

Apparent reduction in recoverable cadmium in agricultural soils exposed to *Moringa oleifera* Lam. during over controlled exposure periods

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Reducción aparente del cadmio recuperable en suelos agrícolas expuestos a *Moringa oleifera* Lam. durante periodos controlados

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Cite as: Jesús Pinedo, K. J. and Hoyos-Alayo, W. M. (2026). «Apparent reduction in recoverable cadmium in agricultural soils exposed to *Moringa oleifera* Lam. during over controlled exposure periods». *South Sustainability*, 7(2), e172.
DOI: 10.21142/SS-0702-2026-e172

Received: 11/7/2026
Peer reviewed
Accepted: 28/9/2026



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Associate editor:
Lorena Ramírez

ABSTRACT

Cadmium contamination of agricultural soils calls for remediation strategies that reduce the recoverable metal fraction without inhibiting plant growth. In this study, we evaluated the variation in recoverable soil Cd during exposure to *Moringa oleifera* Lam. seedlings in accordance with three field-derived initial concentration classes, 1.6, 1.8 and 2.0 mg kg⁻¹, and under three exposure periods, 8, 17 and 39 days. A 3 × 3 factorial arrangement was implemented in a randomized complete block design with five blocks and 45 independent pots. Recoverable Cd was quantified via EPA Method 3050B and digested by ICP-OES using EPA Method 6010D, and seedling morphology was characterized at each destructive harvest. Final Cd was found to have decreased with time in all the soil classes. The 1.8 mg kg⁻¹ class exposed for 39 days produced the lowest residual concentration, 0.30 ± 0.16 mg kg⁻¹, and the greatest apparent reduction, 83.33 ± 8.78%. Initial concentration class, exposure time and their interaction affected final Cd concentration, with $p < 0.001$, $p < 0.001$ and $p = 0.002$, respectively. The full block model explained 89.21% of the variation. The proportion of units at or below the Peruvian agricultural-soil reference value of 1.4 mg kg⁻¹ increased from 26.67% at 8 days to 100% at 39 days. The 1.8 mg kg⁻¹ and 39-day treatment also recorded the highest descriptive plant-growth means. Because unplanted controls, tissue Cd concentrations and a total mass balance were unavailable, the decrease can be interpreted as an apparent reduction in recoverable soil Cd, rather than as direct evidence of phytoextraction.

Keywords: cadmium, exposure time, *Moringa oleifera*, phytoremediation, recoverable metal, soil contamination

RESUMEN

La contaminación por cadmio en suelos agrícolas exige estrategias que reduzcan la fracción recuperable del metal sin limitar el establecimiento vegetal. Este estudio evaluó la variación del Cd recuperable en suelo expuesto a plántulas de *Moringa oleifera* Lam. bajo tres clases iniciales derivadas de campo, 1,6, 1,8 y 2,0 mg kg⁻¹, y tres periodos, 8, 17 y 39 días. Se aplicó un arreglo factorial 3 × 3 en bloques completos al azar, con cinco bloques y 45 macetas independientes. El Cd recuperable se cuantificó después de la digestión EPA 3050B mediante ICP-OES según EPA 6010D. La morfología se caracterizó en cada cosecha destructiva. El Cd final disminuyó con el tiempo en todas las clases. La clase de 1,8 mg kg⁻¹ durante 39 días presentó la menor concentración residual, 0,30 ± 0,16 mg kg⁻¹, y la mayor reducción aparente, 83,33 ± 8,78 %. La clase inicial, el tiempo y su interacción afectaron la concentración final, con $p < 0,001$, $p < 0,001$ y $p = 0,002$. El modelo explicó el 89,21 % de la variación. La proporción de unidades con valores iguales o inferiores a 1,4 mg kg⁻¹ aumentó de 26,67 % a los 8 días a 100 % a los 39 días. El tratamiento de 1,8 mg kg⁻¹ y 39 días registró las mayores medias descriptivas de crecimiento. Ante la ausencia de controles sin plantas, Cd en tejidos y balance total de masa, la disminución se interpreta como reducción aparente del Cd recuperable, no como evidencia directa de fitoextracción.

Palabras clave: cadmio, tiempo de exposición, *Moringa oleifera*, fitorremediación, metal recuperable, contaminación del suelo



1. Introduction

Cadmium (Cd) contamination of agricultural soils threatens environmental and food safety because the metal persists, moves within the rhizosphere, and enters edible plant tissues. The World Health Organization associates chronic exposure to Cd contamination with renal damage, bone disorders and carcinogenic effects, indicating the need for control of soil-to-crop transfer (WHO, 2019). In 29 cacao plots in Bagua, Peru, soil Cd ranged from 1.02 to 3.54 mg kg⁻¹, while mean concentrations were 1.60 mg kg⁻¹ in roots, 2.53 mg kg⁻¹ in leaves, 0.49 mg kg⁻¹ in seed coats and 0.99 mg kg⁻¹ in cotyledons (Oliva *et al.*, 2020). In 95 rice-soil samples from the Amojú river basin, the Cd level was found to reach 2.00 mg kg⁻¹ and 16.8% exhibited slight contamination, with the maximum exceeding Peru's agricultural-soil standard of 1.40 mg kg⁻¹ (Tarrillo *et al.*, 2025).

Cd distribution in Peruvian agroecosystems is heterogeneous. A national dataset of more than 2000 soil and cacao-bean samples found the highest concentrations in Tumbes, Piura, Amazonas and Loreto. Soil Cd was the main predictor of bean content, while geology, pH, texture and rainfall seasonality modified transfer (Thomas *et al.*, 2023). Models for cacao-growing areas indicated that limited soil variables did not reproduce the full spatial variation, highlighting the need for chemical, topographic and management information (Villalaz-Pérez *et al.*, 2024). In Amazonian plantation profiles, mean surface concentrations of 0.61-1.11 mg kg⁻¹ were associated with exchangeable cations, cation-exchange capacity and texture (Vallejos-Torres *et al.*, 2026). In soils cultivated with *Persea americana* Mill., parent material, exchangeable cations and trace elements also explained spatial variation (Solórzano *et al.*, 2025).

