Phytoremediation of cadmium-contaminated *Coffea arabica* coffee-growing soils using *Moringa oleifera* in the Peruvian Andes

Fitorremediación de suelos cafetaleros de *Coffea arabica* contaminados con cadmio mediante *Moringa oleifera* en los Andes peruanos

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ABSTRACT

Cadmium contamination in coffee-growing soils affects soil quality, food safety and the sustainability of Andean coffee systems. In this study, we evaluated changes in total recoverable Cd within a planted, non-draining pot system containing *Moringa oleifera* and *Coffea arabica* coffee-growing soil from Chirinos, Cajamarca, Peru. A 4 × 4 factorial pot experiment was conducted under a randomized complete block design with four blocks. The factors were initial soil Cd, 1.9, 2.4, 2.8 and 3.5 mg kg-1, and development time, 30, 45, 60 and 75 days after transplanting. Residual soil Cd was determined by US EPA 3050B acid digestion and ICP-OES, under US EPA 6010B. Cd removal, Cd removed from soil, plant height, stem thickness, root length and total dry biomass were also evaluated. All initial Cd levels exceeded the Peruvian agricultural soil standard of 1.4 mg kg-1. Residual Cd declined with development time, from 1.603 ± 0.207 mg kg-1 at 30 days to 0.496 ± 0.326 mg kg-1 at 60 days and 0.472 ± 0.192 mg kg-1 at 75 days. The strongest operational response occurred at 3.5 mg kg-1 initial Cd and 60 days, with residual Cd <0.12 mg kg-1, removal ≥96.6% and Cd removed ≥3.380 mg kg-1. Time was the dominant factor in the ANOVA, $F = 81.53$ and $p < 0.001$, while initial Cd was also significant, $F = 3.56$ and $p = 0.020$. The *Moringa oleifera* maintained growth under Cd exposure, with total dry biomass increasing from 0.668 ± 0.087 to 2.945 ± 0.527 g plant-1 between 30 and 75 days. The results obtained support controlled validation of this low-input *Moringa*-soil system for reducing recoverable Cd. The interpretation remains operational because the trial did not include an unplanted contaminated control and Cd was not quantified in plant organs.

Keywords: *cadmium accumulation,* Coffea arabica*,* Moringa oleifera*, coffee-growing soils, phytoremediation, Peruvian Andes*

RESUMEN

La contaminación por cadmio en suelos cafetaleros afecta la calidad del suelo, la inocuidad alimentaria y la sostenibilidad de los sistemas cafetaleros andinos. Este estudio evaluó los cambios del Cd total recuperable dentro de un sistema plantado y sin drenaje que contenía *Moringa oleifera* y suelo cafetalero de *Coffea arabica* procedente de Chirinos (Cajamarca, Perú). Se desarrolló un ensayo factorial 4 × 4 en macetas bajo un diseño de bloques completos al azar con cuatro bloques. Los factores fueron Cd inicial del suelo, 1.9, 2.4, 2.8 y 3.5 mg kg-1, y tiempo de desarrollo, 30, 45, 60 y 75 días después del trasplante. El Cd residual del suelo se determinó mediante digestión ácida US EPA 3050B e ICP-OES bajo US EPA 6010B. También se evaluaron remoción de Cd, Cd removido del suelo, altura de planta, grosor del tallo, longitud de raíz y biomasa seca total. Todos los niveles iniciales de Cd superaron el estándar peruano para suelo agrícola de 1.4 mg kg-1. El Cd residual disminuyó con el tiempo, de 1.603 ± 0.207 mg kg-1 a los 30 días a 0.496 ± 0.326 mg kg-1 a los 60 días y 0.472 ± 0.192 mg kg-1 a los 75 días. La respuesta operacional más alta se registró con 3.5 mg kg-1 de Cd inicial a los 60 días, con Cd residual <0.12 mg kg-1, remoción ≥96.6% y Cd removido ≥3.380 mg kg-1. El tiempo fue el factor dominante en el ANOVA, $F = 81.53$ y $p < 0.001$, mientras que el Cd inicial también fue significativo, $F = 3.56$ y $p = 0.020$. *Moringa oleifera* mantuvo crecimiento bajo exposición a Cd, con aumento de biomasa seca total de 0.668 ± 0.087 a 2.945 ± 0.527 g planta-1 entre 30 y 75 días. Los resultados sustentan la validación controlada de este sistema Moringa-suelo de bajo insumo para reducir Cd recuperable. La interpretación se mantiene operacional porque el ensayo no incluyó un control contaminado sin planta y el Cd no se cuantificó en órganos vegetales.

Palabras clave: *acumulación de cadmio,* Coffea arabica*,* Moringa oleifera*, suelos cafetaleros, fitorremediación, Andes peruanos*

1. Introduction

In Peru, coffee supports a major Andean agro-export chain. Across the country, approximately 427,000 hectares over 15 regions are under coffee cultivation, supporting more than 220,000 producer families and over two million people in direct or indirect employment (MIDAGRI, 2025). For the period 2025-2026, production was projected at 4.2 million 60 kg bags (USDA, 2026). The majority of production is dependent upon smallholder systems, in which *Coffea arabica* is valued for its quality and territorial identity. The importance of coffee production to the national economy makes soil contamination a major production, food-safety and land-management issue, rather than merely an isolated analytical finding.

