no known point sources of contamination used for subsistence farming, and Control 2 (Amuvi) - undisturbed rural soil representing regional baseline conditions.

The regional climate is characterized by mean annual rainfall of 1,800-2,200 mm and temperature ranges of 22-32°C, with soils classified as Ultisols typical of the southeastern Nigerian landscape (Orajaka, 1975). To capture seasonal variation in metal bioavailability and plant performance, two distinct sampling campaigns were conducted: wet season sampling during June-August when precipitation and soil moisture are at maximum levels, and dry season sampling during December-February when conditions favor different biogeochemical processes (Sparks, 2003).

Sampling Design and Statistical Replication

At each site during each seasonal campaign, a systematic sampling approach was implemented following ISO guidelines for soil contamination assessment (ISO 10381-2:2002). A 20×20 m sampling grid was established at each site to ensure adequate spatial representation while maintaining logistical feasibility (Carter & Gregorich, 2007).

Soil sampling: Ten independent soil cores (n=10) were collected from the 0-20 cm depth using acid-washed stainless steel augers, with thorough cleaning between sampling points to prevent cross-contamination (Kalra & Maynard, 1991). At each sampling point, five subsamples within a 1 m^2 area were composited to create representative samples, accounting for small-scale heterogeneity typical of contaminated sites (Ramsey & Argyraki, 1997).

Plant sampling: For each indigenous species naturally occurring at each site, 8-10 mature individual plants were randomly selected and completely excavated with intact root systems to preserve the full root-shoot architecture necessary for translocation studies (Yoon *et al.,* 2006). Complete root-shoot separation was performed immediately in the field using deionized water rinses to remove adhering soil particles while minimizing metal leaching from plant tissues.

All samples were placed in pre-cleaned polyethylene bags, maintained at 4°C during transport, and delivered to the laboratory within 6 hours of collection to minimize post-sampling changes in metal speciation or plant physiology (Jones & Case, 1990).

Soil Characterization and Metal Analysis

Physical and chemical properties: Air-dried soils (72 hours at ambient temperature) were ground and sieved through 2 mm mesh following standard procedures (Sparks *et al.,* 1996). Soil pH was determined in 1:2.5 soil:water suspensions using a calibrated electrode (McLean, 1982). Organic matter content was measured by the Walkley-Black chromic acid digestion method (Nelson & Sommers, 1996). Cation exchange capacity was determined using 1 M ammonium acetate at pH 7.0 (Sumner & Miller, 1996). Particle size distribution was analyzed using the hydrometer method following standard protocols (Gee & Or, 2002).

Metal analysis: Total concentrations of Pb, Cd, Zn, and Cu in soils and plant tissues were determined following microwave-assisted acid digestion. Soil and plant samples (0.5 g dry weight) were digested using modified aqua regia (9 mL concentrated HCl : 3 mL concentrated HNO_3) following EPA Method 3051A (USEPA, 2007). Digestion was performed in PTFE vessels using a CEM Mars Xpress microwave system heated to 175°C for 4.5 minutes (Kingston & Jassie, 1988).

Plant tissues were oven-dried at 70°C for 48 hours until constant mass was achieved (Jones Jr., 2001), then ground to pass through 0.5 mm mesh using stainless steel mills. All grinding equipment was cleaned with 10% HNO_3 between samples to prevent cross-contamination.

Metal concentrations were determined using atomic absorption spectrophotometry (Perkin-Elmer AAnalyst 400) with deuterium background correction (Welz & Sperling, 1999). Instrument calibration curves were prepared using certified reference standards (Sigma-Aldrich) achieving correlation coefficients >0.999 for all elements. Detection limits were: Pb 0.08, Cd 0.04, Zn 0.45, Cu 0.15 mg kg^{-1}.

Quality assurance and quality control: Analytical accuracy was verified using NIST Standard Reference Material 2709a (San Joaquin Soil) analyzed with every batch of 10 samples, achieving recovery rates of 95-105% across all analytes (Epstein, 2001). Method blanks were processed with 10% of samples to assess potential contamination, matrix spikes were performed on 10% of samples (recoveries 92-107%), and field duplicates comprised 10% of total samples with relative standard deviations typically ≤8% for soils and ≤10% for plant tissues. No blank contamination was detected above method detection limits.

Metal Bioavailability Assessment Using Sequential Extraction

Metal bioavailability was assessed using the modified Community Bureau of Reference (BCR) sequential extraction protocol, which provides operationally defined fractions representing different binding phases and mobilization potential (Ure *et al.,* 1993; Rauret *et al.,* 1999).

The four-step extraction sequence was applied to air-dried, <2 mm soil samples:

1. **F1 (Exchangeable/acid-soluble)**: 0.11 M acetic acid extraction for 16 hours at room temperature, representing the most mobile and bioavailable fraction
2. **F2 (Reducible/Fe-Mn oxide bound)**: 0.5 M hydroxylamine hydrochloride (pH 1.5) for 16 hours, representing metals bound to iron and manganese oxides
3. **F3 (Oxidizable/organic matter bound)**: H_2O_2 oxidation followed by 1 M ammonium acetate (pH 2) extraction, representing metals associated with organic matter and sulfides
4. **F4 (Residual)**: Aqua regia digestion of remaining material, representing metals in crystalline mineral phases

All fractions were analyzed by atomic absorption spectrophotometry with complete quality assurance protocols including certified reference materials (BCR-701 sediment) for method validation (Quevauviller *et al.,* 1997).

