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Integrated phytoremediation and MgO Si HAP biochar for heavy metal remediation and maize productivity

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Abstract
Heavy metal contamination in agricultural soils limits crop productivity and presents food safety risks. This study explores an integrated approach combining phytoremediation (PHY) and biochar amendments, specifically a Magnesium Oxide–Silicon–Hydroxyapatite (MOSi-HAP) biochar system, to improve soil health and agricultural output. A 120-day pot experiment was conducted, consisting of two phases: Phase 1 compared different phytoremediation plants (*Brassica juncea* L, *Vetiveria zizanioides* L, *Helianthus annuus* L), MOSi-HAP biochar (3% w/w), and their combination; Phase 2 assessed maize growth in treated soil versus control. Results indicated that the integrated treatment significantly enhanced soil quality by boosting parameters such as porosity (+5.8%), water-holding capacity (+12.9%), organic carbon (to 1.10%), and cation exchange capacity (to 12.0 cmol kg^{-1}), while reducing heavy metals (Cd, Pb, As, Ni) by 93.8%, 94.3%, 94.2%, and 94.1%, respectively. Maize growth improved, with increases in plant height (from 103 ± 2 cm to 137 ± 3 cm), biomass (from 305 ± 5 g to 528 ± 8 g), and grain yield (from 4.2 ± 0.2 t ha^{-1} to 7.1 ± 0.3 t ha^{-1}, a + 69.0% increase). Nutrient uptake of nitrogen, phosphorus, and potassium also rose significantly, alongside enhancements in antioxidant enzyme activities and a reduction in oxidative damage (malondialdehyde levels decreased by 69.1%). Grain concentrations of heavy metals fell by 98.0–98.5%, remaining below international food safety standards. Overall, the PHY + MOSi-HAP biochar method effectively reduced heavy metal bioavailability and accumulation, while improving soil quality and maize productivity under pot-culture conditions.

Keywords Antioxidant enzymes · Biochar · Heavy metal immobilization · Maize productivity · Nutrient uptake · Soil fertility

Abbreviation

%	Percentage
±	Mean ± Standard Deviation
<	Less Than
>	Greater Than
ANOVA	Analysis of Variance
As	Arsenic
B:C Ratio	Benefit-to-Cost Ratio

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BC	Biochar
CAT	Catalase
Cd	Cadmium
CEC	Cation Exchange Capacity
Cm	Centimeter
cm^{-3}	Cubic Centimeter
cmol(+) kg^{-1}	Centimoles of Positive Charge per Kilogram of Soil
Control	Untreated Soil (Without PHY + MOSi-HAP Biochar)
dS m^{-1}	DeciSiemens per Meter
DW	Dry Weight
EC	Electrical Conductivity
F	ANOVA F-statistic
FAO	Food and Agriculture Organization
FW	Fresh Weight
G	Gram
g $plant^{-1}$	Gram per Plant
HAP	Hydroxyapatite
HSD	Honest Significant Difference
ICAR	Indian Council of Agricultural Research
K	Potassium
Kg	Kilogram
MgO	Magnesium Oxide
MDA	Malondialdehyde
Mg	Milligram
mg kg^{-1}	Milligram per Kilogram
mg $plant^{-1}$	Milligram per Plant
MOSi-HAP	Magnesium Oxide–Silicon–Hydroxyapatite
N	Nitrogen
N	Number of Replicates
Ni	Nickel
Nmol	Nanomole
OC	Organic Carbon
P	Phosphorus
Pb	Lead
pH	Potential of Hydrogen (Soil Acidity/Alkalinity)
PHY	Phytoremediation
PHY + BC	Phytoremediation Combined with Biochar
PHY + MOSi-HAP	Phytoremediation Combined with Magnesium Oxide–Silicon–Hydroxyapatite Biochar
POD	Peroxidase
p-value	Probability Value
R	Pearson Correlation Coefficient
SD	Standard Deviation
Si	Silicon
SOD	Superoxide Dismutase
SPSS	Statistical Package for the Social Sciences
t ha^{-1}	Tonnes per Hectare
Total N	Total Nitrogen

U	Enzyme Activity Unit
U mg^{-1} protein	Enzyme Units per Milligram Protein
USD	United States Dollar
WHC	Water-Holding Capacity
WHO	World Health Organization

Introduction

Soil contamination with toxic ions, including heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni), represents a major threat to sustainable agriculture and food safety worldwide. These contaminants originate from industrial effluents, mining activities, excessive agrochemical use, and atmospheric deposition, leading to soil degradation, reduced fertility, and accumulation of metals in edible crops[1]. The transfer of heavy metals through the food chain poses serious risks to human and animal health, making the remediation of contaminated soils a critical environmental and agricultural priority. Conventional remediation methods, including excavation, soil washing, and chemical stabilization, are often expensive, disruptive to soil structure, and environmentally unsustainable. Consequently, increasing attention has been directed toward environmentally friendly and cost-effective remediation approaches that integrate biological and amendment-based technologies[2,3].

