



ROLE OF ORGANIC AND INORGANIC AMENDMENTS IN REMEDIATION OF CONTAMINATED AGRICULTURAL SOIL: A LABORATORY SCALE STUDY

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ABSTRACT:

Heavy metal contamination of agricultural soils poses severe threats to food safety and ecosystem integrity. A bioreactor pot experiment was conducted to evaluate the comparative effectiveness of organic amendments (S2), inorganic amendments (S3), and their combined application (S4) with an unamended control (S1) in remediating heavy metal-contaminated agricultural soil. Key soil parameters, including pH, electrical conductivity (EC), organic carbon (OC), and available nitrogen (N) were assessed at different retention periods, alongside copper (Cu), zinc (Zn), and lead (Pb) concentrations in soil and plant. All amendment treatments progressively raised pH toward neutrality; the inorganic treatment (S3) attained the highest value (7.38 ± 0.035 at harvest) representing $\sim 8.37\%$, while the combined treatment (S4) achieved the greatest EC reduction ($0.17 \pm 0.026 \text{ dS m}^{-1}$) representing $\sim 54\%$. Organic amendments (S2) maximally enriched soil OC ($0.37 \pm 0.040\%$), representing $\sim 12\%$, and all amended soils maintained significantly higher available N than the declining control. At harvest, S4 demonstrated the greatest reductions in Phyto availability of Cu ($2.45 \pm 0.303 \text{ mg kg}^{-1}$), Zn ($3.22 \pm 0.442 \text{ mg kg}^{-1}$), and Pb ($0.96 \pm 0.165 \text{ mg kg}^{-1}$), representing $\sim 75\%$, $\sim 90\%$, and $\sim 80\%$ decreases relative to S1, respectively. Overall, the results indicate that integrating organic and inorganic amendments can provide a synergistic effect, enhancing soil quality while effectively immobilising heavy metals for remediating metal-contaminated agricultural soils.

KEYWORDS:

HEAVY METAL REMEDIATION, ORGANIC AMENDMENTS, BIOCHAR; CHELATING AGENTS, IN SITU IMMOBILISATION, BIOREACTOR POT EXPERIMENT, PHYTO AVAILABILITY.

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1. INTRODUCTION

Heavy metal contamination in agricultural soils has become a serious global concern, affecting ecosystem balance, reducing crop productivity, and posing risks to human health. The World Food and Agriculture Organization (FAO) lists heavy metals as the primary inorganic pollutant in soils worldwide. Major anthropogenic sources include mining and smelting, industrial effluents, phosphate fertilizers, pesticides, and irrigation with contaminated wastewater (Wan et al., 2024; Rahim et al., 2024). Unlike organic pollutants, heavy metals are non-biodegradable and persist indefinitely in the soil-plant system, accumulating progressively in food chains.

Copper (Cu), zinc (Zn), and lead (Pb) are among the most widespread metal contaminants in agricultural soils. Although Cu and Zn are essential micronutrients, phytotoxicity occurs at elevated concentrations; Pb has no known biological function and is toxic at trace levels (Wan et al., 2024). Metal bioavailability is governed principally by soil pH, organic carbon, cation exchange capacity, and

redox potential (Palansooriya et al., 2020). In situ immobilisation using soil amendments is widely recognised as one of the most cost-effective and scalable remediation strategy for reducing metal mobility and bioavailability (Bolan et al., 2014).

Organic amendments reduce metal bioavailability via pH elevation, humic acid complexation, and increased sorption site density (Alvarenga et al., 2009; Walker et al., 2004). Inorganic amendment is expected to influence nitrogen dynamics and microbial activity, while chelating agents and biochar may alter metal mobility through complexation and adsorption processes.

