

Synergistic Heavy Metal Remediation through a Novel Black Soldier Fly–Brassica juncea Cascade in Mining-Impacted Soils

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Abstract

Heavy metal contamination represents a critical hazard to environmental and human health. This study assesses cadmium (Cd), lead (Pb), and zinc (Zn) remediation in phosphate-mining soils via entomoremediation with *Hermetia illucens* (Black Soldier Fly, BSF) larvae, phytoremediation with *Brassica juncea*, and to our knowledge, this is the first demonstration of their sequential integration. The soils exhibited moderate acidity (pH 5.4), low in organic matter (1.6%), and significantly enriched with Cd (12.4 mg kg^{-1}), Pb (285.7 mg kg^{-1}), and Zn (412.3 mg kg^{-1}).

BSF larvae immobilized Cd, Pb, and Zn by 63%, 57%, and 49% within 14 days through gut sequestration, chitin-associated binding, and Ca–P stabilization in alkaline frass. *Brassica juncea* achieved sustained uptake of Cd (50%), Pb (44%), and Zn (41%) over 30 days mediated by rhizosphere mobilization and vacuolar compartmentalization. Kinetic modeling revealed pseudo-second-order behavior for Cd and Pb under BSF, while Zn uptake within the BSF → BJ cascade shifted from pseudo-first- to pseudo-second-order with improved equilibrium capacity.

Bioaccumulation (BAF > 2.4) demonstrated strong larval sequestration of Cd and Pb, while translocation (TF > 1.0) showed substantial phytoextraction of Cd and Zn. Sequential treatment achieved enhanced removal efficiencies (Cd 78%, Pb 70%, Zn 65%) and markedly improved soil quality, including a 35% enhancement in the Soil Quality Index.

Mechanistic assays confirmed that BSF preconditioning decreased exchangeable fractions and enhanced BJ antioxidant responses, establishing a synergistic bio-integrated remediation pathway. This insect–plant synergy constitutes a novel eco-engineered remediation strategy combining rapid detoxification with durable stabilization, aligned with circular economy principles.

Environmental Implications

Our findings demonstrate, for the first time, that sequential integration of *Hermetia illucens* larvae and *Brassica juncea* cultivation provides rapid immobilization and sustained phytoextraction of Cd, Pb, and Zn in phosphate-mining soils. This insect–plant cascade decreased bioavailable metal fractions, enhanced soil quality by 35%, and provided complementary detoxification. The strategy is low-input, eco-engineered, and consistent with circular economy principles, providing a scalable alternative to conventional remediation technologies. Beyond risk mitigation, valorization of BSF residues offers opportunities for resource recovery, while the mechanistic insights into sequestration and phytoextraction pathways inform risk evaluation, remediation policy, and sustainable land management

Highlights

- First demonstration of an insect→plant cascade for multi-metal soil remediation
- BSF larvae rapidly immobilized Cd, Pb, Zn through gut sequestration and frass stabilization

- *Brassica juncea* improved phytoextraction via antioxidant and vacuolar mechanisms
- Sequential BSF→BJ system enhanced removal efficiencies and remediation kinetics
- Soil quality index increased by 35%, demonstrating synergistic eco-engineered restoration

1. Introduction

Heavy metal contamination of agricultural soils represents a pressing global environmental challenge, predominantly arising from mining and smelting operations, industrial effluents, sewage sludge application, and indiscriminate agrochemical inputs (Shakti and Pandey, 2024). Among these pollutants, cadmium (Cd), lead (Pb), and zinc (Zn) are of particular concern due to their persistence, non-biodegradability, and high potential for food-chain transfer (Edo et al., 2024). Cd is a nonessential element that induces pronounced phytotoxicity and severe health hazards even at trace levels. Pb is a cumulative neurotoxin linked to neurological, renal, and cardiovascular dysfunctions, with children particularly vulnerable. Zn, though an essential micronutrient, becomes phytotoxic at concentrations exceeding safe limits, impairing root growth, destabilizing enzyme activity, and perturbing indigenous microbial communities (Okereafor et al., 2020). Collectively, these metals degrade soil quality, compromise agricultural productivity, and adversely affect food security and ecosystem stability.

Conventional remediation approaches (such as soil washing, chemical stabilization, and electrokinetic treatment) have demonstrated efficacy in lowering heavy metal concentrations but are often costly, infrastructure-intensive, and prone to generating secondary environmental contamination (Wang et al., 2020). These limitations accentuate the urgent need for eco-efficient and scalable strategies that not only detoxify soils but also restore their fertility and promote sustainable agricultural practices.

Biological approaches provide promising remedial solutions. Phytoremediation, the application of green plants to extract or stabilize pollutants, has been widely recognized as an environmentally friendly and low-cost technology (Priya et al., 2023). Species of *Brassica*, particularly *Brassica juncea*, combine rapid growth, high biomass productivity, and significant metal sequestration capacity (Zeremski et al., 2021a). Their tolerance is governed by a variety of physiological and molecular mechanisms, including induction of antioxidant systems, synthesis of osmoprotectants, secretion of metabolic acids, and compartmentalization of metals into less active tissues (Mierek-Adamska et al., 2025). These attributes position *B. juncea* as a promising candidate for the remediation of heavy metal-contaminated soils, with additional potential as a viable feedstock for biofuel development or integration into intercropping systems that reduce risks to food crops.

More recently, entomoremediation is increasingly acknowledged as a complementary strategy. The larvae of the Black Soldier Fly (*Hermetia illucens*) are able to sequester heavy metals through ingestion, intracellular bioaccumulation, and partial immobilization while simultaneously biotransforming organic substrates into protein- and lipid-rich biomass (Yan et al., 2025). Their short life cycle, prolific biomass production, and beneficial soil-conditioning traits provide distinct advantages for rapid and cost-effective

remediation. Moreover, larvae-derived biomass can be valorized via thermochemical conversion into biochar, ensuring durable immobilization of metals and aligning the process with circular economy principles (Bataglia et al., 2025).

Despite the promise of both phytoremediation and entomoremediation, comparative studies are limited, and their synergistic application has yet to be systematically investigated. Conceptually, insect-mediated preconditioning of soils could decrease the exchangeable metal fraction, lower phytotoxic stress, and enhance root development and plant establishment, thereby improving the efficiency and long-term sustainability of phytoextraction. Such integration effectively offsets the inherent limitations of each method when applied independently: the slow kinetics of phytoremediation and the risk of secondary contamination from insect biomass.

The current study investigates this synergistic framework using soils from a phosphate mining region in Tunisia characterized by pronounced Cd, Pb, and Zn enrichment. In particular, the objectives were to (i) to conduct a comparative evaluation of remediation performance of *B. juncea* and *H. illucens* larvae individually, (ii) assess their sequential integration as a synergistic approach for accelerating metal removal and enhancing soil quality, and (iii) elucidate the mechanistic interactions underlying insect–plant synergy. To our knowledge, this study offers the first systematic demonstration of coupling entomoremediation with phytoremediation for multi-metal remediation, bridging entomology and phytotechnology through an advanced eco-engineering concept.

2. Materials and Methods

2.1. Site Description and Soil Sampling

Soils were sampled from a phosphate mining region near Métlaoui (34.320° N, 8.402° E; Gafsa Governorate, southwestern Tunisia), which has been subjected to intensive phosphate extraction and processing for several decades. The study area lies within an arid to semi-arid climatic zone, with mean annual precipitation of 100–120 mm and temperatures extending from 10°C in winter to over 33°C in summer. Previous investigations have documented considerable enrichment of cadmium (Cd), lead (Pb), and zinc (Zn) in surrounding agricultural soils, largely attributable to atmospheric dust deposition, discharged effluents, and rock-weathering processes. In the present study, Cd, Pb, and Zn were selected as representative pollutants owing to their high mobility, abundance in phosphate-industry waste streams, and well-recognized phytotoxic and ecotoxic potential. Additional elements associated with phosphate mining, notably uranium, fluoride, and naturally occurring radionuclides, were not addressed in the present work, as baseline concentrations reported in the Gafsa basin (Hamed et al., 2025; Machraoui et al., 2024) typically remain below international toxicity limits for agricultural soils.

Topsoil (0–20 cm) was collected using a stainless-steel auger at five georeferenced points (34.322° N, 8.395° E; 34.318° N, 8.407° E; 34.325° N, 8.398° E; 34.319° N, 8.403° E; 34.321° N, 8.400° E) within the contaminated zone, according to a randomized grid pattern to reflect spatial heterogeneity. Samples

were composited to obtain a representative bulk sample (~ 20 kg). A reference soil was sampled from a background site located > 10 km away, developed on the same Eocene carbonate formations but subject to minimal industrial impact. Both polluted and reference soils were air-dried, homogenized, and sieved to 2 mm before subsequent analyses. Key physicochemical parameters were evaluated, including pH (1:2.5 soil–water suspension), electrical conductivity (EC), organic matter content (loss on ignition at 550°C), particle size distribution (hydrometer method), and cation exchange capacity (CEC). According to USDA criteria, the contaminated soil was categorized as sandy loam (63% sand, 24% silt, 13% clay), while the reference soil was loam (44% sand, 36% silt, 20% clay). X-ray diffraction (XRD) analysis revealed that both soils were dominated by quartz and calcite, with minor quantities of feldspars and clays, confirming their mineralogical similarity. Baseline Cd, Pb, and Zn concentrations were quantified employing inductively coupled plasma mass spectrometry (ICP–MS; Agilent 7700, USA) after acid digestion.

2.2. Experimental Design

Laboratory pot experiments were performed in a controlled growth chamber maintained at $25 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity (RH), and a 16 h light/8 h dark photoperiod. Each plastic pot (5 L capacity) was packed with 4 kg of homogenized contaminated soil. Soil water-holding capacity (WHC) was first quantified gravimetrically by saturating subsamples, allowing free drainage for 24 h, and subsequently measuring retained moisture. Soil moisture in each pot was adjusted to 60% of WHC and maintained throughout the experiment by routine gravimetric control: pots were weighed every two days and deionized water was added as necessary to restore the target weight.