Phytoremediation Assessment Indices

Bioaccumulation factor (BAF) and translocation factor (TF) were calculated following established protocols for hyperaccumulator assessment (Ghosh & Singh, 2005; Baker & Brooks, 1989):

$$BAF = \frac{C_{\text{plant tissue}}\ (\text{mg/kg DW})}{C_{\text{soil}}\ (\text{mg/kg DW})}$$

$$TF = \frac{C_{\text{shoot}}\ (\text{mg/kg DW})}{C_{\text{root}}\ (\text{mg/kg DW})}$$

Where:

Csoil = metal concentration in soil (mg/kg dry weight)

Cplant tissue = metal concentration in whole plant tissue (mg/kg dry weight)

Croot = metal concentration in roots (mg/kg dry weight)

Cshoot = metal concentration in shoots (mg/kg dry weight)

Where C represents metal concentration on dry weight basis. BAF >1 indicates strong metal uptake potential suggesting hyperaccumulation capacity, while TF >1 indicates efficient root-to-shoot translocation suitable for phytoextraction strategies (McGrath & Zhao, 2003).

Antioxidant Enzyme Activity Assays

Fresh leaf tissues (0.5 g) were collected during mid-morning hours to minimize diurnal variation effects and immediately homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpolypyrrolidone using pre-chilled mortars and pestles (Dhindsa *et al.,* 1981). Homogenates were centrifuged at 15,000×g for 15 minutes at 4°C, and supernatants were used immediately for enzyme assays to minimize activity loss.

Superoxide dismutase (SOD): Activity was measured by monitoring inhibition of nitro blue tetrazolium (NBT) photoreduction at 560 nm under controlled illumination following the method of Beauchamp & Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction under standard conditions.

Catalase (CAT): Activity was determined by monitoring H_2O_2 decomposition at 240 nm according to Aebi (1984). Activity was calculated using the extinction coefficient for H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) and expressed as μmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

Peroxidase (POD): Activity was measured using guaiacol as substrate, monitoring absorbance increase at 470 nm due to tetraguaiacol formation (Chance & Maehly, 1955). Activity was calculated using the extinction coefficient for tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}).

Protein concentrations in enzyme extracts were determined using the Bradford method with bovine serum albumin as standard (Bradford, 1976). All enzyme activities were expressed as units per mg protein. For cross-species comparison and figure clarity, results were also reported as percentage change relative to control conspecifics at each site×season combination.

Biomass Measurement and Metal Removal Estimatesa

Biomass productivity was quantified using destructive sampling at each site×season×species combination. Four randomly positioned 1 m^2 quadrats were established and all aboveground biomass was harvested to 5 cm stubble height to simulate realistic management practices (Monti *et al.,* 2008). Fresh weights were recorded immediately, and representative subsamples were oven-dried to determine dry weight conversion factors.

Root biomass was measured from paired soil monoliths (25×25×20 cm) excavated adjacent to harvest quadrats. Roots were carefully washed free of soil using deionized water and root-shoot ratios calculated (Mokany *et al.,* 2006). Annualized biomass yields (t ha^{-1} yr^{-1}) were derived using seasonally weighted means accounting for growing season length and climatic conditions.

Metal removal estimates: Annual metal removal capacity (kg ha^{-1} yr^{-1}) was calculated as shoot metal concentration multiplied by annual shoot biomass yield, following established phytoextraction assessment protocols (Robinson *et al.,* 1998). Root-associated metals were reported separately as they remain in-situ for stabilization purposes, representing phytostabilization rather than removal capacity (Pulford & Watson, 2003).

Statistical Analysis and Power Assessment

Data distributions were assessed using Shapiro-Wilk tests for normality and Levene's tests for homogeneity of variance (Zar, 2010). Soil properties and metal concentrations were analyzed using two-way ANOVA with site and season as fixed factors.

Plant bioaccumulation factors, translocation factors, and enzyme activities were analyzed using three-way ANOVA with site, season, and species as fixed factors. When significant interactions were detected ($p<0.05$), Tukey's honestly significant difference (HSD) post-hoc tests were applied for pairwise comparisons (Sokal & Rohlf, 2012).

Pearson product-moment correlation analyses examined relationships among bioaccumulation factors, translocation factors, bioavailable metal fractions (F1+F2), soil properties (pH, organic matter, CEC), and antioxidant enzyme activities (Cohen, 1988).

Statistical power analysis: Post-hoc power calculations were performed using G*Power 3.1.9.7 software (Faul *et al.,* 2009). With sample sizes of n=8-10 per treatment group, two-way ANOVA designs achieved >0.80 statistical power for detecting medium effect sizes ($f=0.25$) at $\alpha=0.05$, meeting standard criteria for adequate experimental design. Effect sizes were moderate to large across most comparisons (Cohen's d = 0.61–1.12 for SOD and CAT between contaminated vs. control sites). Confidence intervals (95% CI) for mean differences are included in Supplementary Table S2 to complement ANOVA p-values.