Combined application has been advocated as a synergistic strategy (Rahim et al., 2024), yet temporally resolved comparative data under controlled pot conditions are limited. The present study aims to evaluate the effects of different amendment regimes (S1–S4) on soil physicochemical properties and the dynamics of Cu, Zn, and Pb across multiple retention periods under bioreactor pot conditions.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN AND TREATMENTS

A bioreactor pot experiment was established using air-dried, 2-mm-sieved soil from a site with documented Cu, Zn, and Pb contamination. Four treatments were applied in triplicate (n = 3):

| Treatment | Amendment Type | Description |
|-----------|----------------|---|
| S1 | Control | Unamended contaminated soil |
| S2 | Organic | Manures |
| S3 | Inorganic | Inorganic amendment |
| S4 | Combined | Organic & inorganic amendments including biochar & chelating agents |

TABLE 1: EXPERIMENTAL TREATMENT GROUPS USED IN THE BIOREACTOR POT STUDY

2.2 STUDY AREA

Balesar village (26.39°N, 72.47°E), located in the Jodhpur district of Rajasthan, serves as the focal point of the present study. The village has been selected due to its representative socio-economic and environmental characteristics, which align with the objectives of the research. Its geographic location, demographic composition, and local resource dynamics provide a suitable context for examining the key issues addressed in this study.

2.3 SOIL AND PLANT SAMPLING AND ANALYSIS

Samples were collected at different retention periods. Soil pH was determined by direct reading pH meter using glass electrode with saturated KCl-calomel electrode; EC (dS m⁻¹) in 1:5 suspension (Rayment and Higginson, 1992); OC (%) by Walkley-Black oxidation; total available N (kg ha⁻¹) by Kjeldahl (total N) method. Soil Cu, Zn, and Pb were quantified by Atomic Absorption Spectrometer (Perkin Elmer, Model A Analyst 300; AAS); plant metal uptake (Cu, Zn, Pb; mg kg⁻¹) after dry ashing and AAS. All data are expressed as mean ± SD (n = 3).

3. RESULTS AND DISCUSSION

3.1. SOIL PHYSICO-CHEMICAL PROPERTIES

3.1.1 SOIL PH

All the amended treatments produced a sustained and statistically significant (p < 0.05) elevation in soil pH over time. S3 achieved the highest Harvest pH (7.38 ± 0.035), followed closely by S2 (7.22 ± 0.056) and S4 (7.16 ± 0.056). The alkalizing mechanism of organic manures is principally attributed to the liberation of basic cations (Ca²⁺, Mg²⁺, K⁺) during decomposition, OH⁻ release during ammonification, and carbonate/bicarbonate generation from organic matter mineralization (Wong et al., 2009). The comparatively moderate pH elevation in S4 relative to S3, despite its more complex amendment composition, likely reflects pH buffering by biochar — which is known to stabilize soil pH within a range that optimizes nutrient bioavailability without inducing extreme alkalinity (Lehmann and Joseph, 2015). These findings are consistent with Bolan et al. (2014), who reported significant pH elevation in contaminated soils amended with combined organic-mineral inputs.

3.1.2 SOIL ELECTRICAL CONDUCTIVITY (EC)

Soil EC — an index of total dissolved salt load and ionic strength — exhibited clear treatment-dependent dynamics across the experimental period. A progressive decline in EC was observed in all treatments toward Harvest. S4 recorded the lowest final EC (0.17 ± 0.026 dS m⁻¹), significantly below S3 (0.25 ± 0.040), S2 (0.26 ± 0.036), and S1 (0.27 ± 0.030 dS m⁻¹). The pronounced EC reduction in S4 is attributed to biochar's high cation exchange capacity and ion-adsorption potential, which retains soluble ions within the soil matrix and reduces leachable salt concentrations (Laird et al., 2010). All EC values remained below the non-saline threshold of 4 dS m⁻¹, confirming the absence of secondary salinization risk across all treatments.

3.1.3 SOIL ORGANIC CARBON (OC)

Soil OC remained persistently low in S1 throughout the experimental period (0.15–0.17%), underscoring the inherent carbon-depleted status of unmanaged contaminated soil. All amendment treatments significantly elevated OC relative to S1 (p < 0.05). S2 achieved the highest OC across most intervals. The superior OC retention under S2 relative to S3 and S4 suggests that inorganic amendment stimulated microbial N



FIG. 1: LOCATION MAP OF JODHPUR DISTRICT SHOWING THE STUDY AREA (BALESAR VILLAGE)

mineralization in S3 and the biochar-induced priming effect in S4 may have accelerated decomposition of labile organic pools, reducing net OC accumulation (Maestrini et al., 2015). Nevertheless, all amended treatments maintained OC substantially and significantly above S1 throughout, confirming the central role of organic manures in restoring soil carbon stocks — a critical determinant of soil structure, water retention, and microbial activity in degraded and contaminated agricultural soils.