Cadmium (Cd) persists in soil, becoming more mobile under acidic conditions and entering plants through the root system. In humans, chronic exposure affects the kidneys, bones and respiratory system, and Cd is classified as a human carcinogen (WHO, 2024). In response to public health concerns associated with long-term dietary exposure, the European Union tightened maximum Cd levels in foods (European Commission, 2021). In coffee systems, the link between soil Cd and the final product is not linear. Peruvian studies have reported regional differences among soils, roots and beans, with transfer factors below 1 in several systems, while regression analyses from the Upper Huallaga show that bean-metal concentrations also respond to pH, nutrient status, genotype, plantation age and management (Guadalupe *et al*., 2023, 2024; Rofner *et al*., 2025).

This variability supports soil-level intervention. Total Cd, weakly bound fractions and plant-available Cd represent different endpoints. In the soil-coffee system of Pu'er, weakly bound fractions generally accounted for less than 5% of total metal content (Zhou *et al*., 2025), and work on Ethiopian coffee confirmed element-specific differences between soil content and bean accumulation (Berego *et al*., 2023). Soil pH can reduce Cd bioavailability by 27 to 65% and markedly restrict root and shoot uptake (Zakrzewska *et al*., 2023). A remediation trial must therefore report its analytical endpoint precisely and avoid equating a decline in total recoverable soil Cd with confirmed accumulation in plant tissue.

Cd in coffee soils can originate from parent material, fertilizers, atmospheric deposition and earlier land use. Its mobility increases as pH falls and as competition for sorption sites changes, while clay minerals, iron and manganese oxides, organic matter and cation-exchange capacity can retain or release Cd. These controls matter in humid Andean systems, where rainfall, slope and organic inputs vary over short distances. Geogenic enrichment linked to carbonate weathering has produced agricultural soils with high total Cd but variable crop uptake (Xia *et al*., 2020). Remediation must therefore be assessed against a defined standard, an explicit analytical fraction and the physical conditions of the trial. For coffee farms, an intervention is useful when it reduces the soil pool associated with regulatory concern, without creating a second exposure pathway through harvested biomass. This soil-management perspective also connects contamination control with land-use planning and the conservation of ecosystem services in productive territories (Hoyos-Alayo, 2026b).

Phytoremediation offers a low-input option when a species combines contaminant tolerance, root development, biomass production and controlled post-harvest handling (Priya *et al*., 2023; Zheng *et al*., 2024). *Moringa oleifera* grows rapidly and regulates antioxidant and polyphenolic responses under Cd and Pb exposure (Nouman *et al*., 2020). Root-zone interventions have also altered Cd behavior in this species. In one soil experiment, selenite application reduced root Cd by 80% (Fu *et al*., 2020), while chitosan-coated biochar reduced Cd in *Moringa oleifera* shoots, roots and available soil Cd (Zubair *et al*., 2021). These findings support testing of the plant in contaminated agricultural soils, while the possible metal content of harvested biomass calls for precautionary management (Mayfield *et al*., 2026). The low-input characterization is relevant to broader debates on environmental access, decentralized treatment and bio-based adsorptive or remediation options (Hoyos-Alayo, 2026a; Hoyos Alayo, 2025a).

This study aligns with the United Nations Sustainable Development Goals (SDGs) because it addresses soil quality, crop safety and sustainability in an agricultural chain with high social value. The 2030 Agenda defines the SDG 2, target 2.4, for sustainable food production systems and practices that improve land and soil, while SDG 3, target 3.9, addresses illness from hazardous chemicals and pollution. SDG 12, target 12.4, calls for the environmentally sound management of chemicals and wastes, and SDG 15, target 15.3, promotes the restoration of degraded land and soils (United Nations, 2015). The health relevance is associated with the kidney, skeletal and respiratory toxicity of Cd and its classification as a human carcinogen (WHO, 2024). The authors' earlier studies complement this official framework by examining climate action, chemical regulation, land-use change and ecosystem services in related environmental contexts (Hoyos-Alayo, 2025b, 2026b, 2026c). In the case of the Peruvian coffee sector, where the chain involves more than 220,000 families and 427,000 ha of cultivated land, remediation of Cd-affected soil contributes to the protection of exportable production, rural health and edaphic resilience (Guadalupe *et al*., 2024; MIDAGRI, 2025; Rofner *et al*., 2025).

Evidence for the effectiveness of *Moringa oleifera* grown directly in Cd-contaminated *Coffea arabica* soils from the Peruvian Andes remains limited. Previous coffee studies have focused mainly on soil-to-bean transfer and consumer exposure, while *Moringa oleifera* experiments have commonly involved amendments or soils outside the realm of coffee production. In this study, we evaluated the effects of initial soil Cd concentration and plant-development time on total recoverable residual Cd, under a randomized complete block pot design. The analysis incorporated residual Cd, percentage removal, absolute Cd reduction and plant growth, in order to identify a treatment duration that met the Peruvian agricultural-soil standard. Because the experiment lacked an unplanted contaminated control and Cd was not quantified in roots, stems or leaves, the response can be interpreted as an operational change in recoverable Cd within the closed planted soil system, without assigning the decline exclusively to *Moringa oleifera*, or defining a phytoextraction or phytostabilization mechanism.