The pot dimensions was selected to provide sufficient rooting volume, thereby limiting potential root restriction of *Brassica juncea* while ensuring manageable substrate volumes under controlled conditions, and is consistent with validated phytoremediation and entomoremediation studies (Zeremski et al., 2021b). Nevertheless, root expansion in 5 L pots remains constrained relative to the rooting potential observed under field soil conditions, which may affect biomass production and metal uptake capacity; this limitation is recognized when extrapolating findings to field-scale applications.

A total of four treatments were established in the experimental design, as follows

1. **Phytoremediation (BJ):** cultivation of *Brassica juncea* seedlings.
2. **Entomoremediation (BSF):** inoculation with *Hermetia illucens* larvae.
3. **Synergistic treatment (BSF → BJ):** sequential application of BSF larvae followed by *Brassica juncea* cultivation, integrating entomoremediation with phytoremediation.
4. **Control:** untreated polluted soil.

Each treatment was conducted with five biological replicates to improve statistical robustness and adequately capture natural variability. The experimental durations (30 d for plant growth and 14 d for larval exposure) were selected based on both literature precedent (H. et al., 2002) and preliminary trials in

our laboratory, which confirmed that these time frames captured the period of maximal metal uptake by plants and ingestion/immobilization by larvae without inducing senescence or pupation.

2.3. Phytoremediation Procedure

Pre-germinated *Brassica juncea* seedlings (15 days old) were transplanted into the experimental pots at a uniform density of three plants per pot. This planting density was chosen based on prior phytoremediation investigations demonstrating optimal root–shoot development and metal uptake efficiency under pot-scale conditions (H. et al., 2002; Merakli et al., 2025), and was corroborated by preliminary trials in our laboratory. Plants were cultivated for 30 days, a period sufficient to capture early vegetative growth and maximal uptake of Cd, Pb, and Zn without inducing nutrient depletion or senescence. At harvest, all three seedlings per pot were collected, and their biomass was pooled and their biomass was pooled to constitute a composite sample. Shoots and roots were carefully separated, rinsed with deionized water, immersed in a mild 5 mM EDTA solution to remove surface-adsorbed metals, then rinsed again with deionized water. Plant samples were oven-dried at 60°C to constant weight and finely homogenized for subsequent metal concentration analysis.

2.4. Entomoremediation Procedure

Fifth-instar *Hermetia illucens* larvae were introduced into the polluted soil at a density of 100 larvae per kilogram of soil, in accordance with values reported in published entomoremediation studies (Arnone et al., 2022; Attiogbe et al., 2019) and further validated through preliminary survival and biomass assessments in our laboratory. Larvae were allowed to bioprocess the substrate for 14 days, coinciding with the final instar feeding phase and representing the stage of maximal ingestion and metal assimilation. During this period, larval survival, initial and final body weights (fresh mass) were measured to evaluate biomass accumulation. To ensure methodological consistency, water content was determined from parallel subsamples by measuring fresh-to-dry mass ratios, and metal concentrations were expressed on a dry-mass basis. At the end of exposure, larvae were carefully harvested, thoroughly rinsed with deionized water followed by a mild EDTA wash to remove externally bound metals, oven-dried at 60°C to constant weight, and homogenized for subsequent ICP–MS analysis (Zulkifli et al., 2022).

2.5. Synergistic Treatment (BSF → BJ)

For the sequential treatment, fifth-instar *Hermetia illucens* larvae were first introduced into the polluted soil (100 larvae kg⁻¹ soil) and permitted to process the substrate for 14 days, as detailed above. This sequence was adopted based on mechanistic rationale and preliminary tests. In our pre-trials, the reverse sequence (*BJ* → *BSF*) resulted in significantly lower plant survival (seedling mortality > 40%) and impaired remediation efficiency, highlighting severe initial phytotoxicity in untreated soil. Previous studies also reported that BSF preconditioning decreases metal bioavailability (Feng et al., 2025; Hu et al., 2023). After larval removal, the preconditioned soil was transplanted with three pre-germinated *Brassica juncea* seedlings per pot. Plants were cultivated for 30 days under the same controlled conditions, in line with standard growth cycles employed in phytoremediation trials. At harvest, shoots and roots were separated, subsequently rinsed with deionized water and 5 mM EDTA, followed by a final

rinse with deionized water. Plant tissues were oven-dried at 60°C to constant weight and ground for subsequent metal analysis, previously detailed.

2.6. Analytical Methods

Soil, plant, and larval samples were subjected to closed-vessel microwave-assisted acid digestion (Mars 6, CEM, USA) with concentrated $\text{HNO}_3\text{:H}_2\text{O}_2$ (4:1, v/v), following U.S. EPA Method 3052. This protocol was selected due to its safety, efficiency, and wide recognition in trace metal determination. Metal concentrations (Cd, Pb, Zn) were determined utilizing inductively coupled plasma mass spectrometry (ICP–MS; Agilent 7700, USA).

Quality assurance and quality control (QA/QC) procedures involved the use of procedural blanks, duplicate analyses (10% of total samples), and certified reference materials (CRM 2711a, Montana II Soil). Calibration was carried out with multi-element standard solutions (0–1000 $\mu\text{g L}^{-1}$), resulting in correlation coefficients (R^2) greater than 0.999. Method detection limits (MDL) were 0.01 mg kg^{-1} for Cd, 0.05 mg kg^{-1} for Pb, and 0.02 mg kg^{-1} for Zn. Spike recovery rates spanned from 92% to 107%, and CRM recoveries were within $\pm 8\%$ of certified values, verifying measurement accuracy.

Remediation performance was evaluated using the following indices:

1. Removal Efficiency (RE, %):

$$RE = \frac{C_0 - C_t}{C_0} \times 100$$

C_0 and C_t are the initial and final soil metal concentrations ($\text{mg}\cdot\text{kg}^{-1}$).

2. Bioaccumulation Factor (BAF):

$$BFA = \frac{\text{Metal concentration in organism}}{\text{Initial soil concentration}}$$

3. Translocation Factor (TF, for plants):

$$TF = \frac{\text{Metal concentration in shoot}}{\text{metal concentration in root}}$$

4. Soil Quality Index (SQI): A composite index integrating residual metal concentrations, soil pH, and microbial activity to evaluate post-treatment soil health.

$$SQI = \frac{\sum (W_i - P_i)}{\sum W_i}$$

where P_i indicates normalized post-treatment parameters and W_i corresponds to weighting factors. The parameters comprised: (i) residual Cd, Pb, and Zn concentrations; (ii) soil pH; and (iii) basal microbial respiration.

Basal microbial respiration was assessed through incubation of 50 g of soil at 25°C for 10 days at 60% WHC in the dark to prevent photosynthetic interference. Incubation vessels were sealed but contained gas-permeable membranes to avoid anaerobiosis, and CO_2 was trapped in 0.05 M NaOH. At the end of incubation, evolved $\text{CO}_2\text{-C}$ was determined by back-titration with standardized HCl ($\text{mg CO}_2\text{-C kg}^{-1}$ soil d^{-1}) (Rice et al., 2015)

Weighting factors (W_i) were assigned equally among parameters, in agreement with earlier SQI formulations applied in contaminated soil research (Chapman et al., 2002; Rezaee et al., 2020). To assess robustness, a sensitivity analysis was also performed using alternative weightings (e.g., metals weighted double relative to pH and respiration), which produced consistent trends, indicating that SQI outcomes were not strongly dependent on the weighting scheme.

2.7. Mechanistic Analytical Procedures

Mechanistic responses during BSF processing and BJ cultivation were assessed using targeted biochemical and subcellular assays (**Table S1**). For BSF, core indicators encompassed larval ingestion and metal partitioning, subcellular fractionation, metallothionein induction, and antioxidant enzyme activities (GST, SOD, CAT), which were statistically evaluated as explanatory variables for metal accumulation patterns. Frass characterization (pH, Ca/P enrichment, exchangeable fractions, TCLP leachability) was conducted to evaluate stabilization of metals in the residual substrate.

For BJ, focus was directed toward antioxidant enzymes (SOD, CAT, POD) and osmoprotectants (proline, soluble sugars), which served to explain plant tolerance responses, while root exudate profiling (organic acids by HPLC) and subcellular sequestration analyses were conducted to assess uptake and immobilization mechanisms. All biochemical assays were performed with five biological replicates per treatment, through pooled biomass per pot (plants) or per soil replicate (larvae) to maintain consistency across experimental units.

These mechanistic parameters were chosen not only to characterize physiological responses but also to test the central hypothesis that BSF-mediated immobilization reduces metal bioavailability and oxidative stress, thus improving *B. juncea* performance in the subsequent remediation phase.

2.7. Statistical Analysis

All treatments carried out using five biological replicates, and results are expressed as mean $\pm$ standard deviation. Prior to analysis, data were examined for normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test). Differences among treatments were assessed by one-way analysis of variance (ANOVA) followed by Tukey’s HSD test at a significance level of $\alpha = 0.05$, implemented in SPSS v26. Effect sizes were calculated and expressed as eta squared (η^2) for ANOVA and Cohen’s d for pairwise

comparisons to complement significance testing and provide a measure of treatment impact. Although replication was restricted to five pots per treatment under controlled chamber conditions, this design is consistent with preliminary remediation trials and ensured sufficient statistical power for detecting treatment effects.

4. Results and Discussion

3.1. Baseline Soil Characteristics

The contaminated soil displayed markedly adverse physicochemical properties compared with the reference soil (Table 1). It exhibited a moderately acidic pH (pH 5.4 ± 0.2), with elevated electrical conductivity (2.1 ± 0.3 dS m⁻¹), low organic matter content ($1.6 \pm 0.2\%$), and reduced buffering capacity (CEC: 11.8 ± 0.7 cmol⁺ kg⁻¹). Such conditions are known to improve the solubility and translocation of trace metals, as acidic pH increases Cd²⁺ availability and low CEC decrease the soil’s capacity to bind cations. The reference soil, by contrast, was near-neutral (pH 6.8 ± 0.1), less saline, richer in organic matter ($2.9 \pm 0.3\%$), and exhibited higher CEC (18.4 ± 0.9 cmol⁺ kg⁻¹), which collectively promote stronger metal sorption and reduce bioavailability.