Soil pH, organic matter and cation-exchange capacity regulate Cd adsorption, desorption and availability. In 81 paired soil-crop samples, these properties modified extractable metals and grain accumulation (Xu *et al.*, 2022). A database of 31,342 soil-vegetable pairs showed higher Cd bioconcentration in leafy vegetables than in root and solanaceous crops, with pH, organic matter, cation-exchange capacity, Zn and Mn acting as predictors (Pan *et al.*, 2022). Across 30 subtropical soils, Cd reduced barley root elongation by around 50%, and pH explained 44% of toxicity variation (Zhang *et al.*, 2023). Genotypic differences are also relevant. Among 12 *Zea mays* L. cultivars grown at 0.68 mg kg⁻¹ Cd, low-accumulating genotypes displayed bioconcentration factors of 0.016-0.023 and translocation factors of 0.05-0.09 (Shan *et al.*, 2025).

Cd exposure alters plant growth through nutrient imbalances, loss of photosynthetic pigments, membrane damage and the formation of reactive oxygen species. In *Brassica rapa* L. mutants exposed to 100 µmol L⁻¹ CdCl₂, the BraA.cax1a-12 line accumulated more than

three times the Cd recorded in the parental line, while maintaining greater physiological tolerance (Navarro-León *et al.*, 2020b). In *Brassica oleracea* L., the application of 2 mmol L⁻¹ thiosulphate enhanced Cd accumulation, whereas 4 mmol L⁻¹ reduced uptake and affected biomass (Navarro-León *et al.*, 2020a). In *Lepidium sativum* L., CdCl₂ concentrations of 30, 60 and 120 mg L⁻¹ increased lipid peroxidation and H₂O₂, as well as concentration-dependent antioxidant responses (Khalofah *et al.*, 2020). These findings distinguish three components that should not be conflated: tissue concentration, physiological tolerance and the total amount of metal removed. A plant with a high tissue concentration experiences a loss in remediation performance when stress reduces harvestable biomass.

Phytoremediation integrates processes of contaminant extraction, immobilization or restriction of mobility. Its performance is related to biomass production, metal concentration in tissues and distribution between roots and shoots. In a rice intercropping system with *Thalia dealbata* Fraser, the companion species accumulated and stabilized more Cd than rice and reduced transfer to grain (Wang *et al.*, 2020). The combination of *Zea mays* L. with biosurfactants demonstrated that saponins enhanced Cd movement to stems and restricted its arrival at the edible fraction (Mekwichai *et al.*, 2020). In an oilseed rape-rice rotation, endophytic inoculation, soil amendments and a foliar inhibitor integrated remediation with agricultural production (Tang *et al.*, 2020). Rotations with oilseed crops also removed Cd and other metals, although the contaminated biomass required subsequent management in order to prevent its return to the environment (Zhou *et al.*, 2020).

Exposure time and the number of harvests modify cumulative removal, but the response does not follow a constant linear relationship. Sunflower-oilseed rape and peanut-oilseed rape rotations removed 77.24 and 62.09 g Cd ha⁻¹ year⁻¹, respectively, and reduced available Cd in the surface layer by 5.58% and 3.91% (Yang *et al.*, 2022). In a 120-day trial with *Tagetes patula* L. and the biodegradable chelator GLDA, total soil Cd decreased by 7.59% and its transfer to above-ground biomass increased (Li *et al.*, 2022). In an 8-ha field trial with *Sedum plumbizincicola*, the initial soil pH was 5.61 and the Cd concentration was 2.58 mg kg⁻¹. The first harvest produced 1.95 t ha⁻¹ of dry biomass and extracted 339 g Cd ha⁻¹, whereas the second produced 0.91 t ha⁻¹ and extracted 142 g Cd ha⁻¹. After both cycles, total Cd decreased to 1.53 mg kg⁻¹ and more than 70% of the area moved into a safe-use category (Wang *et al.*, 2023). Seasonal trials with ten winter and twelve summer crops confirmed that species selection and annual rotation modify Cd extraction and production safety (Yang *et al.*, 2025).

Functional classification of a species requires differentiation between phytoextraction and

phytostabilization. In Cd-contaminated soils, *Acacia mangium* Willd., *Jatropha curcas* L. and *Manihot esculenta* Crantz concentrated the metal mainly in roots, with values of up to 5.1 mg kg⁻¹ in *A. mangium*, 2.7-4.1 mg kg⁻¹ in *J. curcas* and 2.2-3.9 mg kg⁻¹ in *M. esculenta*, a pattern consistent with immobilization in the root zone (Taepayoon *et al.*, 2022). In *Catharanthus roseus* (L.) G. Don grown in soil affected by battery waste, a kinetic model achieved R² values above 0.992 and estimated maximum uptake rates of 31.94 mg kg⁻¹ in roots and 15.16 mg kg⁻¹ in shoots (AL-Huqail *et al.*, 2025). These examples indicate that a reduction in soil metal content should be interpreted together with tissue distribution and mass balance, when those data are available.

Moringa oleifera Lam. combines rapid growth, biomass production, warm-climate adaptation and antioxidant activity; however, evidence regarding Cd accumulation requires caution. Under Cd- and Pb-enriched conditions, plants accumulated more metal in their roots than stems, maintained translocation factors below 1, and increased their production of antioxidant enzymes and phenolics (Nouman *et al.*, 2020). Soil-applied selenite also modified root Cd uptake. Approximately 2.0 mg kg⁻¹ Na₂SeO₃ reduced root Cd by 80%, whereas around 4.0 mg kg⁻¹ increased root Se by 315% (Fu *et al.*, 2020). These responses are dependent upon rhizosphere chemistry and favor root retention. The evidence supports evaluation of *M. oleifera* as a tolerant remediation candidate, rather than its general classification as a hyperaccumulator.