Phytoremediation (PHY), which utilizes plants to extract, stabilize, or transform contaminants, is considered a sustainable remediation strategy due to its low cost and ecological compatibility. However, its effectiveness is often limited by poor soil fertility, restricted plant growth, and metal-induced stress under contaminated conditions. To overcome these limitations, biochar amendments have been increasingly incorporated into phytoremediation systems. Biochar, a carbon-rich material produced through biomass pyrolysis, improves soil physicochemical properties, enhances nutrient retention, increases cation exchange capacity, and reduces heavy metal bioavailability[4,5]. Biochar-assisted phytoremediation has been shown to enhance heavy metal immobilization, improve soil physicochemical properties, and increase remediation efficiency[1,42]. Biochar application also improves nutrient retention, plant growth, and crop productivity under contaminated conditions[8,9]. Studies have reported that biochar application improves soil structure, decreases metal mobility, and enhances crop productivity in contaminated environments[6,7].

Previous biochar-phytoremediation studies have primarily focused on conventional biochar amendments or modified biochars for metal immobilization[1,23]. These studies have demonstrated improvements in contaminant stabilization, soil quality, and crop growth[8,9], while engineered biochars have been investigated mainly for enhancing heavy metal immobilization[17,23]. While a substantial body of literature has documented the efficacy of biochar amendments in isolating or extracting metals from polluted matrixes[10,11], a majority of these investigations evaluate single-endpoint parameters, primarily targeting soil metal immobilization or plant biomass accumulation under Controlled laboratory conditions[12,13]. Consequently, there remains a critical gap in literature regarding the holistic evaluation of engineered biochars across interconnected ecological and agronomic domains[14,15]. Most existing studies assess contaminant immobilization or soil fertility enhancement as isolated outcomes[16], rather than evaluating how multifunctional engineered biochars simultaneously modulate soil restoration, nutrient uptake efficiency, enzymatic antioxidant defense networks, overall crop productivity, and heavy metal partitioning into edible tissues under field-relevant conditions[17,18]. By shifting the analytical framework from isolated contaminant removal to an integrated soil-plant system, research can better evaluate the multi-target potential of engineered amendments. This gap indicates the need for an integrated remediation approach capable of simultaneously improving contaminant immobilization, nutrient-use efficiency, plant stress tolerance, and crop productivity within a unified soil–plant framework.

To address these limitations, the superiority of the present study lies in evaluating an integrated phytoremediation (PHY) and Magnesium Oxide–Silicon–Hydroxyapatite ((MOSi-HAP) biochar system that employs a MgO–Si–HAP engineered biochar designed to simultaneously enhance contaminant immobilization, nutrient dynamics, and plant stress tolerance[17,23]. This specific engineered biochar was chosen over conventional or single-component biochars because of the synergistic multi-mechanism action of its components: MgO provides abundant basic sites and facilitates cation exchange for heavy metal precipitation, Si enhances plant stress tolerance and cell wall rigidity to restrict metal translocation, and HAP provides phosphate functional groups for strong surface complexation and phosphate-metal precipitation. Unlike previous biochar-phytoremediation studies, the present study simultaneously quantifies soil restoration, nutrient uptake, antioxidant responses, maize productivity, and heavy metal accumulation in edible tissues within a unified soil-plant framework, thereby directly linking remediation efficiency with crop productivity and food safety.