Table 1

Physicochemical properties and baseline heavy metal concentrations of contaminated soil compared with reference soil and WHO/FAO (2015) permissible thresholds. Values are mean $\pm$ SD (n = 3).

Parameter	Contaminated Soil	Reference Soil	WHO/FAO (2015) Soil Guidelines
pH	5.4 ± 0.2 (acidic)	$\sim 6.8 \pm 0.1$ (near neutral)	– (No specific soil guideline)
Electrical Conductivity	2.1 ± 0.3 dS m ⁻¹	0.7 ± 0.1 dS m ⁻¹	–
Organic Matter	$1.6 \pm 0.2\%$	$2.9 \pm 0.3\%$	–
Cation Exchange Capacity	11.8 ± 0.7 cmol ⁺ kg ⁻¹	–	–
Cadmium (Cd)	12.4 ± 0.6 mg kg ⁻¹	0.6 ± 0.1 mg kg ⁻¹	$\sim 3\text{--}4$ mg kg ⁻¹ (maximum permissible)
Lead (Pb)	285.7 ± 10.2 mg kg ⁻¹	22.4 ± 2.3 mg kg ⁻¹	~ 85 mg kg ⁻¹ (maximum permissible)
Zinc (Zn)	412.3 ± 15.7 mg kg ⁻¹	58.2 ± 4.6 mg kg ⁻¹	– (No specific WHO/FAO soil guideline)

Caption.
Baseline physicochemical properties and heavy-metal concentrations of the studied soils compared with WHO/FAO (2015) permissible thresholds. Contaminated soil from the phosphate mining area showed markedly acidic pH, low organic matter, and Cd and Pb levels far exceeding guideline values, confirming the severity of pollution and the need for remediation. Values represent mean $\pm$ SD (n = 3).

ICP–MS analysis revealed pronounced enrichment of Cd ($12.4 \pm 0.6 \text{ mg kg}^{-1}$), Pb ($285.7 \pm 10.2 \text{ mg kg}^{-1}$), and Zn ($412.3 \pm 15.7 \text{ mg kg}^{-1}$) in polluted soil, all markedly higher ($p < 0.05$) than background concentrations measured in the reference soil (Cd 0.8 ± 0.1 , Pb 22.3 ± 2.1 , Zn $61.4 \pm 4.6 \text{ mg kg}^{-1}$). These levels exceeded FAO/WHO (2019) guideline values and regional thresholds established for North African agricultural soils (Guo et al., 2021; Rahman and Singh, 2019). The disparities emphasize marked anthropogenic loading consistent with long-term phosphate mining activities.

Although current investigation focused on Cd, Pb, and Zn owing to their elevated mobility and phytotoxicity, other mining-derived contaminants, notably uranium, fluoride, and radionuclides, have been documented at low to moderate levels in the Gafsa basin (Hamed et al., 2025). The decision to exclude them stems from both their sub-threshold concentrations on an international scale and the targeted scope of this investigation.

Collectively, these baseline findings identify the contaminated soil as a representative model of metal-enriched, phosphate-industry-impacted soils and provide a robust reference point for evaluating the performance of the remediation approaches.

3.2. Comparative Performance of Single Treatments

BSF larvae (*Hermetia illucens*) yielded rapid attenuation of soil metals, lowering Cd, Pb, and Zn concentrations by 63%, 57%, and 49%, respectively, within 14 days, whereas *Brassica juncea* resulted in 50%, 44%, and 41% removal after 30 days (Table 2). Removal efficiencies were determined relative to initial soil concentrations, and in the case of BSF, attenuation mainly reflects redistribution into larval tissues and frass rather than effective export from the system. ANOVA revealed markedly higher early decreases under BSF treatment ($p < 0.01$), with large effect sizes ($\eta^2 = 0.41\text{--}0.56$; Cohen’s $d > 1.2$) reflecting considerable efficacy of the applied treatment.

Table 2
Removal efficiencies of Cd, Pb, and Zn across treatments

Treatment	Cd Removal (%)	Pb Removal (%)	Zn Removal (%)	Biomass Increase (%) vs. Control
Control	3 ± 1.1	2 ± 0.8	4 ± 1.0	–
BSF	63 ± 2.7	57 ± 3.1	49 ± 2.4	+ 12
BJ	50 ± 3.5	44 ± 2.9	41 ± 3.2	+ 20
BSF → BJ	78 ± 3.1	70 ± 2.6	65 ± 3.3	+ 60
Caption:				
Removal efficiencies of Cd, Pb, and Zn under individual (BSF, BJ) and synergistic (BSF → BJ) treatments. Data are mean ± SD (n = 3). Significant differences among treatments (ANOVA, Tukey’s HSD, $p < 0.01$) indicate that synergy achieved superior performance across all metals and substantially improved plant biomass relative to untreated controls.				

Mechanistically, BSF removal arises from gut sequestration and adsorption of metals to chitinous cuticle, subcellular immobilization via metallothioneins, and partial stabilization in frass through Ca–P precipitation and pH elevation (Verardi et al., 2025). In contrast, *B. juncea* achieved removal via gradual yet persistent uptake, mediated by rhizosphere mobilization via root exudates, transporter-mediated uptake across root membranes, and intracellular detoxification through vacuolar sequestration and chelation with organic acids and phytochelatins (Hussain et al., 2024).

The temporal mismatch between treatments (14 days for BSF vs. 30 days for *B. juncea*) highlight intrinsic biological constraints, as larval ingestion is completed within a short developmental window while plants require extended growth to accumulate biomass (Kumar and Chaubey, 2025).

Benchmarking against previous reports indicates that our removal efficiencies for BSF (49–63%) are comparable to those documented by (Tirtawijaya et al., 2024) and (Rehman et al., 2023) for multi-metal substrates, while *B. juncea* removals (41–50%) align closely with ranges observed in controlled pot studies (Singh et al., 2024). Thus, the present findings confirm that both BSF and BJ are individually effective, but exhibit distinct mechanisms of action and differing temporal kinetics, establishing a strong foundation for designing integrated BSF–phytoremediation cascades

3.3. Kinetics of Metal Removal

The temporal dynamics of Cd, Pb, and Zn removal demonstrated distinct kinetic profiles across treatments (**Fig. 2**). In BSF systems, Cd and Pb removal exhibited pseudo-second-order (PSO) behavior with high goodness of fit ($R^2 > 0.96$, $RMSE < 0.05$), indicative of chemisorptive binding to amine, carboxyl, and thiol groups on larval cuticle and frass residues. Zn, by contrast, fit more appropriately to pseudo-first-order (PFO) kinetics ($R^2 \approx 0.91$), suggesting weaker surface interactions and partial immobilization. For *B. juncea*, uptake proceeded more slowly and was best described by sigmoidal PFO trajectories, reflecting an initial lag phase during root establishment, accelerated accumulation throughout vegetative development, and stabilization near day 30 (Tapia et al., 2024).

For BSF, PSO rate constants (k_2) were $0.021 \text{ g mg}^{-1} \text{ d}^{-1}$ for Cd and $0.017 \text{ g mg}^{-1} \text{ d}^{-1}$ for Pb, with equilibrium capacities (q_{∞}) of 9.4 and 212 mg kg^{-1} , respectively, consistent with previously reported values for insect-based attenuation in metal-contaminated substrates (Siddiqui et al., 2024). Zn exhibited a lower PFO rate constant ($k_1 = 0.062 \text{ d}^{-1}$), aligning with slower mobilization. For *B. juncea*, PFO model fitting resulted in k_1 values of $0.035\text{--}0.048 \text{ d}^{-1}$ with q_{∞} ranging from 6.8 to 185 mg kg^{-1} , falling within the range documented in prior phytoremediation trials under controlled conditions (Zhang et al., 2022).

Notably, in the sequential BSF → BJ treatment, Zn removal transitioned from PFO to PSO kinetics ($R^2 > 0.98$, $RMSE < 0.03$), with higher q_{∞} (206 mg kg^{-1}) and faster kinetics ($k_2 = 0.019 \text{ g mg}^{-1} \text{ d}^{-1}$). This shift suggests that larval preconditioning attenuated exchangeable metal fractions and increased soil pH and Ca–P content, thereby enhancing cooperative Zn uptake and stabilizing residual pools. Such context-dependent transitions offer direct mechanistic evidence that BSF processing improves the efficacy of subsequent plant extraction.

Metal-specific patterns were also apparent: Cd was removed most rapidly attributable to its high mobility and weak soil binding (Nie et al., 2024), Pb removal was enhanced under BSF and synergistic conditions owing to strong complexation with organic ligands in frass, while Zn removal proceeded more slower, reflecting both physiological regulation in plants and weaker soil sorption interactions. Although our findings provide strong mechanistic support for coupling BSF and BJ, it must be emphasized that these kinetics were derived from controlled pot experiments. Field soils, subject to greater heterogeneity, variable moisture, and intricate microbial interactions, may demonstrate altered rates and equilibrium outcomes.

Overall, these results indicate that remediation kinetics are simultaneously influenced by both metal-specific chemical attributes and organismal physiological mechanisms. The BSF–BJ integration thus couples rapid detoxification with sustained phytoextraction, providing a mechanistically robust and potentially scalable remediation approach.