Studies of *M. oleifera* extracts indicate biochemical protection but do not quantify Cd removal by living *M. oleifera* plants. In *Triticum aestivum* L. exposed to 500 and 1000 µmol L⁻¹ Cd, a 3% foliar extract increased root dry mass by 39% and 33%, reduced malondialdehyde by 6.16% and 23.50%, and lowered H₂O₂ by 49.20% and 58.70% (Mahmood *et al.*, 2022). In *Lepidium sativum* L., 2-6% extracts mitigated oxidative stress caused by 30-120 mg L⁻¹ CdCl₂ (Khalofah *et al.*, 2020). These protective effects do not demonstrate phytoextraction by *M. oleifera*.

Evidence remains limited on the joint effects of initial Cd concentration and *M. oleifera* exposure time in agricultural soil. Concentration affects availability and stress, while exposure time determines root-soil contact, growth and residual reduction. In field-contaminated soils, Cd classes also retain distinct physicochemical properties. Concentration effects must therefore be interpreted as soil-class responses, rather than as isolated doses, increasing environmental relevance but limiting attribution solely to Cd.

A rigorous phytoextraction assessment integrates Cd levels in roots and shoots, bioconcentration and translocation factors, biomass and mass balance. Without tissue measurements and unplanted controls, the difference between initial and final soil concentrations is an apparent reduction under plant exposure. Here,

phytoremediation was used within this operational scope. Residual recoverable soil Cd and *M. oleifera* morphology were assessed without attributing the decline exclusively to uptake, thereby separating remediation-compatible evidence from proof of mechanism.

This study addresses Sustainable Development Goal targets 2.4, on sustainable agriculture and soil quality, 3.9, on illness from hazardous chemicals, and 15.3 on the restoration of degraded soils (Hoyos-Alayo, 2026a, 2026b). Residual concentration, apparent reduction and biomass response provide verifiable indicators for these targets (Hoyos-Alayo, 2025a, 2025b).

This study evaluated recoverable Cd in agricultural soils exposed to *Moringa oleifera* Lam. across initial classes of 1.6, 1.8 and 2.0 mg kg⁻¹ and exposure periods of 8, 17 and 39 days, while characterizing seedling morphology in a randomized complete block design. We hypothesized that concentration class, time and their interaction would affect final recoverable Cd. Growth served as a complementary tolerance indicator, and the treatment combining the lowest residual Cd with compatible development defined the best integrated response.

2. Materials and methods

2.1. Study area and soil origin

Agricultural soil was collected from the hamlet of San Lorenzo in the Pimentel district of the province of Chiclayo, in Lambayeque, Peru, within a 1000 m² reconnaissance area. After the removal of residues, the 0-30 cm layer was sampled in accordance with the provisions of Ministerial Resolution No. 085-2014-MINAM. Around 50 kg were obtained from field-contaminated sectors. No synthetic Cd was added.

The initial assessment identified three recoverable-Cd contamination classes, 1.6, 1.8 and 2.0 mg kg⁻¹. These values represented the operational initial concentration classes and were used as C_i in the calculation of apparent reduction. All three values exceeded the Peruvian Environmental Quality Standard (EQS) of 1.4 mg kg⁻¹ for agricultural soil established by the provisions of Supreme Decree No. 011-2017-MINAM. Soil corresponding to each class was conditioned separately, air-dried, manually disaggregated, sieved to 2 mm and homogenized before distribution among the experimental units. Because the classes originated from field-contaminated soils, rather than from induced contamination, they retained the soil properties associated with their respective origins.

Field material was obtained from multiple randomly selected surface-soil samples and screened for recoverable Cd before experimental allocation. The material assigned to each operational Cd class was kept separate and homogenized to form one class-specific composite soil lot. Baseline recoverable Cd for each lot was verified from replicate baseline determinations using the EPA 3050B



digestion and EPA 6010D ICP-OES sequence described in Section 2.2; the archived baseline dataset reported means of 1.6, 1.8, and 2.0 mg kg⁻¹ with SD = 0.0 mg kg⁻¹ for each class. Each homogenized lot was then apportioned among 15 experimental units, representing the three exposure periods in each of the five blocks, at approximately 1.11 kg of conditioned soil per pot. This class-specific compositing and homogenization is consistent with the Peruvian Ministry for the Environment (MINAM) surface-soil sampling framework for representative composite samples of non-volatile contaminants (MINAM, 2014, 2017)

2.2. Determination of recoverable cadmium

The recoverable Cd in soil samples was determined by a laboratory accredited by INACAL-DA under registration number LE-178. The samples underwent acid digestion in accordance with U.S. EPA Method 3050B (U.S. EPA, 1996). This procedure quantifies the recoverable fraction through acid digestion and does not represent absolute digestion of the silicate matrix. Measurements were obtained by inductively coupled plasma optical emission spectrometry, ICP-OES, in accordance with U.S. EPA Method 6010D (U.S. EPA, 2018). The results were expressed as mg kg⁻¹ on a dry-soil basis. The Peruvian EQS for agricultural soil was used as the regulatory reference, while recognizing that EPA 3050B quantifies recoverable Cd, rather than the absolute content of the silicate matrix.

2.3. Plant material

Moringa oleifera Lam. seedlings were produced by the research team from purchased seeds sown as a single batch under common pre-experimental temperature, moisture and light conditions. The 12-day age at experimental allocation was counted from seedling emergence, and one seedling was assigned to each pot. Before allocation, seedlings were selected for comparable development and absence of mechanical damage or visible disease. Species selection reflected reported tolerance to metalliferous conditions and differentiated root-shoot Cd responses (Nouman *et al.*, 2020).

2.4. Experimental design and treatment allocation

A randomized complete block design used a 3 × 3 factorial arrangement (Ramos-Galarza, 2021; Cotrina-Urbina *et al.*, 2026; Jiménez-Hernández *et al.*, 2026). Factor A comprised initial Cd classes of 1.6, 1.8 and 2.0 mg kg⁻¹, and factor T comprised 8, 17 and 39 days. The nine combinations appeared once in each of five blocks, giving five replicates and 45 units. Each unit was comprised of one pot with soil and one seedling.