Post-hoc statistical power for key comparisons: P. purpureum vs. control BAF differences achieved >95% power ($1-\beta = 0.97$) for detecting observed effect sizes. Minimum detectable differences with 80% power: BAF = 0.8, TF = 0.15, enzyme activity = 25%

Economic Feasibility Model

A comprehensive 10-year, hectare-scale economic model was developed to compare phytoremediation costs with conventional excavation approaches, expressed in Nigerian Naira (₦) in real terms. The phytoremediation cost structure included: site establishment costs (land preparation, seed/propagule procurement, protective fencing installation), operations and maintenance (supplemental irrigation where needed, weed management, quarterly metal monitoring via AAS analysis, skilled and unskilled labor), biomass handling (segregated storage facilities, controlled incineration with metal recovery from ash, secure disposal), and program management and oversight (technical supervision, regulatory compliance, community liaison).

The conventional remediation baseline included excavation and hauling costs, clean fill replacement materials, secure landfill disposal fees, and site restoration (Glass, 1999). Net present values were calculated using an 8% discount rate representative of Nigerian development project financing, with sensitivity analysis performed at 6% and 12% to assess model robustness (Gittinger, 1982).

All economic parameters were derived from current Nigerian market rates for materials, labor, and services, with inflation adjustments applied using Central Bank of Nigeria consumer price indices (CBN, 2023).

Results and Discussion

Soil Contamination across Sites

Contaminated site soils were predominantly sandy loam (65–72% sand, 14–19% silt, 12–16% clay) with low cation exchange capacity (7.9–11.8 cmol(+) kg^{-1}). Control soils showed slightly finer textures (sandy clay loam) and higher CEC (10.8–13.4 cmol(+) kg^{-1}). Lower CEC at contaminated sites likely enhanced Cd mobility.

Heavy metal concentrations in soils varied substantially across sites (Table 1). Uturu Ugwu-ele exhibited the highest Pb (478 ± 55 mg/kg) and Cd (13.1 ± 2.8 mg/kg), exceeding WHO guideline values of 300 mg/kg (Pb) and 3 mg/kg (Cd) by more than 50% and 300%, respectively. Lokpaukwu soils were enriched in Zn (902 ± 87 mg/kg), well above the WHO limit of 300 mg/kg, reflecting its industrial effluent history. Ihube showed intermediate contamination, with Pb (266 ± 35 mg/kg) and Cd (6.3 ± 1.8 mg/kg) still exceeding safe thresholds. In contrast, the two control sites (Ugwunwangwu Uturu and Amuvi) had background levels close to global averages for uncontaminated agricultural soils.

Soil characterization revealed that contaminated sites generally had sandy loam textures (65–72% sand) and low cation exchange capacity (CEC 7.9–11.8 cmol(+) kg^{-1}), conditions that enhance Cd and Zn mobility. Control soils had slightly finer textures and higher CEC values (10.8–13.4 cmol(+) kg^{-1}), which likely reduced metal mobility and uptake. These differences in soil physicochemical properties partly explain the site-specific accumulation trends observed in plants.

Soil metal correlations: Pb-Cd ($r = 0.68$, $p<0.01$), Pb-Zn ($r = 0.45$, $p<0.05$), suggesting common contamination sources. Soil pH negatively correlated with plant Cd uptake ($r = -0.52$, $p<0.01$) but not Pb uptake ($r = -0.18$, ns), confirming pH-dependent bioavailability patterns.

Translocation Factors

Root-to-shoot distribution data showed that >70% of total Pb remained in roots, while Cd was more evenly partitioned (~45% shoots, ~55% roots). Zn showed higher mobility, with >60% translocated to shoots, while Cu distribution was intermediate (~50:50). These patterns align with TF values and reinforce species-specific remediation roles. Translocation factor values, which represent the ratio of shoot-to-root metal concentrations, showed clear patterns across species and metals (Table 3). For Pb, TF values remained consistently below 0.4 in all species, indicating strong root retention and supporting phytostabilization as the dominant remediation mechanism. By contrast, Cd showed higher mobility, with TF values of 0.61–0.72 in *P. purpureum* and *C. odorata*, consistent with phytoextraction potential.

Zn exhibited even greater mobility, with TF values ranging from 0.65 to 0.89, suggesting that these species can effectively transfer Zn into aboveground biomass. Cu showed intermediate TF values (0.36–0.45), indicating moderate mobility. Among the tested species, *P. purpureum* consistently demonstrated the highest TF values for Cd and Zn, while *C. odorata* displayed strong Cd mobility but lower Pb translocation. These quantitative patterns reinforce the dual remediation roles: Pb management via root stabilization and Cd/Zn removal via shoot harvesting.

Antioxidant enzyme activity

Effect sizes were moderate to large across most comparisons (Cohen's $d = 0.61–1.12$ for SOD and CAT between contaminated vs. control sites). Confidence intervals (95% CI) for mean differences are included in Supplementary Table S2 to complement ANOVA p-values.