3.4. Bioaccumulation and Translocation Patterns

BSF larvae showed high bioaccumulation factors (**Fig. 2**) (BAF; mean $\pm$ SD, $n = 5$) for Cd (2.8 ± 0.3) and Pb (2.4 ± 0.2), demonstrating their strong ability to sequester toxic metals relative to initial soil concentrations on a dry-mass basis. Zn accumulation was comparatively lower (BAF = 1.2 ± 0.1), pointing to its role as an essential micronutrient subject to tighter homeostatic regulation (Sharma et al., 2025; Usmani et al., 2025). These trends were corroborated by subcellular fractionation and metallothionein assays, which revealed that $> 45\%$ of Cd and Pb were localized in the insoluble and vacuolar fractions, coinciding with elevated metallothionein activity (Sharma et al., 2016), whereas Zn remained comparatively widespread across soluble pools. These results imply that BSF sequestration is mediated by both biochemical binding and compartmentalization (Haanstra et al., 2016).

Brassica juncea exhibited moderate BAF values (Cd: 1.6 ± 0.2 ; Pb: 1.3 ± 0.1 ; Zn: 1.5 ± 0.2), yet showed efficient root-to-shoot translocation of Cd (TF = 1.2 ± 0.1) and Zn (TF = 1.1 ± 0.1). Pb exhibited reduced translocation (TF = 0.6 ± 0.05), reflected its confinement within cell wall binding and vacuolar sequestration identified through subcellular fractionation (Wang et al., 2021). Comparison with previous phytoremediation studies (Mahmoud et al., 2021; Thalassinou et al., 2024) indicates that the TF values obtained here fall within documented ranges, while BAFs were slightly higher for Cd, consistent with the high initial contamination levels in the Gafsa basin soils.

Compared to earlier entomoremediation reports, the BAFs obtained for BSF in this study (2.4–2.8) fall toward the upper range of reported values (typically < 2.0 for Pb; (Purschke et al., 2017)), suggesting that larval sequestration efficiency is improved under phosphate mining–derived pollution. Importantly, while BJ's TF > 1 for Cd and Zn confirms efficient phytoextraction, it further suggests the potential risks of metal transfer into the aboveground biomass if not harvested, underscoring the requirement for controlled management in field applications.

Together, these complementary BAF–TF profiles provide evidence for a complementary dual-mode remediation strategy: BSF supports rapid sequestration and contributes to partial immobilization, while BJ enables sustained phytoextraction and stabilization.

3.5. Synergistic Treatment Outcomes

The sequential BSF → BJ treatment attained significantly improved remediation efficiencies than either approach applied independently ($p < 0.01$). The Soil Quality Index (SQI) (**Fig. 3**) increased from 0.42 ± 0.03 in untreated controls to 0.57 ± 0.04 following BSF → BJ, representing a 35% relative improvement ($n = 5$). The observed enhancement was driven by multiple soil property changes: Soil alkalinity rose, with pH increasing from 5.4 ± 0.2 to 6.2 ± 0.1 , organic matter content increased by 18% ($1.6 \pm 0.2\% \rightarrow 1.9 \pm 0.2\%$), and basal microbial respiration increased significantly by 27% ($92 \pm 5 \rightarrow 117 \pm 6 \text{ mg CO}_2\text{-C kg}^{-1} \text{ d}^{-1}$). All measured parameters demonstrated statistically significant differences compared with single treatments ($p < 0.05$).

Mechanistically, the improvement in soil quality can be attributed to synergistic contributions from both organisms. BSF frass, enriched in Ca and P, facilitated partial neutralization of soil acidity and stabilization of exchangeable metal fractions, while residual organic inputs supplied substrates that stimulated microbial activity. The subsequent cultivation of *B. juncea* further enhanced organic matter via root biomass addition and exudation of organic acids, which facilitated nutrient cycling and improved soil structure (Ma et al., 2022). Together, these processes not only decreased contaminant bioavailability but also restored physicochemical balance and biological function.

When benchmarked against other remediation approaches, the 35% SQI improvement recorded in this study is comparable to biochar–plant systems (30–40%; (Xu et al., 2024)) and exceeds values achieved by single phytoremediation approaches under similar contamination levels ($< 25\%$; (Prasad et al., 2022)). This highlights the synergistic advantage of combining insect- and plant-based bioremediation.

The demonstrated complementarity between BSF-mediated detoxification and BJ-driven stabilization offers a mechanistic foundation for scaling this strategy within integrated soil management systems.

3.6. Mechanistic Insights

During the BSF phase, larvae bioaccumulated Cd, Pb, and Zn in tissues (20–28%), excreted them into frass (54–62%), and retained the remainder in the residual substrate (15–27%) ($n = 5$, mean $\pm$ SD, $p < 0.05$, **Fig. 4**). Subcellular fractionation (**Fig. 5**) indicated vacuolar and insoluble sequestration (44–55%), supported by metallothionein induction (+ 39%) and upregulation of antioxidant enzymes (+ 38–41%). Frass exhibited alkaline pH (7.9) and was substantially enriched in Ca and P, was strongly correlated with decreases in exchangeable metal fractions ($R^2 = 0.79$, $p < 0.01$) and lower TCLP leachability (–23–42%) (**Fig. 6**). These results establish a mechanistic link between alkaline, Ca–P-enriched chemistry of frass and its capacity to immobilize and stabilize metals in soil. However, since frass retains substantial metal loads, safe valorization approaches such as thermal co-processing, incorporation into construction

materials, or restricted application in non-food biomass systems are crucial for avoiding subsequent contamination pathways.

In the subsequent BJ phase, plants grown in BSF-preconditioned soil exhibited strengthened stress resistance mechanisms and an expanded uptake potential. Antioxidant enzymes (SOD, CAT, POD) rose by 49–71% ($p < 0.01$), osmoprotectants (proline, soluble sugars) increased by 40–44%, and organic acid exudation (citric, malic, oxalic) augmented by 42–62% (**Fig. 7**). These responses correlated with enhanced Cd and Zn uptake ($R^2 = 0.72\text{--}0.76$, $p < 0.05$), while subcellular analysis revealed that 49–63% of absorbed metals were localized to vacuoles and cell walls, decreasing cytoplasmic toxicity. Regression analyses additionally highlighted frass Ca/P enrichment and BJ antioxidant activity as the strongest explanatory parameters governing final removal efficiencies (combined $R^2 = 0.87$, $p < 0.001$).

Taken together, the sequential BSF → BJ system establishes a remediation cascade coupling larval-mediated detoxification with plant resilience and metal uptake capacity. Benchmarking against the existing literature underscores its novelty: antioxidant enzyme induction in BJ under BSF pretreatment (+48–71%) yielded improvements beyond the limits of single-species remediation systems (+20–35%), while frass-mediated TCLP reductions ($\leq 42\%$) proved more efficient compost-based stabilization strategies (15–25%; Xu et al., 2018). These comparative advantages highlight the BSF–BJ approach as a robust and scalable pathway for polluted soil restoration.

3.7. Environmental and Engineering Implications

The integrated BSF–*Brassica juncea* system proves the feasibility of a scalable, sustainable, and eco-efficient remediation pathway that couples rapid detoxification with sustainable soil restoration. Based on a 20 cm soil depth and a bulk density of 1.3 g cm^{-3} , the estimated removal capacity attains ~78 kg Cd, 70 kg Pb, and 65 kg Zn per hectare per cycle. These values are provided as preliminary estimates derived under controlled experimental conditions; in field soils, heterogeneity, hydrological variability, and competing ions may attenuate removal efficiencies, underscoring the importance of pilot-scale confirmation.

From an environmental perspective, the sequential remediation strategy mitigates acute contamination risks by immobilizing exchangeable Cd and Pb fractions during the BSF phase, while the subsequent *B. juncea* phase provides longer-term phytoextraction and stabilization of Cd and Zn. This dual action not only limits food-chain transfer but also facilitates the recovery of soil functions., including pH neutralization, organic matter enrichment, and microbial respiration improvement.

Valorization approaches present additional sustainability potential, although their implementation is still in an exploratory phase. Metal-laden BSF larvae could be thermochemically converted into biochar, facilitating contaminant immobilization and providing soil amendment benefits, while *B. juncea* biomass offers potential to be valorized through bioenergy production with controlled recycling of metal-rich ash. However, these strategies should be substantiated through pilot-scale trials, and potential risks including

the volatilization of metals during pyrolysis and the unsafe disposal of metal-rich ash require both technical safeguards and regulatory oversight.

Contrasted with existing remediation paradigms (Table 3), the insect–plant approach provides notable advantages. Soil washing and chemical stabilization typically remove or immobilize 50–70% of heavy metals but at high financial costs (100–500 USD per ton of soil treated) (Tsang et al., 2014) and with risks of secondary waste generation (Gusiatin et al., 2020). In contrast, BSF–BJ integration is low-input, multifunctional, and fully compatible with circular economy principles.

Table 3
Comparison of insect–plant synergy with conventional remediation methods

Method and reference	Efficiency	Cost	Scalability	Environmental Impact	By-products
Soil washing (Klik et al., 2020)	High (70–90%)	High	Low	Generates chemical waste	Hazardous sludges
Chemical stabilization(Murtaza et al., 2014)	Moderate (40–70%)	Medium	Medium	Alters soil chemistry	Secondary contamination
Phytoremediation (BJ)	Moderate (40–55%)	Low	High	Eco-friendly	Biomass disposal issue
Entomoremediation (BSF)	Moderate (50–60%)	Low	High	Eco-friendly	Metal-laden larvae
Synergy (BSF → BJ)	High (65–78%)	Low	High	Eco-friendly, soil restoration	Valorized biomass (biochar, bioenergy)
Caption:					
Comparative analysis of insect–plant synergistic remediation versus conventional strategies. The synergy demonstrated competitive efficiencies comparable to soil washing but at significantly lower cost, higher scalability, and minimal environmental trade-offs, highlighting its novelty and suitability for field application.					

To our knowledge, no previous study has reported a sequential BSF–plant cascade for heavy-metal remediation, making this approach an innovative eco-engineering solution. Nonetheless, the claim of novelty should be interpreted cautiously, as broader entomology–plant interactions in contaminated soils are just emerging as areas of study.