3.1.4 SOIL TOTAL NITROGEN (N)

The control (S1) exhibited a continuous decline in soil total N from $77.83 \pm 0.393 \text{ mg kg}^{-1}$ to $66.94 \pm 1.166 \text{ mg kg}^{-1}$ at Harvest, reflecting net N losses through crop removal, gaseous volatilization, and leaching. All amended treatments showed dramatic N accumulation from Day 45 onward. S3 achieved the highest Day 45 value ($149.32 \pm 2.500 \text{ mg kg}^{-1}$), with S2 (129.44 ± 1.857) and S4 ($127.39 \pm 1.314 \text{ mg kg}^{-1}$) slightly lower, reflecting the additional mineral N contribution in S3 and the early immobilization effect of biochar in S4.

By Day 90, all three amended treatments converged at near-peak N levels (S2: 176.82 ± 2.702 ; S3: 177.66 ± 2.732 ; S4: $178.82 \pm 1.827 \text{ mg kg}^{-1}$), with S4 marginally highest — attributable to biochar-mediated reductions in NH_3 volatilization and NO_3^- leaching, which collectively enhance N retention efficiency (Steiner et al., 2008). A moderate decline at Harvest (S2: 172.15; S3: 171.68; S4: $167.86 \text{ mg kg}^{-1}$) is consistent with N uptake by maturing crops. The sustained high N availability under all amendment regimes, far exceeding the control, demonstrates their collective capacity to maintain adequate N nutrition throughout the crop production cycle.

3.2. HEAVY METALS IN SOIL

3.2.1 COPPER (CU) IN SOIL

All amendment treatments produced rapid and sustained reductions in soil Cu: at Harvest, S2, S3 and S4 represented reductions of approximately 82%, 85%, and 87% from respective Day 15 concentrations. The most pronounced decline occurred between Day 15 and Day 45, coinciding with peak chelate mobilization and maximum microbial activity. In S4, the synergistic action of chelating agents (desorbing Cu from exchange sites), biochar (providing high-surface-area adsorptive sequestration of free Cu^{2+}), and organic matter (forming stable Cu-humate complexes) collectively drove the greatest observed reduction (Ahmad et al., 2012; Beesley et al., 2011). S4 was significantly superior to all treatments ($p < 0.05$) at Harvest, confirming the efficacy of the multi-mechanism approach. These results are consistent with Peng et al. (2020), who reported >80% reduction in available Cu with combined biochar-chelate application.

3.2.2 ZINC (ZN) IN SOIL

All amendment treatments achieved substantial Zn reductions: at Harvest, S2, S3 and S4 showed

approximately 76%, 78%, and 80% reductions, respectively. The Zn remediation trajectory closely mirrored that of Cu, with the most rapid decline between Day 15 and Day 45, confirming that chelate-assisted mobilization, phytoextraction, biochar adsorption, and organic complexation govern both metal species concurrently. The slightly lower percentage reduction for Zn relative to Cu, despite comparable initial concentrations, is consistent with the Irving-Williams series, which predicts lower chelate affinity for Zn^{2+} relative to Cu^{2+} (Irving and Williams, 1953). S4 was again significantly superior to all other treatments at Harvest ($p < 0.05$).