The blocks controlled lighting, position and microenvironmental gradients. Within each block, the nine treatment codes were permuted by simple random

allocation without replacement before pot placement, so that each combination occupied one position and occurred once per block. Exposure periods were independent destructive harvests, and each pot was measured once, avoiding pseudoreplication from repeated measurements. Table 1 summarizes the design, and Figure 1 shows unit allocation.

Table 1. Experimental design structure

Component	Levels or number	Specification
Initial Cd concentration class	3	1.6, 1.8 and 2.0 mg kg ⁻¹
Exposure time	3	8, 17 and 39 days
Factorial combinations	9	3 classes × 3 exposure periods
Blocks	5	I, II, III, IV and V
Replicates per treatment	5	One unit per block
Experimental units	45	One pot containing one 12-day-old seedling

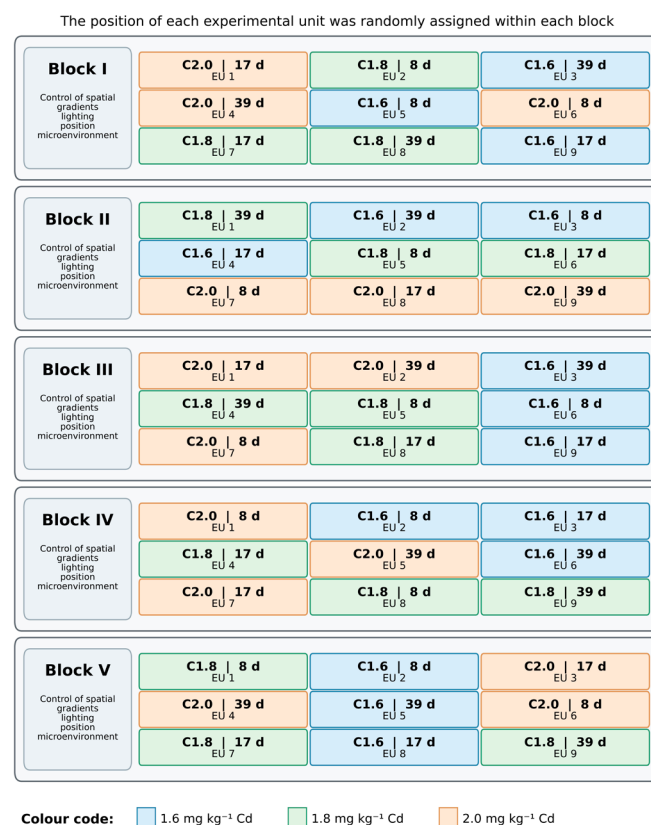


Figure 1. Randomized allocation of the 3 × 3 factorial treatments across five complete blocks

Note. Colors distinguish the three initial Cd classes. Each block contains all nine combinations once.

2.5. Trial establishment and maintenance

The conditioned soil was distributed among 45 pots at approximately 1.11 kg per unit. The pots lacked drainage outlets, in order to restrict percolation. Moisture was maintained by daily weighing and replacement of evapotranspiration losses. All the units received identical management and remained in their assigned block positions.

Without drainage, physical Cd export through water was restricted. The trial lacked unplanted pots and tissue Cd measurements; differences between initial and final concentrations were therefore interpreted as apparent reductions in recoverable soil Cd under plant exposure, rather than as direct evidence of phytoaccumulation or mass balance.

2.6. Harvest, final sampling and response variables

Units assigned to 8, 17 and 39 days were harvested independently. Before seedling removal, plant height was measured from the root collar to the shoot apex using a graduated ruler. Stem diameter was recorded using a Vernier caliper. After extraction, the roots were carefully washed to remove adhering soil, and root length was measured from the collar to the tip of the primary root. Total biomass was dried to constant mass and weighed on a precision balance. Morphological variables were expressed as height in cm, stem diameter in mm, root length in cm and total dry biomass in g plant⁻¹.

After each harvest, the soil remaining in the pot was homogenized and a representative aliquot was collected, in order to determine final recoverable Cd, using the same EPA 3050B digestion and EPA 6010D ICP-OES sequence applied at the baseline. The primary response variable was final Cd concentration, C_f , in mg kg⁻¹. Apparent reduction was calculated using Equation 1, where C_i denotes the operational initial concentration class and C_f the final soil concentration.

$$R_{\text{apparent}} (\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

A lower final Cd concentration with sustained growth was interpreted as compatible with phytoremediation potential within the tested system. Bioconcentration factors, translocation factors and extracted Cd mass were not calculated, because the roots and shoots were not analyzed for Cd.

2.7. Statistical analysis

Data were organized in Microsoft Excel and analyzed in Minitab. Means, standard deviations and coefficients of variation were calculated. Final Cd was evaluated using a general linear model for the 3 × 3 factorial block design, including block, initial concentration class, exposure time and their interaction. Equation 2 defines the model, where Y_{ijk} is the response, μ the mean, B_k the block effect, A_i the concentration-class effect, T_j the time effect, $(AT)_{ij}$ the interaction and ϵ_{ijk} the error.

$$Y_{ijk} = \mu + B_k + A_i + T_j + (AT)_{ij} + \epsilon_{ijk} \quad (2)$$

Random allocation and independent destructive harvests supported independence. Diagnostics examined standardized residuals and unusual observations. Significance was set at $\alpha = 0.05$. Significant interactions were assessed through Tukey simple-effect comparisons at 95% confidence. Growth variables were summarized descriptively without inferential comparisons.

Initial concentration class was treated as a fixed field-soil factor, not as a dose added to a uniform substrate. Unmeasured origin-specific soil properties may have accompanied Cd classes and influenced responses. The effects were therefore interpreted as differences among the tested soil classes.

2.8. Scientific integrity and management of contaminated material

The sampling was conducted with landholder authorization. Data were recorded on field sheets and electronic matrices without excluding units. Cd-exposed biomass was not used for food or feed. Institutional procedures for metal-contaminated material were followed for residual soil, laboratory materials and biomass.