2. Materials and methods

2.1. Soil collection and experimental design

Coffee-growing soil was collected during the 2025 dry-season field campaign from the central coffee-producing sector of Chirinos district, San Ignacio province, Cajamarca, in northern Peru. The sector was referenced at approximately 5°18′ S, 78°54′ W and an altitude of 1830 meters. This district-level reference identifies the sampling area rather than individual farms. Traceability was reconstructed from the field coding scheme as four plot-level composites, one for each baseline Cd stratum. Five subsamples per plot were taken in a zigzag pattern from the 0-30 cm layer, combined in equal proportions to form each composite, and kept separate among strata. Plots were dedicated to agricultural use, with no visible mixing with external residues, and Cd levels above the Peruvian environmental quality standard for agricultural soil (1.4 mg kg-1, in accordance with the provisions of Supreme Decree No. 011-2017-MINAM). The samples were coded, transported under chain of custody, homogenized, cleared of roots and coarse fragments, air-dried, and passed through a 2 mm sieve. Baseline total recoverable Cd defined four levels, 1.9, 2.4, 2.8 and 3.5 mg kg-1, equivalent to 1.36, 1.71, 2.00 and 2.50 times the national standard.

The pot trial used a 4 × 4 factorial arrangement, within a randomized complete block design, composed of four blocks. The factors were initial soil Cd and development time, 30, 45, 60 and 75 days after transplanting. Each block contained the 16 Cd × time combinations once, giving 64 independent experimental units. Each unit contained 1.00 kg of air-dried contaminated soil and one uniform 12-day-old *Moringa oleifera* seedling. With an estimated residual gravimetric moisture of 10%, the pot charge corresponded to approximately 0.90 kg oven-dry soil equivalent. The non-draining polypropylene pots had a reconstructed working volume of 1.5 L, with approximate dimensions of 13 cm upper diameter, 11 cm basal diameter and 14 cm height. Positions were randomized within the blocks. The moisture level was maintained at an estimated 60 ± 5% of water-holding capacity by periodic weighing and replacement with deionized water. These operational estimates were reconstructed from the recorded 1 kg pot charge and the cultivation procedure, in order to establish reproducible soil-water conditions. Each unit was harvested once, and therefore times were analyzed as independent treatments, rather than as repeated measurements. The full randomization layout is shown in Figure S1.

Seedlings were selected for uniform height and visible vigor before transplanting. Cultivation was conducted in a protected, naturally ventilated area under an approximate 12 h light period, a temperature of 23 ± 3°C, and 65 ± 10% relative humidity. Within each block, light exposure and relative pot position were kept comparable, and no chemical chelator, microbial inoculant or organic amendment was applied. The non-draining configuration reduced loss by percolation, while periodic weighing limited systematic moisture differences among pots (Cotrina-Urbina *et al*., 2026; Jiménez-Hernández *et al*., 2026). Harvests at 30, 45, 60 and 75 days after transplanting corresponded to total plant ages of 42, 57, 72 and 87 days from germination. At harvest, the entire soil mass was mixed before subsampling, and the roots were separated carefully, in order to reduce soil carry-over during biometric measurements.

2.2 Analytical and growth measurements

At each harvest, the soil and plant material were separated. The soil was homogenized, and a 1.00 g dry-soil aliquot was analyzed for total recoverable Cd, by US EPA Method 3050B acid digestion, followed by inductively coupled plasma optical emission spectrometry, under US EPA Method 6010B (U.S. EPA, 1996a, 1996b). The instrumental configuration used simultaneous axial-view ICP-OES and the Cd analytical line at 226.502 nm. Results were reported on a dry-soil basis. The accredited laboratory reported a Cd limit of quantification (LQ) of 0.12 mg kg-1. Quality control included analytical blanks, duplicates, multi-element calibration standards, instrumental-linearity verification, and traceable codes for block, Cd level and harvest time. The acceptance criteria comprised a calibration coefficient r ≥ 0.995, initial and continuing calibration verification within 90-110% of the expected value, calibration blanks not exceeding three times the instrumental detection limit, post-digestion spike recovery of 75-125%, and duplicate relative percent difference ≤20%.

The primary response was residual soil Cd concentration. Cd removal was calculated as (Ci − Cf)/ Ci × 100, and Cd removed from soil as Ci − Cf, where Ci and Cf are the initial and residual concentrations. These quantities describe reduction within the non-draining planted system. They do not represent a tissue mass balance, because plant organs were not analyzed for Cd. Plant height was measured from the collar to the main apex, stem thickness at collar height with a Vernier caliper, root length after soil separation and total dry biomass after drying to constant mass. All concentrations were linked to the original experimental-unit code before calculation. Means and standard deviations were obtained from the four blocks for each Cd × time combination. A result below the limit of quantification (LQ) was treated as censored data in descriptive reporting and was never presented as a measured zero. Dry biomass represented the combined harvested plant material per pot. Growth variables were treated as complementary indicators of tolerance and establishment, not as direct surrogates for Cd uptake.

2.3. Statistical analysis and safe handling

Minitab v22.3.1 was used for descriptive and inferential analysis. Four of the 64 observations were censored, all of which were from the 3.5 mg kg-1 × 60-day combination. They were reported descriptively as <0.12 mg kg-1 and replaced consistently with 0.12 mg kg-1 before fitting the statistical model. This full-LQ substitution was selected as a conservative upper-bound convention, because it maximized the residual concentration and minimized the calculated removal for the affected treatment. It was not interpreted as a measured concentration below the analytical limit. Because substitution methods can introduce bias, inequality notation was retained and inference for the censored combination was restricted accordingly (George *et al*., 2021). A general linear model included block, initial Cd and time as fixed effects. The pre-specified model estimated marginal main effects and did not estimate the Cd × time interaction. Treatment-combination means were therefore interpreted descriptively, without inferential claims regarding interaction. ANOVA used α = 0.05, and significant factor means were separated by Tukey's test, at 95% confidence. Model adequacy was reviewed from studentized residuals, residuals versus fitted values and the normal probability plot. Traceable observations were retained, unless a coding, sampling or analytical error was identified.