MOSi-HAP biochar, enriched with magnesium oxide (MgO), silicon (Si), and hydroxyapatite (HAP), offers additional advantages through its high adsorption capacity and strong metal-binding characteristics. The multifunctional performance of MOSi-HAP biochar relies on distinct chemical and physiological mechanisms: MgO provides abundant alkaline functional groups and hydroxyl species that elevate soil pH and facilitate heavy metal precipitation as insoluble hydroxides; HAP supplies active phosphate groups that promote surface complexation, ion exchange, and the formation of highly stable metal-phosphate precipitates (such as pyromorphite-like structures); and Si reduces toxic ion mobility via surface co-precipitation while simultaneously upregulating plant antioxidant enzymes, reinforcing cell wall physical barriers, and suppressing root-to-shoot metal translocation. The combined action of these components promotes heavy metal immobilization while improving soil fertility and nutrient availability. When integrated with phytoremediation, MOSi-HAP biochar can enhance rhizosphere functioning, nutrient-use efficiency, and plant stress tolerance, thereby improving both soil restoration and crop productivity. The effectiveness of integrated biochar-phytoremediation systems is influenced by soil characteristics, crop species, and amendment properties. Sandy soils generally require amendments that improve water retention and nutrient-holding capacity, whereas calcareous and clay-rich soils require materials capable of optimizing nutrient dynamics and contaminant stabilization[8,9]. Understanding these soil-specific dynamics is directly relevant to this study, as the physical and chemical behavior of the engineered MOSi-HAP biochar, particularly its metal binding, ion exchange, and nutrient-release efficiency, varies depending on the surrounding soil texture and pH. Furthermore, interactions among soil, plants, and microorganisms play an important role in determining remediation efficiency and long-term sustainability.

The novelty of the present study lies in evaluating an integrated PHY + MOSi-HAP biochar system that employs a MgO–Si–HAP engineered biochar designed to simultaneously enhance contaminant immobilization, nutrient dynamics, and plant stress tolerance[17,23]. This specific engineered biochar was chosen over conventional or single-component biochars because of the synergistic multi-mechanism action of its components: MgO provides abundant basic sites and facilitates cation exchange for heavy metal precipitation, Si enhances plant stress tolerance and cell wall rigidity to restrict metal translocation, and HAP provides phosphate functional groups for strong surface complexation and phosphate-metal precipitation. Unlike previous biochar-phytoremediation studies, the present study simultaneously quantifies soil restoration, nutrient uptake, antioxidant responses, maize productivity, and heavy metal accumulation in edible tissues within a unified soil–plant framework, thereby directly linking remediation efficiency with crop productivity and food safety. The objectives of this study were to: (i) assess the effects of PHY + MOSi-HAP biochar on soil physical properties, including bulk density, porosity, moisture content, and water-holding capacity; (ii) evaluate improvements in soil chemical fertility through changes in pH, organic carbon, cation exchange capacity, and nutrient availability (N, P, and K); (iii) quantify reductions in soil heavy metal concentrations and bioavailability; (iv) determine the effects on maize growth, biomass production, and grain yield; and (v) investigate nutrient uptake, antioxidant enzyme responses, and heavy metal accumulation in maize tissues.

To address these challenges, this study aims to evaluate an integrated phytoremediation system using engineered MOSi-HAP biochar by quantifying its simultaneous impacts on heavy metal immobilization, soil restoration, plant antioxidant defense, maize yield, and metal partitioning into edible tissues (objective). Unlike previous studies focused solely on single-endpoint contaminant removal, this work establishes a holistic soil–plant framework that directly links rhizosphere chemical stabilization with plant physiological health and crop safety under field-relevant conditions (significance). Ultimately, by combining soil remediation, crop performance, food safety, and economic evaluation, this study provides a comprehensive strategy for the sustainable agricultural reuse of contaminated soils while safeguarding food security (expected outcomes).

Materials and methods

Study area and soil sampling

The experimental soil was collected from Kadachanendhal, Madurai, Tamil Nadu, India. Surface soil samples (0–20 cm depth) were collected after removing surface debris. Multiple subsamples were composited to obtain a representative sample. The soil was air-dried at room temperature, gently crushed, and passed through a 2-mm sieve prior to laboratory analyses.

Physico-chemical and heavy metal properties of soil

The experimental study evaluated the physico-chemical and heavy metal properties of the soil prior to remediation, summarized in Table 1 provides observed physico-chemical and heavy metal properties of experimental soil along with permissible and optimum reference limits. This assessment included key physical parameters such as bulk density, particle density, porosity, moisture content, and water-holding capacity, which indicated

Table 1 Physical, chemical, and heavy metal characteristics of the investigated soil.