3.2.3 LEAD (PB) IN SOIL

Baseline soil Pb was uniform across treatments. S1 exhibited negligible remediation. In contrast, amendment treatments produced marked reductions: at harvest, S2, S3 and S4 produced reductions of approximately 68%, 72%, and 78%, respectively. The more modest absolute Pb reductions compared to Cu and Zn reflect Pb's inherently lower soil mobility and its tendency to form stable phosphate, carbonate, and hydroxide precipitates (Kabata-Pendias, 2010). Nevertheless, the reductions achieved in S4 are notable and are attributed to chelating agents converting residual Pb into bioavailable forms amenable to phytoextraction, while biochar simultaneously adsorbs mobilized Pb^{2+} through electrostatic interactions and surface complexation, preventing re-mobilization (Beesley et al., 2011). S4 was significantly superior to all treatments at Day 90 and Harvest ($p < 0.05$), affirming the indispensability of the combined amendment approach for effective Pb remediation.

3.3. HEAVY METAL ACCUMULATION IN PLANT TISSUE

3.3.1 COPPER (CU) IN PLANT

Plant tissue Cu at Day 15 showed minor treatment differences, reflecting initial soil-plant equilibrium. S1 declined moderately at Harvest, consistent with slow passive phytoextraction. S4 exhibited the most dramatic decline — a 74% reduction from its Day 15 value. The sharp reduction in plant Cu under S4, despite concomitant soil Cu immobilization, implicates biochar-mediated phytoavailability suppression as the dominant mechanism: biochar's strong Cu adsorption affinity restricts root apoplastic and symplastic uptake, outcompeting chelate-enhanced solubilization under

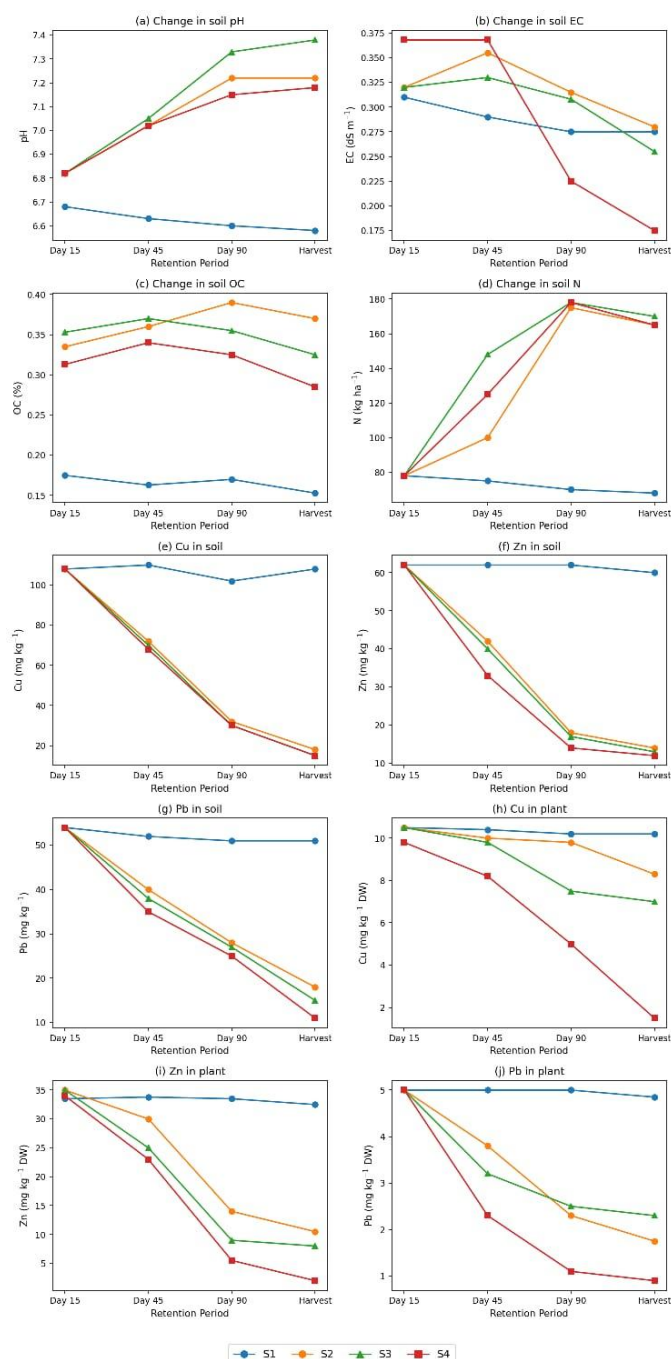


Fig.2.: Temporal variation in soil physicochemical properties and heavy metal concentrations in soil and plants under different treatments (T1-T4) during the retention period. Values presented as Mean \pm SD (n = 3).

the applied dosages (Namgay et al., 2010). This mechanistic interpretation is supported by the consistently inverse relationship between soil Cu reduction and plant Cu accumulation across treatments, concordant with findings reported by Ahmad et al. (2012).