The statistical model was selected to preserve the four-block structure and estimate the main effects supported by the available analysis. Model fit was reported with S, R2, adjusted R2 and predicted R2. Tukey groupings were interpreted within each factor, and no causal mechanism was inferred from Pearson correlations among residual Cd and growth variables. The omission of the Cd × time interaction limits inference to average effects across factor levels and prevents formal testing to determine whether or not temporal response differs among initial Cd concentrations. This restriction also avoids the treating of the four censored observations in one treatment combination as precise evidence of interaction. Development time simultaneously increased biomass and changed the recoverable soil-Cd response, and therefore correlations were interpreted as descriptive associations.

Soils and harvested biomass were handled using gloves and labelled containers. Harvested roots, stems and leaves were collected intact, placed in sealed double bags or rigid labelled containers, and stored in a covered area pending controlled disposition. The material was treated as potentially Cd-containing waste and was excluded from food, feed, compost, mulch, unrestricted soil application and open burning. Tools were cleaned over a contained surface, and the washings were retained, rather than being discharged to drains or soil. Until organ-specific Cd analysis confirms its classification, the biomass should be transferred through an authorized waste-management route. Soil collection was authorized by plot managers and did not interrupt coffee production.

Table 1. Experimental design and analytical framework

Component	Specification	Levels or unit	Role
Design	4 × 4 factorial RCBD	4 blocks, 64 pots	Controls spatial gradients and tests Cd and time effects
Initial soil Cd	Total recoverable Cd	1.9, 2.4, 2.8 and 3.5 mg kg^{-1}	Contamination factor
Development time	Destructive harvest after transplanting	30, 45, 60 and 75 days	Temporal factor
Experimental unit	One 12-day-old seedling in a 1.5 L non-draining pot containing 1.00 kg air-dried soil	1 plant pot-1, approximately 0.90 kg dry-soil equivalent, 60 ± 5% water-holding capacity	Closed planted soil system with controlled water balance
Primary response	Residual total recoverable Cd	mg kg^{-1}	US EPA 3050B digestion and US EPA 6010B ICP-OES at 226.502 nm
Derived responses	Cd removal and Cd removed	% and mg kg^{-1}	(Ci − Cf)/Ci × 100 and Ci - Cf
Growth responses	Height, stem thickness, root length and dry biomass	cm, mm, cm and g $plant^{-1}$	Plant establishment and tolerance

Note. RCBD, randomized complete block design. Ci and Cf denote initial and residual soil Cd, respectively. Each of the 16 Cd × time combinations occurred once in every block. Pot mass, dry-soil equivalent, working volume, moisture target and cultivation conditions are reported as reconstructed operational estimates based on the recorded 1 kg soil charge and cultivation procedure.

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3. Results

3.1. Residual Cd and removal performance

All initial Cd levels exceeded the Peruvian agricultural-soil standard. Residual Cd decreased with development time across the contamination gradient (Table 2). The mean across Cd levels fell from 1.603 ± 0.207 mg kg^{-1} at 30 days to 1.088 mg kg^{-1} at 45 days, 0.496 ± 0.326 mg kg^{-1} at 60 days and 0.472 ± 0.192 mg kg^{-1} at 75 days. Every experimental unit harvested at 60 or 75 days was below 1.4 mg kg^{-1}.

The highest operational response occurred at 3.5 mg kg-1 initial Cd and 60 days. All four observations in this combination were below the 0.12 mg kg-1 LQ, corresponding descriptively to removal ≥96.6% and Cd reduction ≥3.380 mg kg-1. The true treatment mean was therefore below 0.12 mg kg-1, while 0.12 mg kg-1 was used only as a conservative upper-bound value in ANOVA. At 75 days, the same initial level had 0.439 ± 0.210 mg kg-1 residual Cd and 87.5 ± 6.0% removal. The temporal profiles in Figure 1 show the sharp decline up to 60 days and the smaller difference between 60 and 75 days.

Regulatory compliance was reached by 45 days for all treatment means, although the 1.9 mg kg^{-1} treatment showed the widest dispersion. At 30 days, the four means remained above 1.4 mg kg^{-1}. Absolute reduction increased with the starting pool, reaching 2.546 ± 0.177 mg kg^{-1} at 3.5 mg kg^{-1} and 45 days, before the maximum at 60 days. These patterns show why residual concentration, percentage removal and absolute reduction must be interpreted together.