3. Results

3.1. Dynamics of recoverable Cd and apparent reduction

Final Cd decreased over time in every soil class, but the magnitude differed and was not monotonic with initial concentration. Means ranged from 1.36 to 1.58 mg kg⁻¹ at 8 days, 0.86 to 1.42 mg kg⁻¹ at 17 days, and 0.30 to 0.88 mg kg⁻¹ at 39 days. The 1.8 mg kg⁻¹ class at 39 days displayed the lowest residual, 0.30 ± 0.16 mg kg⁻¹, and the greatest apparent reduction, $83.33 \pm 8.78\%$ (Table 2).



Table 2. Final concentration, apparent reduction and frequency of units at or below the reference value by treatment

Initial Cd class (mg kg ⁻¹)	Time (days)	Final Cd (mg kg ⁻¹)	CV of final Cd (%)	Apparent reduction (%)	Units ≤ 1.4 mg kg ⁻¹ n (%)
1.6	8	1.44 ± 0.22	15.21	10.00 ± 13.69	2 (40)
1.6	17	1.14 ± 0.24	21.13	28.75 ± 15.05	5 (100)
1.6	39	0.88 ± 0.22	24.64	45.00 ± 13.55	5 (100)
1.8	8	1.36 ± 0.24	17.71	24.44 ± 13.38	2 (40)
1.8	17	0.86 ± 0.09	10.40	52.22 ± 4.97	5 (100)
1.8	39	0.30 ± 0.16	52.70	83.33 ± 8.78	5 (100)
2.0	8	1.58 ± 0.18	11.32	21.00 ± 8.94	0 (0)
2.0	17	1.42 ± 0.16	11.57	29.00 ± 8.22	4 (80)
2.0	39	0.58 ± 0.18	30.84	71.00 ± 8.94	5 (100)

Note. Values are means ± standard deviations, n = 5. The last column shows the frequency of units with final recoverable Cd at or below the Peruvian EQS for agricultural soil, 1.4 mg kg⁻¹.

Units at or below 1.4 mg kg⁻¹ increased from 26.67% at 8 days to 93.33% at 17 days and 100% at 39 days. Across the trial, 33 of 45 units, 73.33%, met this reference. Figure 2 shows the temporal divergence among classes.

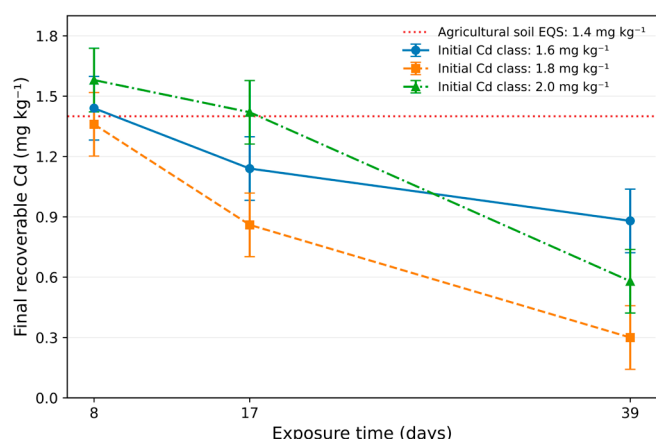


Figure 2. Final recoverable Cd concentration as a function of exposure time and initial concentration class

Note. Points represent adjusted means, and error bars show 95% confidence intervals calculated from the residual error of the factorial block model. The dotted line represents the Peruvian EQS for agricultural soil, 1.4 mg kg⁻¹.

Apparent reduction increased over time in each class. At 39 days, the means were 45.00% for 1.6 mg kg⁻¹, 83.33%

for 1.8 mg kg⁻¹ and 71.00% for 2.0 mg kg⁻¹. Figure 3 shows within-treatment variability.

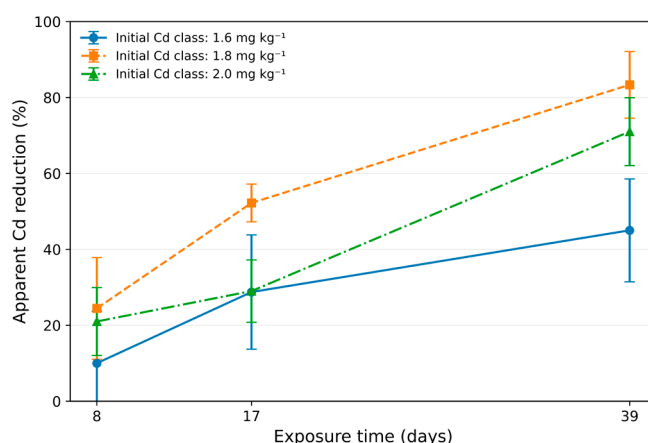


Figure 3. Apparent reduction in recoverable soil Cd according to initial concentration class and exposure time

Note. Points represent means, and error bars represent standard deviations, n = 5.

3.2. Factorial effects on final Cd concentration

The factorial block model explained 89.21% of final-Cd variation, with adjusted R² of 85.16%, predicted R² of 78.66%, and S = 0.1737 mg kg⁻¹. Initial class, exposure time, their interaction and block were significant (Table 3).

Table 3. Analysis of variance for the 3 × 3 factorial randomized complete block model

Source	DF	Adjusted SS	Adjusted MS	F	p
Block	4	0.3747	0.0937	3.10	0.029
Initial Cd concentration class	2	1.1231	0.5616	18.62	<0.001
Exposure time	2	5.8564	2.9282	97.07	<0.001
Concentration class × time	4	0.6262	0.1566	5.19	0.002
Error	32	0.9653	0.0302		
Total	44	8.9458			

Note. S = 0.1737 mg kg⁻¹, R² = 89.21%, adjusted R² = 85.16%, predicted R² = 78.66%. The largest absolute standardized residual was 1.94; therefore, no observations with |r| ≥ 2 were identified.