3.3.2 ZINC (ZN) IN PLANT

Initial plant Zn was comparable across treatments. S1 maintained elevated plant Zn at Harvest while S4 declined at Harvest, representing a reduction exceeding 90% from Day 15. While Zn is an essential plant micronutrient, the

Harvest concentration under S4 (3.22 mg kg⁻¹ DW) approaches the deficiency threshold for many crops (15–20 mg kg⁻¹ DW; Marschner, 2012), indicating that prolonged S4 application at these dosages may require supplemental Zn nutrition in subsequent cropping cycles. The greater proportional reduction in plant Zn relative to Cu under S4 reflects Zn's higher native soil bioavailability and more active apoplastic transport, providing a larger pool amenable to biochar-mediated suppression.

3.3.3 LEAD (PB) IN PLANT

Plant tissue Pb concentrations were low across all treatments at Day 15, consistent with Pb's restricted phloem mobility and limited shoot translocation. S1 declined marginally at Harvest. Amendment treatments produced progressively greater reductions, an 80% reduction relative to S4 at Day 15. The food safety significance of plant Pb accumulation is considerable; the WHO/FAO Codex Alimentarius stipulates maximum permissible levels of 0.2 mg kg⁻¹ fresh weight for cereal grain. The trend across treatments unequivocally demonstrates that S4 is the most effective strategy for minimizing Pb entry into the food chain from contaminated soils. The reduction in plant Pb under S4 — despite chelate-mediated soil Pb mobilization — confirms that biochar's competitive adsorption of Pb²⁺ outpaces chelate-facilitated root uptake under the experimental conditions, consistent with findings reported by Beesley and Marmiroli (2011).

4. CONCLUSIONS

Across all ten measured parameters, the consistent treatment gradient S4 > S3 > S2 > S1 was maintained for soil fertility indicators (pH, OC, N) and for heavy metal reduction in both soil and plant matrices (Fig.2). S4 demonstrated the most comprehensive multifunctional performance: improving all soil fertility parameters while achieving the greatest reductions in soil Cu (87%), Zn (80%), and Pb (78%), and most effectively curtailing plant tissue metal accumulation. S3 closely rivalled S4 in nutrient enrichment — achieving the highest soil pH at Harvest (7.38) — but was consistently less effective in metal remediation, underscoring the indispensable contributions of chelating agents and biochar to immobilization and phytoextraction outcomes. S2 provided moderate improvements over S1 across all parameters, affirming the foundational importance of organic matter inputs, but was insufficient as a standalone remediation approach. S1 deteriorated systematically across all parameters, confirming the unsustainability of unmanaged contaminated soil without active intervention.

Mechanistically, the superiority of S4 is attributable to four synergistic processes: (i) organic manures raise soil pH, enhance CEC, and complex metals; (ii) inorganic amendment for nitrogen provides soluble N that stimulates plant growth and biomass-mediated phytoextraction; (iii) chelating agents desorb tightly-bound metals from exchange sites, increasing Phyto availability; and (iv) biochar provides a high-surface-area

sorbent that sequesters mobilized metals, prevents leaching, and buffers against secondary salinization — collectively achieving both stabilization and extraction outcomes within a single treatment cycle. These findings provide robust empirical support for the integrated organic–mineral amendment system as a practical, cost-effective, and scalable strategy for heavy metal remediation in contaminated agricultural soils. For practical remediation of Cu, Zn, and Pb co-contaminated agricultural soils, a combined amendment strategy is recommended. Future investigations should address long-term immobilisation stability across multiple growing seasons and field-scale performance under variable soil types and climatic conditions.

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