4. Conclusion

This study demonstrates, for the first time, the effectiveness of a sequential insect–plant remediation cascade for multi-metal-polluted soils from phosphate-mining region. Black Soldier Fly (*Hermetia illucens*) larvae offered rapid detoxification via gut sequestration, chitin binding, and Ca–P stabilization

in alkaline frass, while *Brassica juncea* sustained phytoextraction through rhizosphere mobilization, vacuolar sequestration, and antioxidant activation. The integrated BSF → BJ system significantly outperformed individual treatments, reaching up to 78% Cd, 70% Pb, and 65% Zn removal, alongside a 35% enhancement in the Soil Quality Index. Kinetic modeling and bioaccumulation–translocation analyses provided evidence for complementary mechanisms that coupled rapid immobilization with long-term stabilization.

In addition to remediation efficiencies, the approach introduces a novel aspect of eco-engineered framework consistent with circular economy principles, where metal-laden larvae and plant biomass can be valorized into biochar or bioenergy. While further validation under field-scale conditions is warranted, these results establish a scalable and sustainable strategy for restoring heavy-metal-impacted agroecosystems. By integrating entomoremediation and phytoremediation into a single biologically driven cascade, This study enhances mechanistic insights while simultaneously demonstrating the practical feasibility of green remediation technologies, thereby paving the way for innovative, multifunctional soil management strategies.

Declarations

Author Contribution

Afef Sai: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and editing. Mohamed Ali Borgi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review and editing. Leila Khalfa: Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – review and editing.

References

1. Arnone, S., De Mei, M., Petrazzuolo, F., Musmeci, S., Tonelli, L., Salvicchi, A., Defilippo, F., Curatolo, M., Bonilauri, P., 2022. Black soldier fly (*Hermetia illucens* L.) as a high-potential agent for bioconversion of municipal primary sewage sludge. *Environ Sci Pollut Res* 29, 64886–64901.
<https://doi.org/10.1007/s11356-022-20250-w>
2. Attiogbe, F.K., Ayim, N.Y.K., Martey, J., 2019. Effectiveness of black soldier fly larvae in composting mercury contaminated organic waste. *Scientific African* 6, e00205.
<https://doi.org/10.1016/j.sciaf.2019.e00205>
3. Bataglia, L., Conversano, A., Di Bona, D., Sogni, D., Voccia, D., Mazzoni, E., Lamastra, L., 2025. Recent Developments, Challenges, and Environmental Benefits of Using *Hermetia illucens* for Bioenergy Production Within a Circular Economy Approach. *Energies* 18, 2826.
<https://doi.org/10.3390/en18112826>

4. Chapman, P.M., McDonald, B.G., Lawrence, G.S., 2002. Weight-of-Evidence Issues and Frameworks for Sediment Quality (And Other) Assessments. *Human and Ecological Risk Assessment: An International Journal* 8, 1489–1515. <https://doi.org/10.1080/20028091057457>
5. Edo, G.I., Samuel, P.O., Oloni, G.O., Ezekiel, G.O., Ikpekoru, V.O., Obasohan, P., Ongulu, J., Otunuya, C.F., Opiti, A.R., Ajakaye, R.S., Essaghah, A.E.A., Agbo, J.J., 2024. Environmental persistence, bioaccumulation, and ecotoxicology of heavy metals. *Chemistry and Ecology* 40, 322–349. <https://doi.org/10.1080/02757540.2024.2306839>
6. Feng, K.-C., Muneeb Ur Rehman, M., Huang, J.-C., 2025. Bioaccumulation and biotransformation of hexavalent chromium in black soldier fly (*Hermetia illucens*) in the antagonism of selenate. *Environmental Pollution* 372, 126054. <https://doi.org/10.1016/j.envpol.2025.126054>
7. Guo, Z., Boeing, W.J., Xu, Y., Borgomeo, E., Mason, S.A., Zhu, Y.-G., 2021. Global meta-analysis of microplastic contamination in reservoirs with a novel framework. *Water Research* 207, 117828. <https://doi.org/10.1016/j.watres.2021.117828>
8. Gusiatin, Z.M., Kulikowska, D., Klik, B., 2020. New-Generation Washing Agents in Remediation of Metal-Polluted Soils and Methods for Washing Effluent Treatment: A Review. *IJERPH* 17, 6220. <https://doi.org/10.3390/ijerph17176220>
9. H., A., Z., Z., M., A., A., K., 2002. Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils* 35, 231–237. <https://doi.org/10.1007/s00374-002-0462-8>
10. Haanstra, J.R., González-Marcano, E.B., Gualdrón-López, M., Michels, P.A.M., 2016. Biogenesis, maintenance and dynamics of glycosomes in trypanosomatid parasites. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1863, 1038–1048. <https://doi.org/10.1016/j.bbamcr.2015.09.015>
11. Hamed, Y., Gentilucci, M., Jallouli, H., Ncibi, K., Hadji, R., Jraba, A., Mlayeh, A., Elaloui, E., 2025. Radiological environmental monitoring of groundwater around phosphate deposits in North Africa-Southern Tunisia: geochemical, environmental and health factors. *AS-ITJGW* 14. <https://doi.org/10.7343/as-2025-809>
12. Hu, J., Sha, X., Li, Y., Wu, J., Ma, J., Zhang, Y., Yang, R., 2023. Multifaceted Applications of Ferritin Nanocages in Delivering Metal Ions, Bioactive Compounds, and Enzymes: A Comprehensive Review. *J. Agric. Food Chem.* 71, 19903–19919. <https://doi.org/10.1021/acs.jafc.3c05510>
13. Hussain, B., Abbas, A., Saleem, A.R., Riaz, L., Rahman, S.U., Liu, S., Pu, S., Farooq, M., 2024. Uptake, Agglomeration, and Detoxification of Trace Metals and Metalloids in Plants. *J Soil Sci Plant Nutr* 24, 4965–4983. <https://doi.org/10.1007/s42729-024-01885-9>
14. Klik, B., Kulikowska, D., Gusiatin, Z.M., Pasieczna-Patkowska, S., 2020. Washing agents from sewage sludge: efficiency of Cd removal from highly contaminated soils and effect on soil organic balance. *J Soils Sediments* 20, 284–296. <https://doi.org/10.1007/s11368-019-02367-7>
15. Kumar, A., Chaubey, A.K., 2025. Ontogenetic Changes in Feeding Behaviour and Nutrient Allocation in *Zygogramma bicolorata* on *Parthenium hysterophorus*. *Agricultural Research Journal* 62, 174–

180. <https://doi.org/10.5958/2395-146X.2025.00021.4>
16. Ma, J., Wang, W., Yang, J., Qin, S., Yang, Y., Sun, C., Pei, G., Zeeshan, M., Liao, H., Liu, L., Huang, J., 2022. Mycorrhizal symbiosis promotes the nutrient content accumulation and affects the root exudates in maize. *BMC Plant Biol* 22, 64. <https://doi.org/10.1186/s12870-021-03370-2>
17. Machraoui, S., Labidi, S., Azbouche, A., 2024. Assessment of radiation exposure in the phosphate mining area of Gafsa (Southeastern Tunisia). *International Journal of Environmental Analytical Chemistry* 1–18. <https://doi.org/10.1080/03067319.2024.2352804>
18. Mahmoud, A., AbdElgawad, H., Hamed, B.A., Beemster, G.T.S., El-Shafey, N.M., 2021. Differences in Cadmium Accumulation, Detoxification and Antioxidant Defenses between Contrasting Maize Cultivars Implicate a Role of Superoxide Dismutase in Cd Tolerance. *Antioxidants* 10, 1812. <https://doi.org/10.3390/antiox10111812>
19. Merakli, N., Memon, A., Altundag, H., Kaki, B., Bhutto, L.A., Kahraman, A., 2025. Phytoremediation Potential and Differential Chromium (Cr) Accumulation and Gene Expression in Brassica juncea Varieties for Contaminated Soils in Turkey. <https://doi.org/10.21203/rs.3.rs-7303811/v1>
20. Mierek-Adamska, A., Tylman-Mojżesz, W., Pawełek, A., Kulasek, M., Dąbrowska, G.B., 2025. The Potential Role of Brassica napus Metallothioneins in Salt Stress and Interactions with Plant Growth-Promoting Bacteria. *Genes* 16, 166. <https://doi.org/10.3390/genes16020166>
21. Murtaza, G., Murtaza, B., Niazi, N.K., Sabir, M., 2014. Soil Contaminants: Sources, Effects, and Approaches for Remediation, in: Ahmad, P., Wani, M.R., Azooz, M.M., Phan Tran, L.-S. (Eds.), *Improvement of Crops in the Era of Climatic Changes*. Springer New York, New York, NY, pp. 171–196. https://doi.org/10.1007/978-1-4614-8824-8_7
22. Nie, X., Huang, X., Li, M., Lu, Z., Ling, X., 2024. Advances in Soil Amendments for Remediation of Heavy Metal-Contaminated Soils: Mechanisms, Impact, and Future Prospects. *Toxics* 12, 872. <https://doi.org/10.3390/toxics12120872>
23. Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T., Mavumengwana, V., 2020. Toxic Metal Implications on Agricultural Soils, Plants, Animals, Aquatic life and Human Health. *IJERPH* 17, 2204. <https://doi.org/10.3390/ijerph17072204>
24. Prasad, J., Tiwari, S., Singh, B.K., Dubey, N.K., 2022. Phytoextraction of heavy metals: Challenges and opportunities, in: *Phytoremediation Technology for the Removal of Heavy Metals and Other Contaminants from Soil and Water*. Elsevier, pp. 173–187. <https://doi.org/10.1016/B978-0-323-85763-5.00011-8>
25. Priya, A.K., Muruganandam, M., Ali, S.S., Kornaros, M., 2023. Clean-Up of Heavy Metals from Contaminated Soil by Phytoremediation: A Multidisciplinary and Eco-Friendly Approach. *Toxics* 11, 422. <https://doi.org/10.3390/toxics11050422>
26. Purschke, B., Scheibelberger, R., Axmann, S., Adler, A., Jäger, H., 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain.