The significant interaction showed that temporal response was dependent on soil class. Classes did not differ at 8 days. At 17 days, the 1.8 mg kg⁻¹ class was lower than the 1.6 and 2.0 mg kg⁻¹ classes. At 39 days, all differed in the order 1.8 < 2.0 < 1.6 mg kg⁻¹. Within 1.8 mg kg⁻¹, all the times differed. Within 1.6 mg kg⁻¹, 17 and 39 days did not differ, while within 2.0 mg kg⁻¹, 8 and 17 days did not differ (Table 4).

Table 4. Tukey comparisons of the simple effects of the interaction between initial concentration class and exposure time

Initial Cd class (mg kg ⁻¹)	8 days	17 days	39 days
1.6	1.44 aA	1.14 bB	0.88 aB
1.8	1.36 aA	0.86 cB	0.30 cC
2.0	1.58 aA	1.42 aA	0.58 bB

Note. Values are mean final Cd concentrations in mg kg⁻¹. Lower-case letters compare classes within each exposure period, and upper-case letters compare exposure periods within each class. Means sharing a letter do not differ according to Tukey's test at 95% confidence. Tukey's honestly significant difference (HSD) = 0.270 mg kg⁻¹.

3.3. Morphological response of *Moringa oleifera* Lam.

All morphological variables increased with time in every class. The 1.8 mg kg⁻¹ class exhibited the highest descriptive means at each assessment. At 39 days, the seedlings reached 64.02 ± 0.87 cm in height, with a 5.60 ± 0.06 mm stem diameter, 28.02 ± 0.50 cm root length and 4.45 ± 0.08 g plant⁻¹ dry biomass. The 2.0 mg kg⁻¹ class displayed the lowest mean height and biomass throughout (Table 5).

Dry biomass separated descriptively as exposure progressed. From 8 to 39 days, means rose from 0.75 to 3.60 g plant⁻¹ at 1.6 mg kg⁻¹, 0.85 to 4.45 g plant⁻¹ at 1.8 mg kg⁻¹, and 0.66 to 3.02 g plant⁻¹ at 2.0 mg kg⁻¹ (Figure 4).

Table 5. Morphological variables of *Moringa oleifera* Lam. by treatment

Initial Cd class (mg kg ⁻¹)	Time (days)	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Dry biomass (g plant ⁻¹)
1.6	8	20.48 ± 1.11	2.40 ± 0.07	12.20 ± 0.69	0.75 ± 0.11
1.6	17	35.00 ± 1.20	3.40 ± 0.07	17.00 ± 0.72	1.55 ± 0.12
1.6	39	58.02 ± 0.45	5.00 ± 0.03	23.52 ± 0.24	3.60 ± 0.04
1.8	8	21.84 ± 1.11	2.55 ± 0.07	13.22 ± 0.65	0.85 ± 0.11
1.8	17	37.00 ± 0.65	3.71 ± 0.04	19.22 ± 0.34	1.85 ± 0.05
1.8	39	64.02 ± 0.87	5.60 ± 0.06	28.02 ± 0.50	4.45 ± 0.08
2.0	8	18.94 ± 0.70	2.30 ± 0.05	12.78 ± 0.41	0.66 ± 0.06
2.0	17	31.36 ± 1.02	3.20 ± 0.06	17.96 ± 0.58	1.33 ± 0.10
2.0	39	49.74 ± 0.82	4.64 ± 0.05	24.36 ± 0.45	3.02 ± 0.07

Note. Values are means ± standard deviations, n = 5. Morphological variables were analyzed descriptively.

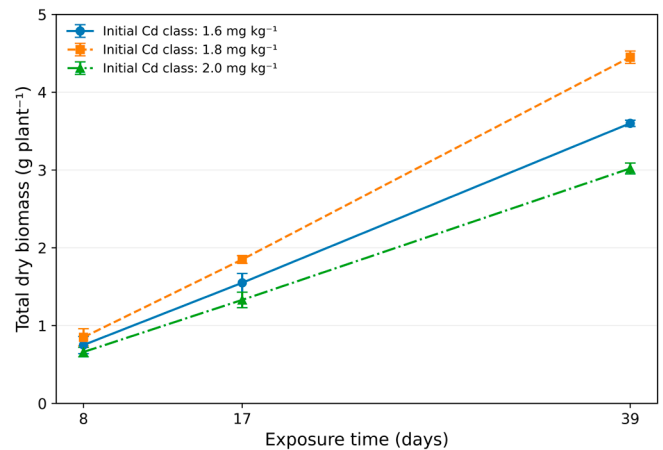


Figure 4. Total dry biomass of *Moringa oleifera* Lam. according to initial Cd concentration class and exposure time.

Note. Points represent means, and error bars represent standard deviations, n = 5.

3.4. Integrated treatment performance

The combination of 1.8 mg kg⁻¹ and 39 days produced the lowest residual Cd, the greatest apparent reduction and the highest mean plant-growth values. All five units recorded final Cd at or below the agricultural-soil EQS, with concentrations ranging from 0.10 to 0.50 mg kg⁻¹. The combination of 2.0 mg kg⁻¹ and 39 days ranked second for residual Cd, with 0.58 ± 0.18 mg kg⁻¹ and 71.00 ± 8.94% reduction, although it exhibited lower mean biomass than the 1.6 and 1.8 mg kg⁻¹ classes. Within the 1.6 mg kg⁻¹ class, final Cd did not differ between 17 and 39 days, although mean biomass increased. The findings represent an apparent reduction in recoverable Cd under plant exposure and do not demonstrate transfer of the metal to plant tissues.