Table 2. Residual soil Cd and removal by initial Cd level and development time

Initial Cd (mg kg^{-1})	30 days	45 days	60 days	75 days
1.9	1.647 ± 0.105 (13.3 ± 5.5%)	1.062 ± 0.407 (44.1 ± 21.4%)	0.901 ± 0.070 (52.6 ± 3.7%)	0.666 ± 0.153 (64.9 ± 8.0%)
2.4	1.434 ± 0.323 (40.3 ± 13.5%)	1.164 ± 0.193 (51.5 ± 8.0%)	0.505 ± 0.271 (79.0 ± 11.3%)	0.408 ± 0.111 (83.0 ± 4.6%)
2.8	1.640 ± 0.189 (41.4 ± 6.7%)	1.171 ± 0.392 (58.2 ± 14.0%)	0.458 ± 0.212 (83.7 ± 7.6%)	0.377 ± 0.186 (86.5 ± 6.6%)
3.5	1.690 ± 0.104 (51.7 ± 3.0%)	0.954 ± 0.177 (72.7 ± 5.1%)	<0.12 (≥96.6%)	0.439 ± 0.210 (87.5 ± 6.0%)

Note: Each cell gives residual Cd in mg kg-1 as mean ± standard deviation, with Cd removal in parentheses, n = 4. LQ = 0.12 mg kg-1. The four observations at 3.5 mg kg-1 × 60 days were censored and are retained as <0.12 mg kg-1. The corresponding removal and reduction values are lower bounds. All 60- and 75-day units were below the Peruvian agricultural-soil standard of 1.4 mg kg-1. Detailed heatmaps are provided in Figures S2 and S3.

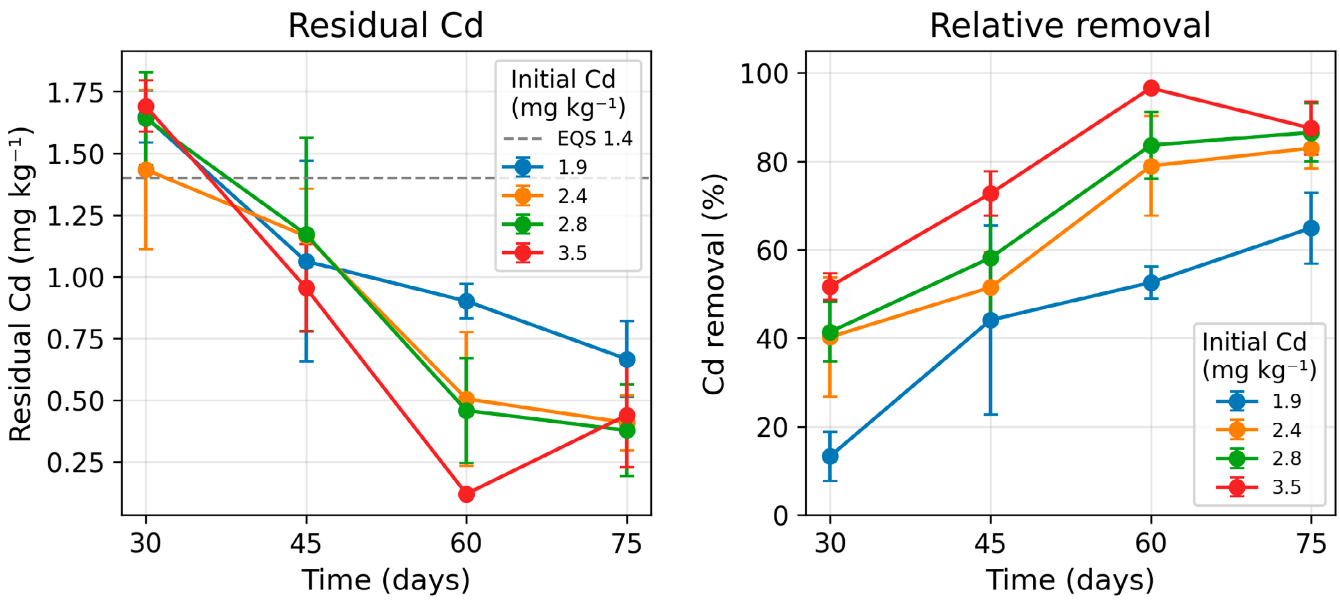

Figure 1. Temporal trends in residual Cd and Cd removal by initial contamination level

3.2. Statistical effects

Development time was the dominant factor in the model, $F = 81.53$ and $p < 0.001$, while initial Cd also affected residual concentration, $F = 3.56$ and $p = 0.020$. Block was not significant, $F = 2.19$ and $p = 0.100$. The model explained 82.90% of observed variation, with adjusted $R^2 = 80.05\%$ and predicted $R^2 = 75.98\%$ (Table 3).

Tukey separation placed 30, 45 and 60 days in distinct groups, while 60 and 75 days shared the lowest-residual group. Adjusted means were 1.603, 1.088, 0.496 and 0.472 mg kg^{-1} for 30, 45, 60 and 75 days, respectively. For initial Cd, the 1.9 mg kg^{-1} level differed from 3.5 mg kg^{-1}, with the intermediate concentrations overlapping both groups.