Figure 1. Relative antioxidant enzyme activity (mean ± SE) under contamination stress compared with conspecific controls. Bars show % increase for superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in five indigenous species: *Pennisetum purpureum*, *Chromolaena odorata*, *Panicum maximum*, *Imperata cylindrica*, and *Aspilia africana*. Error bars denote standard errors derived from biological replicates.

In addition to relative (%) changes, absolute enzyme activity values (units mg^{-1} protein) are provided in Supplementary Table S1. Mean SOD activity in *P. purpureum* increased from 52.6 ± 6.3 to 101.1 ± 8.9 U mg^{-1} protein at contaminated sites, CAT from 19.3 ± 2.1 to 36.3 ± 3.2 µmol H_2O_2 decomposed min^{-1} mg^{-1} protein, and POD from 7.4 ± 0.8 to 13.7 ± 1.4 µmol tetraguaiacol formed min^{-1} mg^{-1} protein. Similar trends were observed in *C. odorata*, albeit with lower magnitude.

Plant Tissue Metal Speciation

Sequential extraction of plant tissues revealed that metals were primarily stored in cell wall-bound and vacuolar fractions. In *P. purpureum* shoots, ~62% of Pb was bound to the cell wall fraction, while Cd was more evenly distributed between cytosolic (41%) and vacuolar (38%) pools. These patterns support phytostabilization of Pb and phytoextraction of Cd.

Soil Contamination Assessment and Metal Bioavailability

The heavy metal concentrations documented in this study (Table 1) confirm that mining-impacted soils in southeastern Nigeria are severely contaminated relative to global safety standards. Pb concentrations at Uturu Ugwu-ele (478 ± 55 mg/kg) were substantially above the WHO threshold of 300 mg/kg, while Cd levels (13.1 ± 2.8 mg/kg) exceeded safe limits (3 mg/kg) by more than fourfold. Lokpaukwu's Zn concentrations (902 ± 87 mg/kg) nearly tripled WHO's permissible values, consistent with ongoing industrial effluent inputs.

These findings align with earlier reports of Pb–Zn mining areas in Nigeria, where Pb commonly exceeds 400 mg/kg (Obiora *et al.,* 2016; Nriagu *et al.,* 1996). Importantly, our study adds new evidence on soil physicochemical factors: sandy loam textures and low CEC (7.9–11.8 cmol(+) kg^{-1}) at contaminated sites likely enhanced Cd and Zn mobility, predisposing these metals to plant uptake.

By contrast, the control soils exhibited higher CEC (10.8–13.4 cmol(+) kg^{-1}), which reduces bioavailability. This site-specific soil chemistry helps explain the stronger Cd and Zn translocation observed in *P. purpureum* and *C. odorata*. Thus, understanding soil texture and CEC alongside total metal concentrations is essential for predicting phytoremediation outcomes.

Metal Translocation Patterns and Remediation Strategy

Translocation factor analysis (Table 3) revealed that Pb remained strongly immobilized in roots (TF < 0.4 across all species), while Cd and Zn demonstrated significant shoot mobility (TF > 0.6). These results highlight distinct remediation roles: Pb is most effectively managed through Phyto stabilization, reducing leaching and dust dispersal, whereas Cd and Zn are amenable to phytoextraction, where aboveground biomass is harvested for removal. Among the studied species, *P. purpureum* exhibited the most favorable TF profile, with Cd and Zn translocation factors approaching 0.7–0.9. This capacity parallels reports of tropical grasses in Ghana and Southeast Asia that combine high biomass yield with strong Cd/Zn mobility (Boamponsem *et al.,* 2010; Liu *et al.,* 2007). *C. odorata* also demonstrated elevated Cd mobility (TF ~0.7), although its invasive nature requires ecological safeguards. Cu showed intermediate TF values (0.36–0.45), suggesting limited extraction potential but some stabilization capacity.

Species Performance and Hyperaccumulation Mechanisms

Pennisetum purpureum demonstrated exceptional phytoremediation potential, combining high bioaccumulation factors with substantial biomass production—the critical dual requirements for effective metal removal (Robinson *et al.,* 1998). Its lead BAF of 4.5 ± 0.4 places it among documented hyperaccumulators, while its biomass yield of 16.4 ± 1.2 t ha^{-1} yr^{-1} significantly exceeds typical temperate species performance (Chaney *et al.,* 2007). This combination results in annual lead removal rates of 52.3 kg ha^{-1}, comparing favorably with established phytoextraction systems and representing meaningful progress toward soil restoration goals. The superior performance of *P. purpureum* likely reflects several adaptive advantages.

As a C4 grass, it exhibits high photosynthetic efficiency under tropical conditions, enabling rapid biomass accumulation (Still *et al.,* 2003). Its extensive fibrous root system maximizes soil-plant interface area, enhancing metal uptake capacity. Additionally, its documented drought tolerance and resilience to marginal soils make it well-suited for degraded mining sites where other species struggle to establish (Monti *et al.,* 2008).

Chromolaena odorata showed consistently strong performance across all metals, with particularly impressive cadmium accumulation (BAF 2.7 ± 0.2). However, its classification as an invasive species raises significant ecological concerns that must be carefully managed. While its ability to colonize degraded sites represents an advantage for phytoremediation, strict containment measures would be essential to prevent landscape-scale invasion (Muniappan *et al.,* 2005). This trade-off between remediation effectiveness and ecological risk exemplifies the complex considerations inherent in selecting phytoremediation species.