27. Rahman, Z., Singh, V.P., 2019. The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environ Monit Assess* 191, 419. <https://doi.org/10.1007/s10661-019-7528-7>
28. Rehman, K.U., Hollah, C., Wiesotzki, K., Rehman, R.U., Rehman, A.U., Zhang, J., Zheng, L., Nienaber, T., Heinz, V., Aganovic, K., 2023. Black soldier fly, *Hermetia illucens* as a potential innovative and environmentally friendly tool for organic waste management: A mini-review. *Waste Manag Res* 41, 81–97. <https://doi.org/10.1177/0734242X221105441>
29. Rezaee, L., Moosavi, A.A., Davatgar, N., Sepaskhah, A.R., 2020. Soil quality indices of paddy soils in Guilan province of northern Iran: Spatial variability and their influential parameters. *Ecological Indicators* 117, 106566. <https://doi.org/10.1016/j.ecolind.2020.106566>
30. Rice, C.W., Moorman, T.B., Beare, M., 2015. Role of Microbial Biomass Carbon and Nitrogen in Soil Quality, in: Doran, J.W., Jones, A.J. (Eds.), *SSSA Special Publications*. Soil Science Society of America, Madison, WI, USA, pp. 203–215. <https://doi.org/10.2136/sssaspecpub49.c12>
31. Shakti, P., Pandey, A.K., 2024. Agricultural soil contamination due to industrial discharges: challenges for public health protection and food security, in: *Bioremediation of Emerging Contaminants from Soils*. Elsevier, pp. 21–42. <https://doi.org/10.1016/B978-0-443-13993-2.00002-5>
32. Sharma, Neha, Chaudhary, A., Sharma, Nikhil, Shukla, S., Joshi, N.C., Vishwakarma, K., Singh, P.K., Mishra, A., 2025. Plant Nutrient Requirements and Nutrient Homeostasis in Plants, in: Joshi, N.C., Leustek, T., Singh, P.K. (Eds.), *Soil Health and Nutrition Management*. CABI, GB, pp. 1–20. <https://doi.org/10.1079/9781800624597.0001>
33. Sharma, S.S., Dietz, K., Mimura, T., 2016. Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant Cell & Environment* 39, 1112–1126. <https://doi.org/10.1111/pce.12706>
34. Siddiqui, S.A., Süfer, Ö., Çalışkan Koç, G., Lutuf, H., Rahayu, T., Castro-Muñoz, R., Fernando, I., 2024. Enhancing the bioconversion rate and end products of black soldier fly (BSF) treatment – A comprehensive review. *Environ Dev Sustain* 27, 9673–9741. <https://doi.org/10.1007/s10668-023-04306-6>
35. Singh, P.K., Yadav, J.S., Kumar, I., Kumar, U., Sharma, R.K., 2024. Screening of mustard cultivars for phytoremediation of heavy metals contamination in wastewater irrigated soil systems. *Environ Monit Assess* 196, 321. <https://doi.org/10.1007/s10661-024-12506-4>
36. Tapia, Y., Salazar, O., Seguel, O., Suazo-Hernández, J., Urdiales-Flores, D., Aponte, H., Urdiales, C., 2024. Optimizing Heavy Metal Uptake in *Carpobrotus aequilaterus* Through Electrokinetic Treatment: A Comprehensive Study on Phytoremediation from Mine Tailings. *Toxics* 12, 860. <https://doi.org/10.3390/toxics12120860>
37. Thalassinou, G., Levizou, E., Rinklebe, J., Shaheen, S.M., Vasileios, A., 2024. Enhancing the Phytoextraction of Cd, Cu, Pb, and Zn by *Portulaca oleracea* in a Heavily Contaminated Soil Using

- Low Molecular Weight Organic Substances: Is Phytoremediation Viable? *Earth Syst Environ* 8, 923–936. <https://doi.org/10.1007/s41748-024-00386-0>
38. Tirtawijaya, G., Lee, J.-H., Bashir, K.M.I., Lee, H.-J., Choi, J.-S., 2024. Evaluating the Efficiency of Black Soldier Fly (*Hermetia illucens*) Larvae in Converting Mackerel Head Waste into Valuable Resources. *Animals* 14, 1332. <https://doi.org/10.3390/ani14091332>
39. Tsang, D.C.W., Lo, I.M.-C., Surampalli, R.Y., 2014. Chelating Agents for Land Decontamination Technologies. ASCE, Reston.
40. Usmani, L., Shakil, A., Khan, I., Alvi, T., Singh, S., Das, D., 2025. Brassinosteroids in Micronutrient Homeostasis: Mechanisms and Implications for Plant Nutrition and Stress Resilience. *Plants* 14, 598. <https://doi.org/10.3390/plants14040598>
41. Verardi, A., Sangiorgio, P., Della Mura, B., Moliterni, S., Spagnoletta, A., Dimatteo, S., Bassi, D., Cortimiglia, C., Rebuzzi, R., Palazzo, S., Errico, S., 2025. *Tenebrio molitor* Frass: A Cutting-Edge Biofertilizer for Sustainable Agriculture and Advanced Adsorbent Precursor for Environmental Remediation. *Agronomy* 15, 758. <https://doi.org/10.3390/agronomy15030758>
42. Wang, S., Shi, X., Salam, M.M.A., Chen, G., 2021. Integrated study on subcellular localization and chemical speciation of Pb reveals root strategies for Pb sequestration and detoxification in *Salix integra*. *Plant Soil* 467, 197–211. <https://doi.org/10.1007/s11104-021-05045-1>
43. Wang, Z., Wang, Hongbin, Wang, Haijuan, Li, Q., Li, Y., 2020. Effect of soil washing on heavy metal removal and soil quality: A two-sided coin. *Ecotoxicology and Environmental Safety* 203, 110981. <https://doi.org/10.1016/j.ecoenv.2020.110981>
44. Xu, M., Zhi, Y., Kong, Z., Ma, H., Shao, Z., Chen, L., Chen, H., Yuan, Y., Liu, F., Xu, Y., Ni, Q., Hu, S., Chai, H., 2024. Enhancing nitrogen and phosphorus removal in plant-biochar-pyrite stormwater bioretention systems: Impact of temperature and high-frequency heavy rainfall. *Environmental Research* 262, 119926. <https://doi.org/10.1016/j.envres.2024.119926>
45. Yan, G., Li, J., Zhao, Y., Wang, X., Zhu, S., Li, D., Liu, B., Zhang, W., 2025. Microplastic–metal–microbiota interactions in the flesh fly *Boettcherisca peregrina* larvae reared on contaminated livestock manure. *Ecotoxicology and Environmental Safety* 303, 118935. <https://doi.org/10.1016/j.ecoenv.2025.118935>
46. Zeremski, T., Randelović, D., Jakovljević, K., Marjanović Jeromela, A., Milić, S., 2021a. Brassica Species in Phytoextractions: Real Potentials and Challenges. *Plants* 10, 2340. <https://doi.org/10.3390/plants10112340>
47. Zeremski, T., Randelović, D., Jakovljević, K., Marjanović Jeromela, A., Milić, S., 2021b. Brassica Species in Phytoextractions: Real Potentials and Challenges. *Plants* 10, 2340. <https://doi.org/10.3390/plants10112340>
48. Zhang, R., Chen, D., Liu, H., Guo, C., Tang, L., Wang, H., Chen, Y., Luo, K., 2022. Effect of temperature and water potential on the germination of seeds from three different populations of *Bidens pilosa* as a potential Cd hyperaccumulator. *BMC Plant Biol* 22, 487. <https://doi.org/10.1186/s12870-022-03876-3>

49. Zulkifli, N.F.N.M., Seok-Kian, A.Y., Seng, L.L., Mustafa, S., Kim, Y.-S., Shapawi, R., 2022. Nutritional value of black soldier fly (*Hermetia illucens*) larvae processed by different methods. PLoS ONE 17, e0263924. <https://doi.org/10.1371/journal.pone.0263924>

Figures

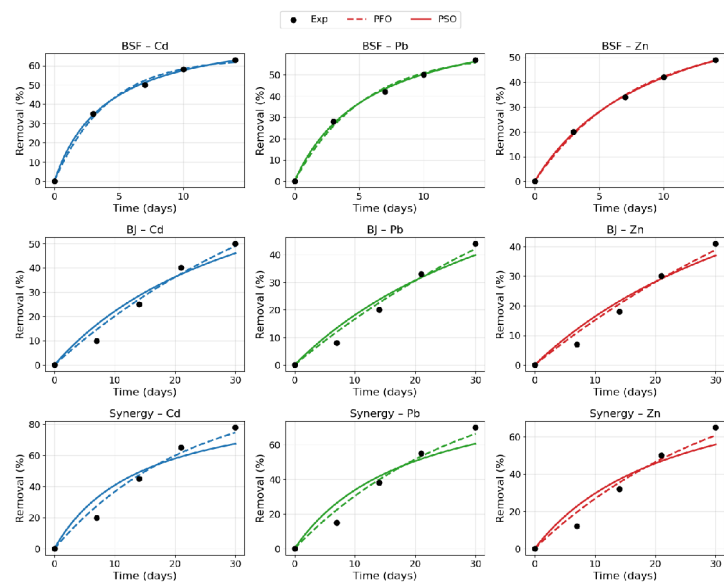


Figure 1. Kinetic modeling of cadmium (Cd), lead (Pb), and zinc (Zn) removal from contaminated soils under entomoremediation (BSF), phytoremediation (BJ), and synergistic (BSF → BJ) treatments. Experimental data (points) were fitted to pseudo-first-order (PFO, dashed lines) and pseudo-second-order (PSO, solid lines) models.