Table 3. ANOVA and adjusted means for total recoverable residual soil Cd

Analysis	Factor or level	df or n	Estimate	F	p	Group or 95% CI
ANOVA	Block	3	Adj. MS 0.12535	2.19	0.100	
ANOVA	Initial Cd	3	Adj. MS 0.20380	3.56	0.020	
ANOVA	Time	3	Adj. MS 4.66549	81.53	<0.001	
ANOVA	Error	54	MS 0.05723			
Tukey, Cd	1.9 mg kg^{-1}	16	1.069 mg kg^{-1}			A, 0.949 to 1.189
Tukey, Cd	2.4 mg kg^{-1}	16	0.878 mg kg^{-1}			AB, 0.758 to 0.998
Tukey, Cd	2.8 mg kg^{-1}	16	0.911 mg kg^{-1}			AB, 0.792 to 1.031
Tukey, Cd	3.5 mg kg^{-1}	16	0.801 mg kg^{-1}			B, 0.681 to 0.921
Tukey, time	30 days	16	1.603 mg kg^{-1}			A, 1.483 to 1.723
Tukey, time	45 days	16	1.088 mg kg^{-1}			B, 0.968 to 1.208
Tukey, time	60 days	16	0.496 mg kg^{-1}			C, 0.376 to 0.616
Tukey, time	75 days	16	0.472 mg kg^{-1}			C, 0.353 to 0.592

Note: The model used conservative substitution of 0.12 mg kg-1 for the four censored observations, all from the 3.5 mg kg-1 × 60-day combination. This upper-bound substitution was applied consistently before ANOVA and yielded a conservative comparison because it overstated residual Cd and understated removal in that combination. S = 0.239222, R2 = 82.90%, adjusted R2 = 80.05% and predicted R2 = 75.98%. Means with different letters within a factor differed by Tukey at 95% confidence. The model estimates main effects only. Treatment combinations and the Cd × time pattern are descriptive. Diagnostics are shown in Figure S4.

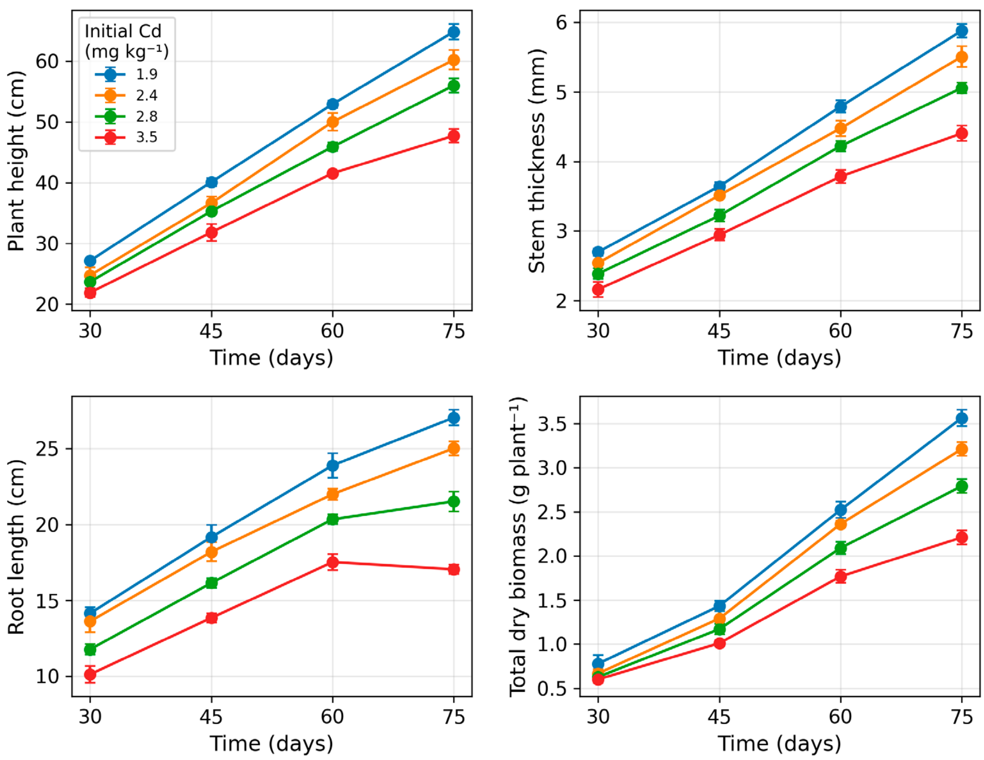

Figure 2. Growth trajectories of *Moringa oleifera* by initial Cd level

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3.3. Plant growth

Moringa oleifera became established grew at every Cd level. Across treatments, height increased from 24.37 ± 2.13 cm at 30 days to 57.20 ± 6.61 cm at 75 days, stem thickness from 2.45 ± 0.22 to 5.21 ± 0.58 mm, root length from 12.42 ± 1.71 to 22.66 ± 3.95 cm, and dry biomass from 0.668 ± 0.087 to 2.945 ± 0.527 g plant-1. Higher initial Cd reduced the magnitude of growth, especially at 3.5 mg kg-1, but did not prevent establishment. These variables document tolerance within the planted system and do not establish Cd uptake.

Residual Cd was negatively associated with plant height, r = −0.740, and dry biomass, r = −0.720. Removal was positively associated with biomass, r = 0.558, while root length and biomass were strongly related, r = 0.937. These associations describe coincident responses and do not establish Cd allocation to plant organs. The complete correlation matrix and biomass plots are provided in Table S2 and Figure S5.

4. Discussion

4.1. Regulatory and coffee-system relevance

The trial produced a clear soil-level response. All baseline concentrations were above the national agricultural-soil standard, whereas all units reached compliance by 60 days and remained compliant at 75 days. The 3.5 mg kg^{-1} × 60-day combination achieved the lowest reportable residual concentration and the greatest absolute reduction. This result addresses contamination before uptake by *Coffea arabica*, which is relevant because metal transfer to coffee beans varies across regions, management systems and edaphic conditions (Guadalupe *et al*., 2023, 2024; Rofner *et al*., 2025). A soil-treatment endpoint therefore complements, rather than replaces, the surveillance of beans and beverages.