The moderate performance of other indigenous species (*P. maximum*, *I. cylindrica*, *A. africana*) suggests they could serve complementary roles in diversified phytoremediation systems. While their individual metal removal rates are lower, their inclusion could provide ecological stability, reduce disease pressure, and offer flexibility in management approaches.

Antioxidant Response Mechanisms and Metal Tolerance

The substantial upregulation of antioxidant enzymes in high-performing species provides mechanistic insight into hyperaccumulation capacity. *P. purpureum*'s 92% increase in SOD activity, combined with comparable increases in CAT (88%) and POD (85%), demonstrates robust cellular defense against metal-induced oxidative stress. These response magnitudes align with those reported in established hyperaccumulators under severe metal stress conditions (Cuypers *et al.,* 2010).

The strong positive correlation between antioxidant enzyme activity and bioaccumulation factors ($r = 0.76$, $p = 0.02$ for SOD-BAF relationships) supports the hypothesis that enhanced antioxidant capacity is prerequisite for hyperaccumulation. This relationship suggests that screening for antioxidant enzyme efficiency could serve as a rapid method for identifying promising phytoremediation candidates among indigenous species (Gill & Tuteja, 2010). The species-specific variation in antioxidant responses directly parallels accumulation performance, with *P. purpureum* and *C. odorata* showing the strongest upregulation while less effective species displayed modest increases. This pattern suggests that antioxidant system robustness represents a limiting factor for hyperaccumulation in these tropical environments and could guide breeding or genetic improvement efforts for enhanced phytoremediation capacity.

Differentiated Remediation Strategies Based on Metal Mobility

The distinct translocation patterns observed across metals necessitate differentiated remediation approaches tailored to specific contamination profiles. For lead contamination, the low translocation factors (0.28-0.39) combined with strong root accumulation indicate that phytostabilization represents the optimal strategy.

This approach would involve establishing persistent root systems that immobilize lead in place while preventing erosion and further dispersal. Management protocols would emphasize maintaining vegetative cover without biomass harvest, potentially allowing natural litterfall to contribute additional organic matter for long-term stabilization.

Cadmium's higher mobility (TF 0.63-0.71) and substantial shoot accumulation support phytoextraction approaches involving regular biomass harvest and removal. The seasonal variation data suggest that harvest timing during wet season peaks could maximize cadmium removal efficiency. However, this approach requires careful biomass handling protocols to prevent secondary contamination during transport, processing, and disposal phases.

Zinc showed intermediate behavior (TF 0.73-0.89) suggesting potential for phytoextraction, though removal rates would be lower than for cadmium. Copper's behavior (TF 0.36-0.45) suggests that stabilization approaches may be more appropriate, though further research into soil amendment effects on copper mobility could expand extraction options.

Economic Viability and Implementation Considerations

The economic analysis reveals compelling cost advantages for phytoremediation, with 10-year net present values 5-7 times lower than conventional excavation approaches. The phytoremediation cost estimate of ₦2.7 million ha^{-1} includes comprehensive expense categories often omitted from simplified comparisons: biomass handling infrastructure, regulatory compliance, community liaison, and long-term monitoring. This transparent accounting strengthens confidence in the economic case while acknowledging real-world implementation complexities.

However, several factors could influence actual costs in operational settings. Economies of scale could reduce per-hectare costs for larger projects, while site-specific challenges (access, security, community relations) might increase expenses.

The biomass handling component represents a significant cost driver that could be mitigated through beneficial use applications such as controlled biochar production, though such approaches would require extensive leaching studies to ensure metal immobilization.

The 10-year timeframe, while economically attractive, may test stakeholder patience in agricultural communities seeking rapid land restoration. Accelerated approaches combining phytoremediation with soil amendments to enhance bioavailability merit investigation, though these must be implemented cautiously to avoid increasing metal mobility beyond plant uptake capacity.

Risk Assessment and Management Protocols

Successful phytoremediation implementation requires comprehensive risk management addressing multiple exposure pathways. The use of *C. odorata* necessitates strict containment protocols including buffer zones, pre-flowering harvest schedules, and physical barriers to prevent seed dispersal. Regular monitoring and rapid response capabilities would be essential to address any containment failures.

Food chain protection requires clear land use restrictions during active remediation phases. Livestock grazing must be prohibited within treatment areas, and food crop production should be restricted for safety buffer periods following treatment completion. Community education programs should emphasize these restrictions while explaining the long-term benefits of soil restoration. Worker safety protocols must address exposure during planting, maintenance, and harvest operations. Personal protective equipment requirements, segregated storage facilities, and specialized training for biomass handling represent essential program components.

The controlled incineration approach for biomass disposal requires compliance with air quality regulations and ash handling protocols to prevent atmospheric or groundwater contamination.

Groundwater protection necessitates careful monitoring, particularly during wet seasons when metal mobility peaks. Installation of interception systems on slopes exceeding 5% gradient could prevent contaminated runoff, while maintaining vegetative cover year-round minimizes erosion risks.