4. Discussion

4.1. Time-dependent reduction and concentration-class interaction

The principal finding of the study was the progressive decline in final recoverable Cd across all three soil classes, accompanied by a significant concentration class $\times$ time interaction. Exposure time produced the largest F statistic in the model ($F = 97.07$), while the interaction remained significant ($p = 0.002$). Time exposure was therefore found to govern the general direction of the response, but the rate and magnitude of change were dependent upon soil class. The 1.8 mg kg⁻¹ class showed the clearest temporal separation, decreasing from 1.36 ± 0.24 mg kg⁻¹ at 8 days to 0.30 ± 0.16 mg kg⁻¹ at 39 days. By contrast, the 1.6 mg kg⁻¹ class did not differ between 17 and 39 days, and the 2.0 mg kg⁻¹ class did not differ between 8 and 17 days. These plateaus show that extending exposure did not produce a constant incremental response in every soil class.

The time effect matches the evidence indicating that cumulative Cd removal is dependent upon duration, biomass and harvest frequency. Oilseed rotations removed 62.09–77.24 g Cd ha⁻¹ year⁻¹ and reduced available Cd by 3.91–5.58% (Yang *et al.*, 2022). Over three years, repeated flowering-stage harvests of *Solanum nigrum* L. reduced extractable Cd by 36.4%, compared with 27.6% after one annual mature harvest (Dou *et al.*, 2022). Digestate increased *Pennisetum hybridum* biomass, rhizosphere availability and root-shoot transfer, indicating that time is beneficial when growth and bioavailability persist (He *et al.*, 2021).

Additional time or harvests do not ensure proportional Cd export. In an 8-ha *Sedum plumbizincicola* trial, the second harvest extracted less Cd, due to the decline in biomass (Wang *et al.*, 2023). *Pennisetum hybridum* column and lysimeter studies have shown simultaneous biomass removal and migration between soil layers, requiring separation of plant export from vertical redistribution (Chen *et al.*, 2023). Accordingly, the 83.33% apparent reduction here does not reflect field extraction efficiency; rather, it represents a change in concentration in closed pots, and not Cd mass recovered from tissues.

The non-monotonic ordering of the soil classes is environmentally relevant. At 39 days, final concentrations followed the order $1.8 < 2.0 < 1.6$ mg kg⁻¹, despite the higher initial Cd assigned to the 2.0 mg kg⁻¹ class. The classes originated from field-contaminated soils, rather than from a common soil amended with graded Cd doses. Therefore, initial Cd co-varied with unmeasured edaphic properties. Soil pH, organic matter, cation-exchange capacity, texture and competing elements regulate Cd sorption, extractability and crop accumulation (Pan *et al.*, 2022; Xu *et al.*, 2022). In Peru, geology, texture, pH and rainfall seasonality modify soil-to-plant Cd transfer (Thomas *et al.*, 2023). Also, experiments across soils

containing 0.19–2.91 mg kg⁻¹ Cd have found that plant growth, root accumulation, exudation and microbial responses change with the soil contamination level (Zhang *et al.*, 2024). The superior response of the 1.8 mg kg⁻¹ class should therefore be interpreted as the behavior of that soil class within this trial, and not as evidence that 1.8 mg kg⁻¹ is an intrinsically optimal Cd concentration.

4.2. Growth response and functional interpretation of *Moringa oleifera*

All morphological variables increased between 8 and 39 days, which demonstrates seedling establishment in every soil class. The 1.8 mg kg⁻¹ class maintained the highest descriptive means and reached 64.02 ± 0.87 cm in height and 4.45 ± 0.08 g plant⁻¹ in dry biomass at 39 days. The 2.0 mg kg⁻¹ class consistently recorded the lowest height and biomass, indicating a descriptive growth disadvantage associated with that field-derived soil class. Cd toxicity cannot be isolated as the sole cause, because the classes also retained distinct soil properties. Across subtropical agricultural soils, pH explained 44% of the variation in Cd-related barley root inhibition (Zhang *et al.*, 2023). Cd exposure can also alter root length, diameter, tissue organization and nutrient relations before visible mortality occurs (Sabella *et al.*, 2022). The morphology recorded here is consistent with short-term tolerance, but it does not establish the absence of physiological stress.

Cd tolerance involves root-surface exclusion, cell-wall and vacuolar sequestration, chelation, antioxidants and regulated organ transport (Luo and Zhang, 2021). In Cd- and Pb-enriched substrates, *M. oleifera* retained more metal in the roots than the stems, had translocation factors below 1, and increased antioxidant enzymes and phenolics (Nouman *et al.*, 2020). In the same species, approximately 2.0 mg kg⁻¹ Na₂SeO₃ reduced root Cd by 80% (Fu *et al.*, 2020). These results support a root-centered, chemically responsive strategy, but not classification as a Cd hyperaccumulator.

The coinciding of the lowest residual Cd and greatest biomass in the 1.8 mg kg⁻¹ and 39-day treatment supports its selection as the best integrated response under the tested conditions. Greater root length and biomass increase contact with the soil matrix and the area available for sorption, exudation and microbial interactions. Biomass production alone does not demonstrate extraction. *Miscanthus $\times$ giganteus* maintained biomass on Cd-contaminated soil but removed modest quantities in shoots, leading to its interpretation as a phytostabilization candidate, rather than as an efficient phytoextractor (Zgorelec *et al.*, 2020). *Acacia mangium* Willd., *Jatropha curcas* L., and *Manihot esculenta* Crantz also retained most Cd in their roots under phytostabilization conditions (Taepayoon *et al.*, 2022). By contrast, willow NJU513 combined high biomass with measured leaf and stem Cd concentrations, bioconcentration factors and extracted mass, while rhizosphere compounds contributed to Cd

activation or passivation (Li *et al.*, 2024). Studies that have demonstrated phytoextraction quantify Cd separately in roots and shoots and integrate concentration with biomass or uptake kinetics (AL-Huqail *et al.*, 2025). The present data identify remediation potential and establishment, but not the dominant mechanism.