The threshold-based result is relevant for a production system dominated by small farms, but pot-scale compliance does not establish farm-scale recovery. The trial controlled water movement, soil mass and plant density, conditions that reduce the variability found in coffee fields. Its strength lies in its analytical comparability across 64 units and four baseline concentrations. Its limitation is the external validity under rainfall, erosion, root competition and repeated fertilization. The 60-day treatment should therefore be considered as a testable management hypothesis, rather than as a direct recommendation for unrestricted field use.

The endpoint calls for a restricted interpretation. Acid digestion and ICP-OES quantify total recoverable soil Cd, not the labile fraction or tissue accumulation. Although the pots had no drainage, changes in extractability, root retention, rhizosphere reactions, analytical variability and transfer to unmeasured biomass could all contribute to the lower residual concentration. Coffee-soil evidence shows that total and weakly bound metals differ substantially (Zhou *et al*., 2025), while soil-to-bean accumulation is dependent upon element-specific biological controls (Berego *et al*., 2023). The data support an operational decline within the planted soil system, but the absence of an unplanted contaminated control prevents attribution of that decline exclusively to *Moringa oleifera* and does not demonstrate conclusively that phytoextraction was the dominant mechanism.

4.2. Temporal response and plant establishment

Time accounted for the largest share of statistical variation. The separation of 30, 45 and 60 days, followed by equivalent adjusted means at 60 and 75 days, identified 60 days as the efficient operational duration within the tested range. This timing coincided with substantial root and biomass development. By 60 days, dry biomass had reached 2.186 ± 0.303 g $plant^{-1}$ and root length 20.94 ± 2.46 cm. Greater root-soil contact and plant establishment were therefore present when residual Cd reached its lowest mean.

Moringa oleifera tolerance is consistent with its reported antioxidant and polyphenolic regulation under metalliferous stress (Nouman *et al*., 2020). Studies that altered the root environment with selenite or biochar also changed Cd concentrations in roots, shoots and soil, indicating that rhizosphere chemistry influences the species' response (Fu *et al*., 2020; Zubair *et al*., 2021). The rise from <0.12 mg kg-1 at 60 days to 0.439 ± 0.210 mg kg-1 at 75 days in the highest-Cd treatment should be interpreted with caution, given that the 60-day observations were censored and both treatment values remained below the standard. A plausible biochemical hypothesis is that aging fine roots at 87 days underwent greater turnover or early senescence, releasing cell solutes and altering rhizosphere chemistry. Cd stress can also modify exudation of low-molecular-weight organic acids such as citrate, malate and oxalate. These ligands, together with local acidification and metal-complexation reactions, can desorb part of the previously sorbed Cd pool and increase the fraction recovered by acid digestion (Seregin and Kozhevnikova, 2024). Changes in root exudates can restructure rhizosphere microbial communities and their metal-transforming activity, creating a further pathway for temporary Cd remobilization (Lin *et al*., 2025; Hu *et al*., 2024). This mechanism remains a hypothesis, however, given that root senescence, organic acids, pH and microbial composition were not measured.

The correlation pattern supports, but does not demonstrate conclusively, a biological contribution. Residual Cd declined as height and dry biomass increased, and removal rose with biomass. These associations are consistent with greater root exploration, rhizodeposition and contact between soil particles and the developing root system. They also contain a temporal component, because both growth and removal increased during the same period. Direct evidence of uptake would require organ-specific Cd concentrations and total plant-Cd mass. Without those measurements, the correlations strengthen internal coherence, but they cannot resolve the mechanism.

4.3. Initial contamination and edaphic controls

Initial Cd had a significant but non-monotonic effect. The adjusted residual mean at 3.5 mg kg^{-1} was lower than at 1.9 mg kg^{-1}, and the highest starting concentration produced the largest absolute reduction. This pattern does not demonstrate proportional uptake. A larger initial pool can increase contact with labile Cd, while pH, mineralogy, cation-exchange capacity, organic matter and root-zone chemistry regulate the fraction recovered after treatment. In controlled systems, soil pH has reduced Cd bioavailability by 27 to 65% (Zakrzewska *et al*., 2023), and geogenic mineral weathering can create high total Cd without equivalent plant risk (Xia *et al*., 2020).

The coffee context strengthens this interpretation. Peruvian soil-root-grain studies have reported transfer factors below 1, and data for Upper Huallaga link bean metals with soil chemistry, genotype, plantation age and agronomic management (Guadalupe *et al*., 2024; Rofner *et al*., 2025). The results of this study therefore reflect an integrated plant-soil response under controlled moisture and no drainage. Field recommendations require direct measurement of soil properties and Cd fractions, rather than extrapolation from initial total concentration alone.

4.4. Position within phytoremediation evidence

The removal range compares favorably with assisted Cd-remediation studies, but direct numerical comparisons require caution. Earthworms and organic residues increased Cd extraction by *Solanum nigrum* in one experiment (Luo *et al*., 2024), while arbuscular mycorrhizal fungi, citric acid, chelators and growth regulators have altered metal availability and plant performance in other systems (Yang *et al*., 2024; Li *et al*., 2025). Those approaches included amendments or microbial assistance. In this trial, we used *Moringa oleifera* without chelators, inoculants or biochar, which is relevant in the context of resource-limited coffee farms.