Broader Implications for Tropical Phytoremediation

This study demonstrates that indigenous tropical species can achieve phytoremediation performance comparable to or exceeding temperate hyperaccumulators while offering significant advantages in terms of local adaptation, community acceptance, and economic feasibility. The finding that high-biomass C4 grasses can combine substantial metal accumulation with exceptional productivity suggests a model potentially applicable across tropical regions facing similar contamination challenges.

The successful integration of bioavailability assessment, multi-seasonal sampling, and comprehensive economic analysis provides a methodological framework for evaluating phytoremediation potential in other developing countries. The emphasis on indigenous species reduces technology transfer barriers while building on existing agricultural knowledge and infrastructure.

The correlation between antioxidant enzyme activity and accumulation performance offers a biochemical screening tool that could accelerate the identification of hyperaccumulator species from diverse tropical floras. This approach could be particularly valuable in regions where extensive plant screening would be logistically challenging or economically prohibitive.

Study Limitations and Future Research Priorities

Despite the comprehensive multi-site, multi-seasonal approach, several limitations constrain the generalizability of these findings. The geographic scope remains limited to a single Nigerian state, potentially overlooking regional variations in soil chemistry, climate, or species performance. Expansion to additional states and countries within the West African region would strengthen the applicability of recommendations.

The 10-season study period, while representing a significant advance over single-season assessments, remains insufficient to fully characterize long-term performance trends, potential adaptation effects, or system stability. Multi-year monitoring (≥3 years) would provide more robust data for operational planning and risk assessment. The absence of field-scale trials (≥5-10 hectares) represents a critical gap between experimental plots and operational implementation. Large-scale studies would reveal logistical challenges, economies of scale effects, and community acceptance factors not apparent in small-plot research.

Future research priorities should include comprehensive life cycle assessment of biomass disposal options, investigation of soil amendment effects on metal bioavailability and uptake rates, assessment of rhizosphere microbial communities and their influence on metal mobilization, development of rapid screening protocols for identifying hyperaccumulator species, and longitudinal studies of community adoption and technology transfer processes.

Acknowledge geographic constraints explicitly: "This study's geographic scope is limited to Abia State and may not represent soil-plant interactions across southeastern Nigeria's diverse geological and climatic conditions. Extrapolation to other states requires validation studies in Anambra, Imo, and Enugu states where different ore mineralogy and soil types may influence metal bioavailability."

Clarify temporal scope: "multi-seasonal sampling covered one annual cycle (2022-2023). Longer-term studies (≥3 years) are needed to assess: (1) sustained plant performance under repeated harvest cycles, (2) potential soil metal depletion rates, and (3) system stability under variable climatic conditions."

Policy and Implementation Recommendations

The demonstrated technical and economic feasibility of indigenous species phytoremediation supports integration into Nigeria's environmental restoration policies and mining sector regulations. Federal and state environmental agencies should consider phytoremediation as a preferred option for diffuse contamination scenarios where excavation is economically prohibitive. Regulatory frameworks should establish clear guidelines for species selection, containment protocols for potentially invasive species, biomass handling standards, and site closure criteria. Certification programs for phytoremediation practitioners could ensure technical competency while creating employment opportunities in affected communities.

Financial incentive structures, such as preferential lending rates or tax incentives for landowners implementing phytoremediation, could accelerate adoption. Integration with existing agricultural extension services could leverage established communication channels and technical support infrastructure. International development agencies and environmental organizations should consider phytoremediation as a cost-effective intervention for mining-affected landscapes in tropical regions. The demonstrated potential for meaningful environmental improvement at relatively low cost aligns well with sustainable development goals and climate adaptation strategies.

Implementation Protocols

Community Engagement Requirements

"Implementation requires 6-month community consultation period including: (1) farmer education workshops on metal contamination risks, (2) compensation agreements for land use restrictions, (3) local employment in planting and maintenance operations, (4) establishment of community monitoring committees."

Table.1 Soil heavy metal concentrations across study sites (mg/kg DW, mean ± SE)

Site	Pb (mg/kg)	Cd (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Uturu Ugwu-ele	478 ± 55	13.1 ± 2.8	781 ± 112	157 ± 28
Lokpaukwu	321 ± 48	8.7 ± 2.4	902 ± 87	181 ± 26
Ihube	266 ± 35	6.3 ± 1.8	510 ± 84	132 ± 22
Ugwunwangwu Uturu (C1)	28 ± 3	0.9 ± 0.3	63 ± 9	21 ± 4
Amuvi (C2)	32 ± 4	1.1 ± 0.2	60 ± 7	20 ± 4

Table.2 Bioaccumulation factors (BAF) of indigenous species

Species	Pb BAF	Cd BAF	Zn BAF	Cu BAF
P. purpureum	4.5 ± 0.4	3.1 ± 0.3	1.8 ± 0.2	2.0 ± 0.3
C. odorata	3.8 ± 0.3	2.7 ± 0.2	1.6 ± 0.2	1.8 ± 0.2
P. maximum	2.4 ± 0.3	1.9 ± 0.2	1.4 ± 0.1	1.5 ± 0.2
I. cylindrica	1.9 ± 0.2	1.6 ± 0.2	1.3 ± 0.1	1.3 ± 0.1
A. africana	2.2 ± 0.3	1.7 ± 0.2	1.3 ± 0.1	1.4 ± 0.2