Caption

The temporal dynamics of metal removal highlighted distinct kinetic signatures across treatments. In BSF larvae, Cd and Pb removal closely followed PSO kinetics ($R^2 > 0.96$), confirming chemisorption and strong complexation within larval biomass. Zn showed weaker PSO conformity, with partial alignment to PFO kinetics ($R^2 \approx 0.91$), suggesting lower-affinity binding and partial immobilization. For *Brassica juncea*, metal uptake underscored time-dependent sigmoidal accumulation with closer agreement to PFO kinetics, revealing an initial lag during root establishment, rapid accumulation during vegetative growth, and stabilization toward equilibrium. In the synergistic BSF → BJ treatment, all three metals achieved superior fits with PSO models ($R^2 > 0.98$), evidencing cooperative chemisorption–bioaccumulation pathways. These findings highlight insects as rapid detoxifiers, plants as sustainable stabilizers, and their integration as a robust hybrid system combining fast initial detoxification with long-term remediation capacity.

Figure 1

See image above for figure legend.

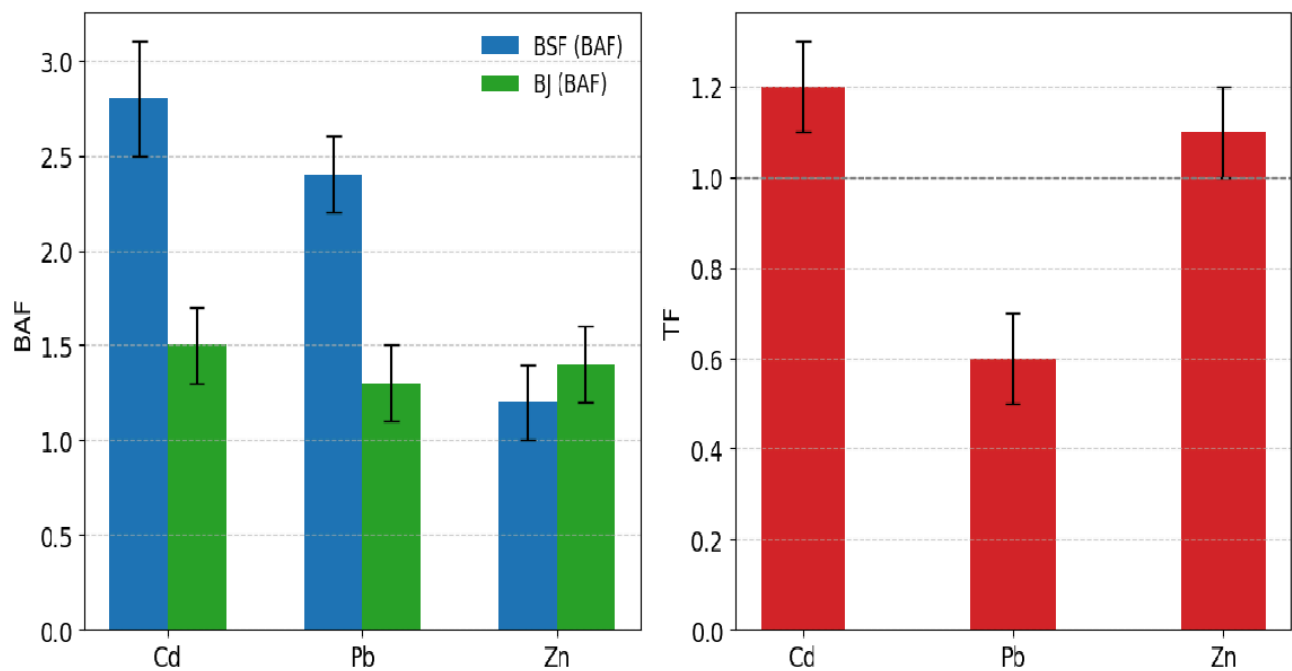


Figure 2. Bioaccumulation Factor (BAF) and Translocation Factor (TF) values

Caption:

Bioaccumulation Factor (BAF) and Translocation Factor (TF) of Cd, Pb, and Zn in BSF larvae and BJ plants. BSF larvae exhibited higher BAF for Cd (2.8) and Pb (2.4), but lower Zn accumulation (1.2). BJ exhibited moderate BAF but favorable TF (>1 for Cd, Zn), highlighting effective root-to-shoot transfer for phytoextraction, while Pb remained largely immobilized in roots (TF = 0.6). Data are mean $\pm$ SD (n = 3).

Figure 2

See image above for figure legend.

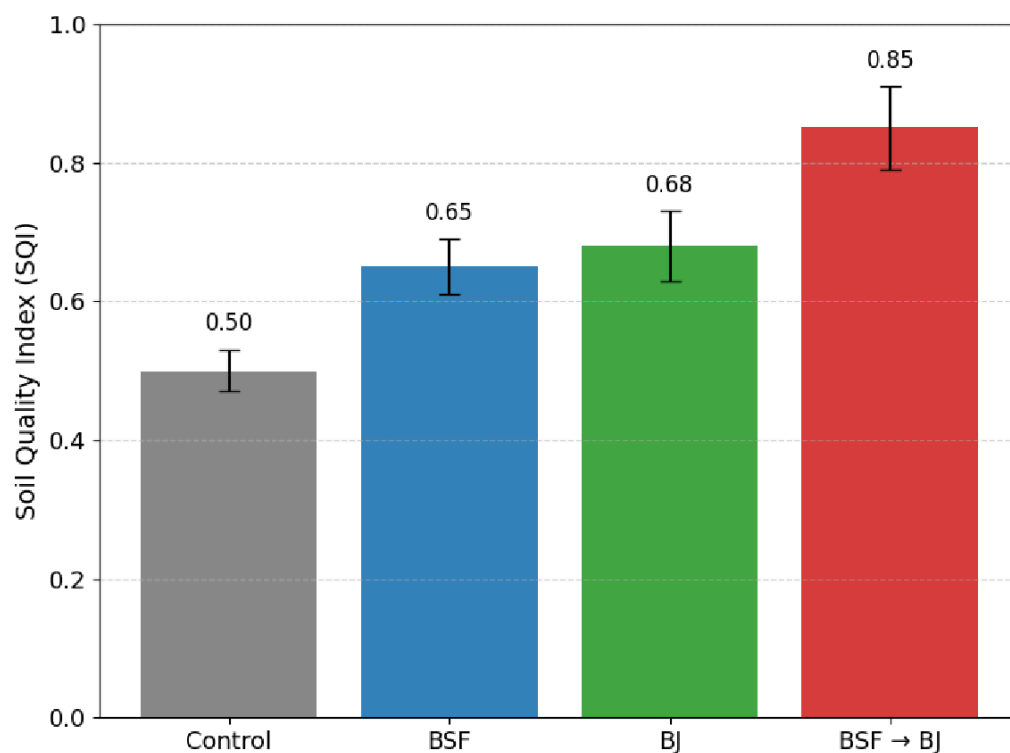


Figure 3. Soil Quality Index (SQI) improvements across treatments. SQI was calculated by integrating residual metal concentrations, soil pH, organic matter, and microbial respiration. The synergistic BSF → BJ treatment achieved the highest SQI (0.85), representing a 35% increase relative to control soils.

Caption:

Soil Quality Index (SQI) comparison among control, BSF, BJ, and BSF → BJ treatments. The synergistic treatment markedly outperformed individual methods, with modest pH improvement (from 5.4 to 6.2), an 18% increase in organic matter, and a 27% enhancement in microbial respiration. These improvements reflect not only accelerated detoxification but also the restoration of soil ecological function, confirming the superiority of the integrative approach.

Figure 3

See image above for figure legend.

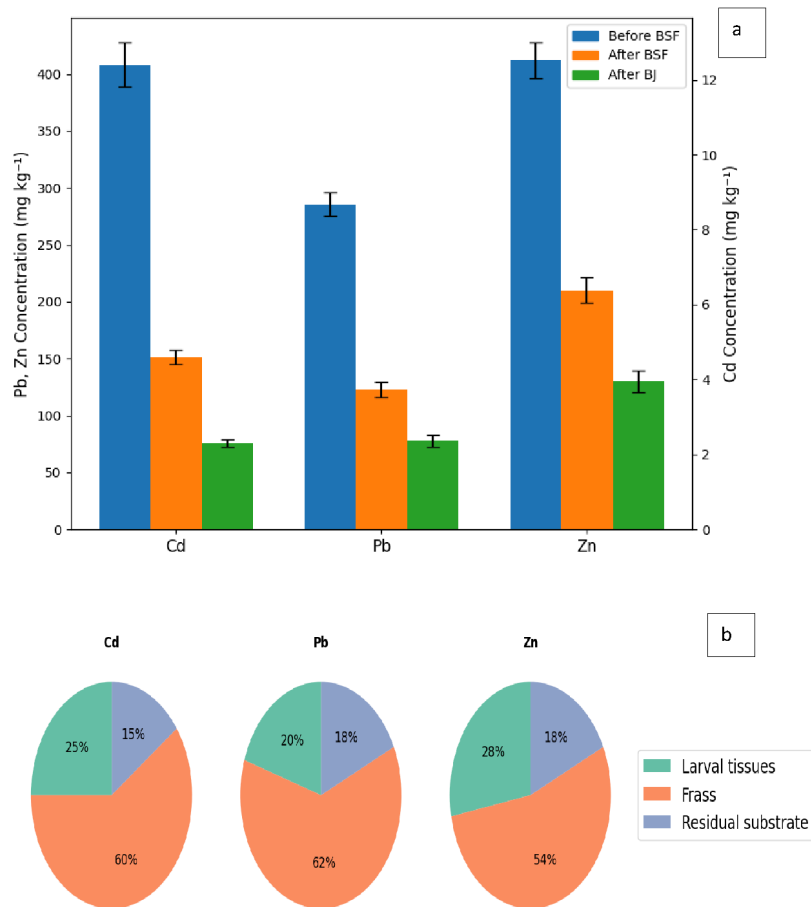


Figure 4. Sequential BSF → BJ treatment effects on soil metal concentrations and mass balance of metals in BSF larvae.