Compared with amendment-assisted systems, the experimental treatment was operationally simple. This simplicity reduces input requirements and avoids uncertainty regarding residual chelators or amendment-derived changes in soil chemistry. It also narrows the causal interpretation, given that the trial did not include an unplanted contaminated control or a treatment with an alternative species. The planted, non-draining soil system showed a decline in total recoverable Cd under the tested conditions, but the decline cannot be attributed exclusively to *Moringa oleifera*. Natural aging, redistribution among soil fractions, root-associated retention and analytical variability could not be isolated completely.

Plant growth also supports the feasibility of repeated harvest. Phytoremediation depends on both tissue concentration and biomass, as shown in *Solanum lycopersicum* and *Cannabis sativa* studies that assessed uptake together with growth and translocation (Testa *et al*., 2023; Jia *et al*., 2025). Here, growth persisted under all Cd levels, and lower residual Cd coincided with greater height and biomass. The contribution of this study lies in the applying of this tolerance to *Coffea arabica* soil from the Peruvian Andes and identifying a 60-day response window. While the study does not establish a field protocol, it does provide a controlled basis for testing one.

4.5. Application limits and validation requirements

A short *Moringa oleifera* cycle could be tested as a pre-planting or rehabilitation step for contaminated soil batches, particularly where excavation or chemical washing is impractical. Implementation must include controlled harvesting and a documented biomass-management chain. Because tissue Cd was not measured, roots, stems and leaves should be treated as potentially contaminated material, excluded from food, feed, compost, mulch and unrestricted disposal, and stored in sealed labelled containers on an impermeable covered surface. Biomass should not be openly burned, because ash can concentrate metals and facilitate dispersal. Organ-specific Cd analysis should precede classification, reuse or final treatment, and material that exceeds applicable thresholds should be transferred to an authorized hazardous-waste manager. Evidence from *Moringa oleifera* grown in metal-affected environments supports this precaution (Mayfield *et al*., 2026).

A field validation program should begin with bounded microplots or contained soil beds, rather than with direct planting across productive farms. It should include untreated contaminated soil, unplanted controls and, where feasible, an established phytoremediation species as a comparator. Repeated measurements at 30, 45, 60 and 75 days would test whether the apparent optimum persists under open drainage. Agronomic endpoints should include survival, water demand, root recovery and the labor required to remove biomass without dispersing contaminated material.

The main limitation of this study is the absence of a Cd mass balance. The experiment conducted did not measure Cd in roots, stems or leaves, sequential soil fractions, pH, cation-exchange capacity, dissolved organic carbon, microbial activity or rhizosphere chemistry. It also lacked an unplanted contaminated control, meaning that the observed decline cannot be attributed exclusively to *Moringa oleifera*. The fixed-effects model did not estimate the Cd × time interaction, which restricts inferential interpretation to marginal main effects and renders as a

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descriptive the treatment combinations. Four censored observations were concentrated in one combination, and full-LQ substitution provided a conservative upper bound rather than precision below the analytical limit. In future trials, the retrospectively reconstructed pot moisture, dry-soil equivalent and environmental conditions will also require confirmation. Validation should quantify organ-specific Cd, soil fractions and physicochemical changes, then test the 60-day treatment in field microplots exposed to rainfall, slope, heterogeneous organic matter and native microbiota. These measurements would distinguish uptake from immobilization or altered extractability and define safe biomass management.

5. Conclusions

The planted, non-draining *Moringa*-soil system showed a decline in total recoverable Cd in contaminated *Coffea arabica* soil under the 4 × 4 randomized complete block pot trial. Development time was the dominant factor, and 60 days produced an adjusted residual concentration equivalent to 75 days with a shorter treatment period. At 3.5 mg kg-1 initial Cd and 60 days, residual Cd was <0.12 mg kg-1, removal was ≥96.6% and operational Cd reduction was ≥3.380 mg kg-1. All treatments harvested at 60 and 75 days were below the Peruvian agricultural-soil standard. *Moringa oleifera* maintained root, shoot and biomass growth across the contamination gradient, although growth was lower at the highest initial Cd level. The evidence supports controlled validation of a low-input planted soil treatment, rather than confirmed phytoextraction or exclusive causation by *Moringa oleifera*, given that the trial lacked an unplanted contaminated control, Cd was not measured in plant organs, and the model did not estimate the Cd × time interaction. Future field studies will need to include planted and unplanted controls, Cd mass balance, soil speciation, physicochemical monitoring and safe handling of harvested biomass, before the method can be considered for coffee-farm rehabilitation.

Supplementary material

Figures S1-S5 and Tables S1-S2 contain the randomization layout, treatment heatmaps, model diagnostics, detailed growth means, biomass associations and the complete correlation analysis.

Authorship contribution

David Antonio Ruiz Salazar: Conceptualization, investigation, methodology, data curation, writing (original draft).

Aleyda Brigitte Sánchez Hidrogo: Investigation, formal analysis, validation, visualization.

Walter Manuel Hoyos-Alayo: Supervision, writing (revision and editing), project administration, corresponding author.

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Conflicts of interest

The authors declare that they have no potential conflicts of interest.

Artificial Intelligence use statement

The authors declare that no artificial intelligence tool was used in any part of the manuscript preparation process.

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