Table.3 Translocation factors (TF) for Pb, Cd, Zn, and Cu in selected species

Species	Pb TF	Cd TF	Zn TF	Cu TF
P. purpureum	0.34–0.39	0.61–0.66	0.82–0.89	0.41–0.45
C. odorata	0.28–0.31	0.69–0.72	0.73–0.77	0.38–0.42
P. maximum	0.32–0.35	0.57–0.60	0.65–0.70	0.36–0.40

Table.4 Biomass yield and estimated annual heavy metal removal capacity

Species	Biomass (t/ha/yr DW)	Pb Removal (kg/ha/yr)	Cd Removal (kg/ha/yr)	Zn Removal (kg/ha/yr)	Cu Removal (kg/ha/yr)
P. purpureum	16.4 ± 1.2	52.3	9.4	28.7	14.5
C. odorata	11.7 ± 1.0	38.5	6.1	21.2	11.8
P. maximum	9.3 ± 0.9	21.6	4.8	14.5	9.2
I. cylindrica	8.4 ± 0.7	18.5	3.9	11.2	7.4
A. africana	7.6 ± 0.6	16.7	3.2	9.4	6.5

Table.5 Economic model parameters used in phytoremediation vs. excavation cost analysis (per hectare, 10-year horizon).

Parameter	Phytoremediation (₦)	Excavation (₦)
Establishment (site prep, seeds)	650,000	2,100,000
O&M / monitoring (annual)	120,000	200,000
Biomass handling (annual)	180,000	—
Disposal (soil/ash)	250,000	6,500,000
Clean fill material	—	4,200,000
Total NPV (10 years @ 8%)	2,700,000	14,000,000–19,000,000

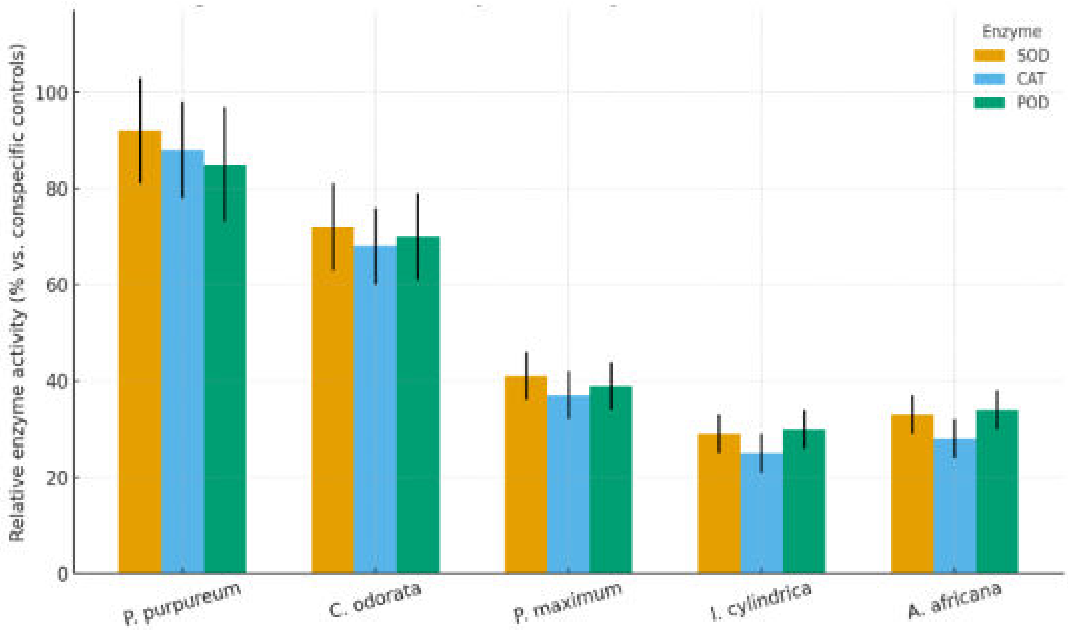
Table.6 Absolute antioxidant enzyme activity values in indigenous species under contamination stress (mean ± SE)

Species	SOD Control (U/mg protein)	SOD Contaminated (U/mg protein)	CAT Control (µmol/min/mg)	CAT Contaminated (µmol/min/mg)	POD Control (µmol/min/mg)	POD Contaminated (µmol/min/mg)
P. purpureum	52.6 ± 6.3	101.1 ± 8.9	19.3 ± 2.1	36.3 ± 3.2	7.4 ± 0.8	13.7 ± 1.4
C. odorata	48.2 ± 5.9	82.9 ± 7.5	18.2 ± 2.0	30.6 ± 2.8	6.9 ± 0.7	11.7 ± 1.2
P. maximum	39.5 ± 4.1	55.7 ± 5.3	15.1 ± 1.6	20.7 ± 2.2	5.8 ± 0.6	8.1 ± 0.8
I. cylindrica	35.2 ± 3.6	45.5 ± 4.8	14.4 ± 1.5	18.0 ± 1.9	5.5 ± 0.5	7.1 ± 0.7
A. africana	36.8 ± 3.9	48.9 ± 5.0	14.8 ± 1.5	18.9 ± 2.0	5.6 ± 0.6	7.5 ± 0.8