4.3. Meaning of the apparent reduction in recoverable Cd

Apparent reduction defines the evidential scope. Drainage-free pots restricted percolation, and the same EPA 3050B and EPA 6010D ICP-OES sequence was used at baseline and harvest, strengthening internal comparison. However, the design lacked unplanted controls, organ-specific Cd and mass balance. Initial-final differences may combine uptake, root-surface adsorption, rhizosphere immobilization, redistribution among operational soil fractions and analytical or sampling variation. Chelator-assisted cultivation can alter soil Cd fractions and plant subcellular distribution simultaneously (Li *et al.*, 2022). Soil columns also show that loss from a sampled layer can coexist with downward migration undetected by one final bulk-soil measurement (Chen *et al.*, 2023).

Rhizosphere processes provide a credible explanation for the class-specific trajectories, although these were not measured. Microbial inoculation can modify Cd uptake and restructure rhizosphere communities during phytoremediation (You *et al.*, 2023). A high-Cd-accumulating *Salix* ecotype selected a rhizosphere microbiome associated with greater Cd mobilization and extraction (Song *et al.*, 2024). In *Houttuynia cordata*, Cd level altered root exudates, bacterial and fungal communities and the balance between Cd mobilization and passivation (Zhang *et al.*, 2024). Similarly, willow experiments linked amino-group compounds with passivation and carboxyl-group compounds with activation of soil Cd (Li *et al.*, 2024). Such processes could reduce the recoverable fraction around *M. oleifera* roots without proportional transfer to above-ground biomass; however, this remains a mechanistic hypothesis, rather than a demonstrated result.

Taken together, the significant concentration class × time interaction is consistent with class-dependent evolution of rhizosphere conditions, rather than with a uniform temporal decline. As the roots expanded, differences among the field-derived soil classes in sorption behavior, pH buffering, organic matter, competing ions, root exudation and microbial activity may have altered Cd mobilization or immobilization at different rates, producing the divergent trajectories observed at 17 and 39 days (Pan *et al.*, 2022; Xu *et al.*, 2022; Zhang *et al.*, 2024; Li *et al.*, 2024); however, these mechanisms remain untested, because soil physicochemical properties, rhizosphere microbial communities and Cd concentrations in plant tissues were not measured.

The block model explained 89.21% of variation, adjusted R^2 was 85.16% and the largest absolute standardized residual was 1.94. Predicted R^2 of 78.66% indicates moderate prediction and residual uncertainty. The significant block effect, $p = 0.029$, confirms spatial or microenvironmental influence and supports blocking, while treatment effects remained significant. Because morphology was analyzed descriptively, consistent rankings support integrated interpretation, but not inferential differences.

4.4. Regulatory relevance, application and study boundaries

Units at or below the Peruvian agricultural-soil reference increased from 26.67% at 8 days to 100% at 39 days, giving the 39-day treatment screening relevance in closed pots; however, this does not establish field compliance, because EPA 3050B measures recoverable Cd, soil may re-equilibrate, and persistence after plant removal remained untested. Field validation requires independent composite samples, unplanted controls, post-treatment equilibration and replication across seasons and soils.

Practical phytoremediation is dependent upon Cd mass removed per area, productive harvests and contaminated-biomass management. Crop-rotation flux studies quantify annual inputs and outputs to avoid overestimating remediation from concentration alone (Yang *et al.*, 2022). Digestate-assisted *Pennisetum hybridum* shows that biomass and shoot transfer may shorten remediation, while agronomic inputs alter rhizosphere availability (He *et al.*, 2021). Field-scale *Sedum plumbizincicola* assessments integrated tissue concentration, biomass, extracted mass and post-treatment soil Cd, before assigning safer land use (Wang *et al.*, 2023). Contaminated biomass requires containment or controlled treatment, in order to prevent Cd return (Zhou *et al.*, 2020).

Further validation of *M. oleifera* should quantify Cd in roots, stems and leaves, determine organ biomass, calculate bioconcentration and translocation factors, and close the soil-plant-residue mass balance. Each field class will also require pH, organic matter, cation-exchange capacity, texture, redox conditions and operational Cd fractions. Unplanted pots would separate plant-associated change from abiotic ageing and analytical variation. Repeated harvests with post-harvest equilibration would test persistence and safe biomass export, rather than soil-profile redistribution.

In this study, exposure time and field-derived soil class jointly controlled recoverable Cd during early *M. oleifera* establishment. The 1.8 mg kg⁻¹ and 39-day treatment combined the lowest residual with the strongest descriptive growth. These findings support further evaluation of *M. oleifera* in warm Cd-affected agricultural



soils, while retaining claims consistent with the measured variables.

5. Conclusions

Exposure time dominated final recoverable Cd, and its effect varied by field-derived soil class. The concentration class $\times$ time interaction rejected a uniform temporal response. At 39 days, the 1.8 mg kg⁻¹ class produced the lowest final concentration, 0.30 ± 0.16 mg kg⁻¹, and greatest apparent reduction, $83.33 \pm 8.78\%$, with the highest descriptive means for height, stem diameter, root length and biomass. The 2.0 mg kg⁻¹ class ranked second at 0.58 ± 0.18 mg kg⁻¹ and $71.00 \pm 8.94\%$. All 15 units harvested at 39 days were at or below 1.4 mg kg⁻¹. Thus, under controlled conditions, 1.8 mg kg⁻¹ was the best-performing field-soil class, not an optimal Cd dose.

Moringa oleifera Lam. established and increased biomass in every treatment, a response consistent with short-term tolerance under the tested conditions. This study does not demonstrate phytoextraction, because it lacked unplanted controls, Cd measurements in plant tissues and a total mass balance. The decrease in soil concentration should remain defined as an apparent reduction in the recoverable fraction under plant exposure. Field-scale confirmation, tissue partitioning, soil-property characterization, post-harvest equilibration

and repeated-harvest mass balances will be required before assigning a remediation mechanism or predicting long-term performance.

Authors' contributions

Kary Jakely Jesús Pinedo: Conceptualization, investigation, data curation, formal analysis, visualization and writing (original draft).

Walter Manuel Hoyos-Alayo: Methodology, validation, supervision, project administration and writing (revision and editing).

Both authors approved the final manuscript.

Research funding

This research was self-funded by the authors.

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 tools were used in any part of the manuscript preparation process.

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