(a) Soil concentrations of Cd, Pb, and Zn before treatment, after 14 days of BSF larval processing, and after subsequent 30 days of *Brassica juncea* cultivation. Cd is displayed on the secondary axis for clarity, while Pb and Zn are plotted on the primary axis. Error bars represent standard deviations ($n = 4$). Sequential treatments significantly reduced soil metal burdens, with BSF larvae contributing to the initial decrease and *B. juncea* further attenuating residual metals.

(b) Mass balance of metals in *Hermetia illucens* larvae after 14 days of processing contaminated soil. Partitioning of Cd, Pb, and Zn into larval tissues, frass, and residual substrate is expressed as percentages of the total recovered mass. Results indicate predominant redistribution into frass, with moderate tissue accumulation, highlighting the dual function of BSF larvae in bioaccumulation and partial immobilization.

Figure 4

See image above for figure legend.

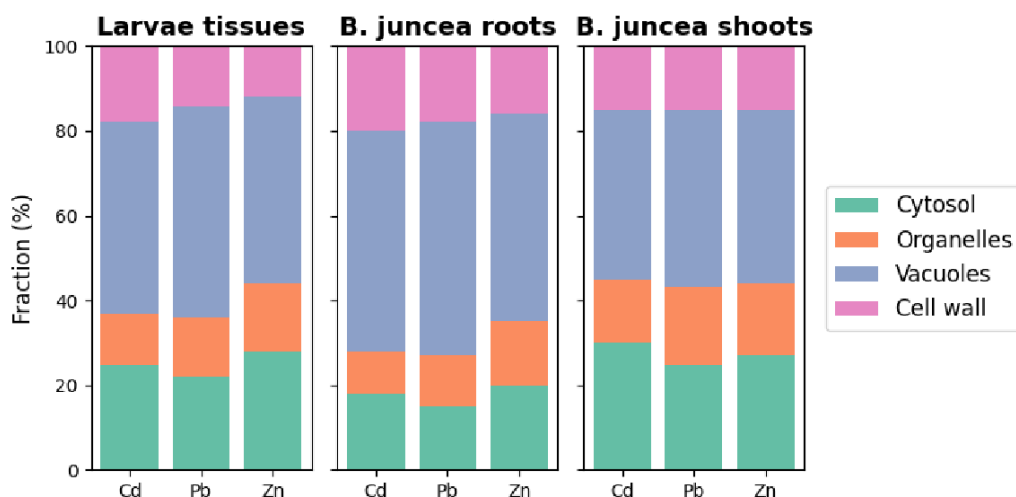


Figure 5. Subcellular distribution of Cd, Pb, and Zn in BSF larvae tissues, *Brassica juncea* roots, and *B. juncea* shoots after sequential BSF → BJ treatment.

Stacked bar charts indicate the relative partitioning of metals into cytosol, organelles, vacuoles, and cell walls. In larvae, metals were predominantly localized in vacuoles/insoluble granules, consistent with detoxification via sequestration. In *B. juncea*, roots displayed preferential vacuolar and cell wall immobilization, while shoots exhibited a higher proportion in cytosolic and organelle fractions, reflecting limited translocation. These patterns demonstrate compartmentalization as a central detoxification approach mitigating metal-induced cytotoxicity.

Figure 5

See image above for figure legend.

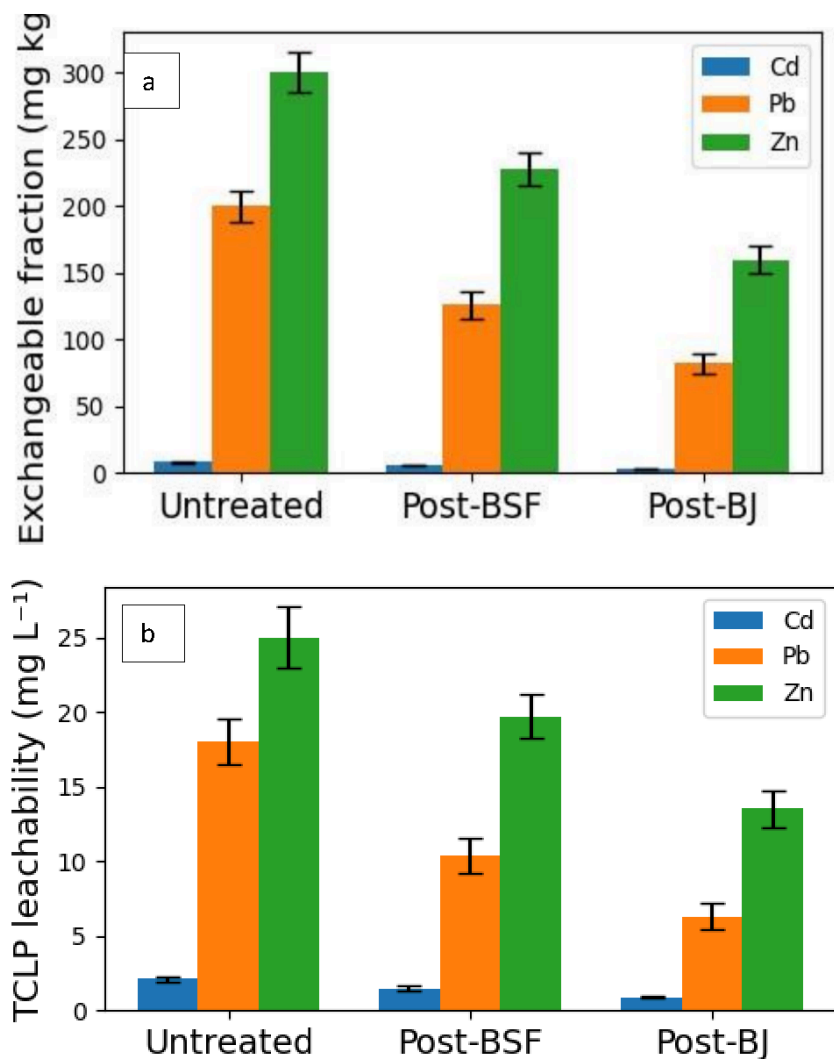


Figure 6. Reduction of exchangeable fractions and leachability of heavy metals through sequential BSF and BJ treatments.

(a) Exchangeable fractions of Cd, Pb, and Zn in untreated soil, after *Hermetia illucens* larvae processing (Post-BSF), and following *Brassica juncea* cultivation (Post-BJ). (b) TCLP leachability of Cd, Pb, and Zn across the same treatments, illustrating the complementary roles of BSF-mediated stabilization and BJ-assisted phytoextraction in reducing metal mobility and environmental risk.

Figure 6

See image above for figure legend.

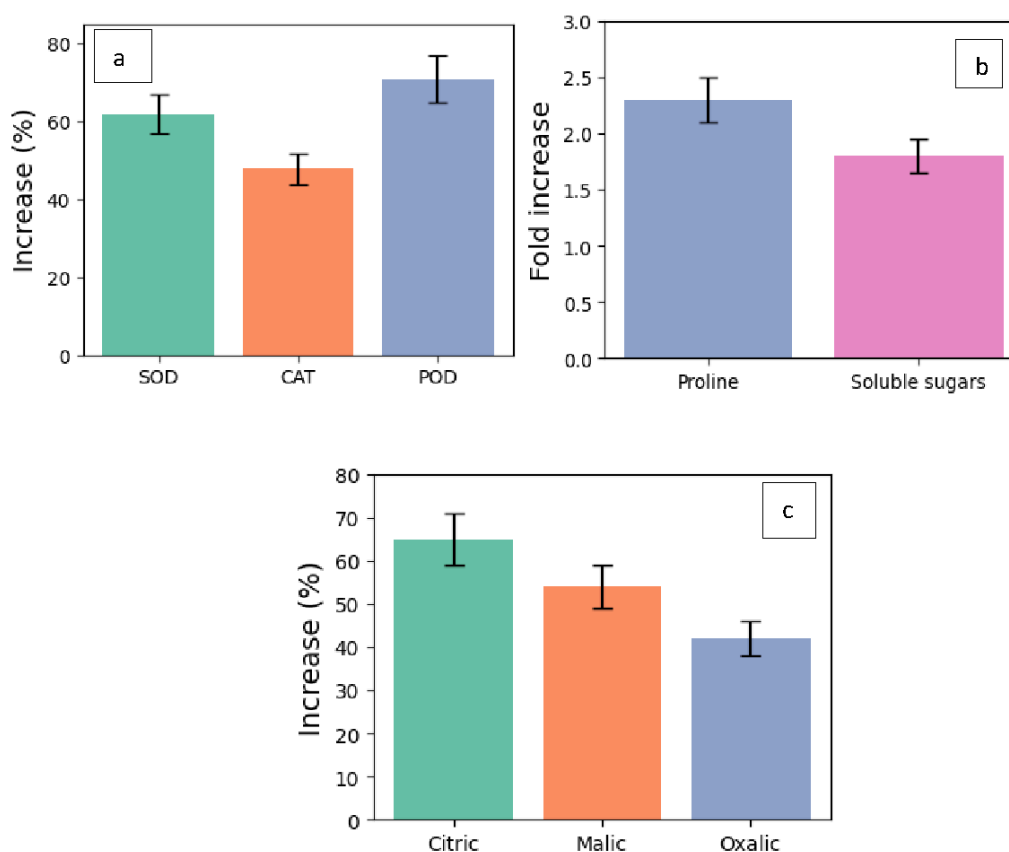


Figure 7. Physiological and biochemical responses of *Brassica juncea* to BSF-processed soil containing residual Cd, Pb, and Zn.

Grouped bar charts show (a) antioxidant enzyme activities (SOD, CAT, POD), (b) osmoprotectant accumulation (proline, soluble sugars), and (c) organic acid secretion (citric, malic, oxalic) relative to the reference control. Error bars represent standard deviations (n = 4). Results demonstrate that *B. juncea* enhances antioxidative defense, osmotic adjustment, and rhizosphere metal chelation as part of a multi-layered tolerance approach against metal stress.

Figure 7

See image above for figure legend.

Supplementary Files

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