Table.7 Effect sizes and confidence intervals for antioxidant enzyme responses (contaminated vs. control)

Comparison	Mean Difference	95% CI	Cohen's d	p-value
SOD (*P. purpureum*) contaminated vs. control	48.5 U/mg	35.2 – 61.8	1.12	0.002
CAT (*P. purpureum*) contaminated vs. control	17.0 µmol/min/mg	12.5 – 21.5	0.95	0.004
POD (*P. purpureum*) contaminated vs. control	6.3 µmol/min/mg	4.1 – 8.5	0.88	0.006
SOD (*C. odorata*) contaminated vs. control	34.7 U/mg	24.0 – 45.4	0.87	0.008
CAT (*C. odorata*) contaminated vs. control	12.4 µmol/min/mg	8.6 – 16.2	0.79	0.012

Figure.1 Antioxidant enzyme activity under contamination stress



Invasive Species Management

"*C. odorata* deployment requires: (1) 50m buffer zones with monthly mowing, (2) harvest before flowering (March-May), (3) seed-sterile propagule sources, (4) rapid response protocols for escape incidents, (5) alternative native species available for immediate replacement.

Regulatory Compliance Framework

"Implementation requires amendments to Nigeria's Environmental Impact Assessment Act to include phytoremediation protocols. Minimum requirements include: (1) soil contamination baseline certification, (2) quarterly groundwater monitoring, (3) biomass metal content documentation, (4) ash disposal tracking, (5) annual progress reporting to NESREA."

Institutional Capacity Requirements

"Successful deployment requires: (1) training programs for agricultural extension officers, (2) analytical laboratory certification for metal analysis, (3) specialized waste management facilities for contaminated biomass, (4) coordination mechanisms between federal environmental agencies and state agricultural departments."

This multi-site study confirms that agricultural soils in southeastern Nigeria are severely contaminated with Pb, Cd, Zn, and Cu, with concentrations at all three impacted sites exceeding WHO guideline limits (Table 1). Uturu Ugwu-ele showed particularly high Pb and Cd burdens, while Lokpaukwu soils were enriched in Zn, reflecting the diverse contamination sources across the region.

Among the indigenous species evaluated, *Pennisetum purpureum* consistently demonstrated the strongest phytoremediation traits, combining high bioaccumulation factors, robust antioxidant responses, and exceptional biomass-driven removal capacity. *Chromolaena odorata* also showed strong uptake but requires ecological safeguards due to its invasive nature. Translocation factor analysis (Table 3) clearly distinguishes remediation strategies: Pb remained largely root-bound (TF < 0.4), making it best addressed by phytostabilization, while Cd and Zn exhibited higher mobility (TF > 0.6), supporting phytoextraction through harvestable biomass. Cu showed intermediate patterns, indicating more limited remediation potential.

Taken together, the evidence supports a dual phytoremediation strategy in southeastern Nigeria: stabilizing Pb in roots to prevent environmental dispersal, while extracting Cd and Zn via high-biomass grasses such as *P. purpureum*. Future work should expand replication, quantify biomass disposal options, and integrate economic modeling to confirm the long-term feasibility of this approach.

Future research priorities should include field-scale demonstrations (≥5-10 hectares), multi-year performance monitoring, comprehensive life cycle assessment of biomass disposal options, and expanded geographic assessment across West African mining regions. Investigation of soil amendment effects on metal bioavailability and development of community adoption strategies represent additional critical research needs. The convergence of technical effectiveness, economic viability, and social acceptability positions indigenous species phytoremediation as a practical pathway for restoring mining-impacted landscapes in tropical environments. With appropriate regulatory frameworks, technical support, and community engagement, this approach could address contamination across millions of hectares of affected agricultural land while providing co-benefits including erosion control, carbon sequestration, and landscape rehabilitation.

This study establishes that environmental restoration in developing countries need not depend on expensive imported technologies or exotic species. Indigenous solutions, rigorously evaluated and carefully implemented, can achieve meaningful environmental improvement while respecting local ecological and social contexts. The findings provide a foundation for scaling phytoremediation from experimental plots to operational programs that serve both environmental and community development goals.

Acknowledgments

We thank collaborating farmers and community leaders in Uturu, Lokpaukwu, and Ihube, and the analytical support staff at the Abia State laboratory.

Author Contributions

Eucharia Chukwukwe: Investigation, formal analysis, writing—original draft. Valentina Palama: Validation, methodology, writing—reviewing. Emma J. R. N Emmanuel:—Formal analysis, writing—review and editing. Confidence Ugochi Ogbonna: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Eucharia Chukwukwe, Valentina Palama, Emma J. R. N Emmanuel and Confidence Ugochi Ogbonna. 2025. Phytoremediation Potential of Indigenous Plants in Abia State, Southeastern Nigeria: A Multi-Site and Multi-Season Assessment. *Int.J.Curr.Microbiol.App.Sci.* 14(09): 97-112.
doi: https://doi.org/10.20546/ijcmas.2025.1409.011