Parameter	Observed Value	Permissible/Optimum Limit	Reference
Physical Properties			
Bulk Density (g cm^{-3})	1.46 ± 0.02	< 1.60	USDA-NRCS[10]
Particle Density (g cm^{-3})	2.65 ± 0.03	2.65	FAO[11]
Porosity (%)	44.9 ± 0.5	40–60	Weil and Brady[12]
Moisture Content (%)	12.4 ± 0.3	Site-specific	FAO[11]
Water-Holding Capacity (WHC, %)	32.6 ± 0.7	25–45	Weil and Brady[12]
Chemical Properties			
Soil pH	6.52 ± 0.10	6.0–7.5	FAO[11]
Electrical Conductivity (EC, dS m^{-1})	0.31 ± 0.05	< 2	FAO[11]
Organic Carbon (OC, %)	0.92 ± 0.04	0.75–1.5	ICAR[13]
Cation Exchange Capacity (CEC, cmol(+) kg^{-1})	12.3 ± 0.6	10–20	ICAR[13]
Total Nitrogen (N, %)	0.10 ± 0.01	280–560	ICAR[13]
Available Phosphorus (P, mg kg^{-1})	8.5 ± 0.4	10–25	ICAR[13]
Exchangeable Potassium (K, mg kg^{-1})	110 ± 9	110–280	ICAR[13]
Heavy Metal Concentrations			
Cadmium (Cd, mg kg^{-1}) – Total	4.8 ± 0.2	5	WHO[14]
Cadmium (Cd, mg kg^{-1}) – Bioavailable	2.9 ± 0.1	3	Kumar and Mani[15]
Lead (Pb, mg kg^{-1}) – Total	78.4 ± 3.2	100	WHO[14]
Lead (Pb, mg kg^{-1}) – Bioavailable	24.6 ± 1.4	84	Kumar and Mani[15]
Arsenic (As, mg kg^{-1}) – Total	12.1 ± 0.5	20	WHO[14]
Arsenic (As, mg kg^{-1}) – Bioavailable	5.8 ± 0.3	10	WHO[14]
Nickel (Ni, mg kg^{-1}) – Total	28.7 ± 1.7	50	WHO[14]
Nickel (Ni, mg kg^{-1}) – Bioavailable	9.4 ± 0.5	30	WHO[14]

a moderately compacted soil with adequate water retention. Chemical properties, including pH, electrical conductivity, organic carbon, cation exchange capacity, and available macro-nutrients (N, P, K), reflected moderate fertility suitable for maize cultivation. Heavy metal analyses included both total and bioavailable fractions, in which total concentrations were determined using standard acid digestion procedures and bioavailable fractions were determined using standard extraction methods to assess the readily available metal pool for plant uptake and remediation evaluation. The heavy metal analysis revealed elevated concentrations of cadmium, lead, arsenic, and nickel, indicating potential risks for crop safety and the need for effective remediation strategies.

The soil exhibits moderate compaction, adequate porosity, and suitable fertility levels, but the presence of heavy metals, particularly Pb and As, approaches or exceeds safe limits. This highlights the importance of PHY + MOSi-HAP biochar amendment to improve soil quality, enhance nutrient availability, and ensure safe maize production.

Novel phytoremediation system

A Controlled pot-culture phytoremediation system was established to maintain uniform heavy metal concentrations, stable moisture levels, and consistent nutrient availability, allowing precise evaluation of plant uptake performance under defined contamination conditions[16–18]. A three-species intercropping strategy was adopted to combine rapid metal translocation, deep-root stabilization, and high biomass accumulation, thereby enhancing overall remediation efficiency compared with single-species cultivation. Seeds of *Brassica juncea*, *Vetiveria zizanioides*, and *Helianthus annuus* used for plant establishment and subsequent phytoremediation experiments were purchased from Government of Tamil Nadu authorized seed-selling shop: Sri Hari Fertilizer Shop, Srivilliputhur, Tamil Nadu, India. Healthy and viable seeds of uniform size and appearance were selected prior to sowing to ensure consistent plant establishment across all experimental treatments. Each pot with a volume of 20 L and dimensions of 35 cm diameter and 35 cm height accommodated all three species in an alternating triangular arrangement with 10 to 12 cm spacing. This layout maximized root-zone coverage, minimized interspecific competition, and promoted rhizosphere interactions, leading to improved metal uptake under confined pot conditions. Table 2 presents the characteristics of the plant species used in the pot-scale phytoremediation system, including their key traits, target metals, root architecture, and suitability for Controlled cultivation.

MOSi-HAP biochar development

A dual-feedstock biochar was developed using Moringa oleifera seed husk and rice husk to optimize structural and chemical properties for heavy metal remediation. Moringa seed husk provided high lignin content and abundant surface functional groups (–COOH, –OH), enhancing adsorption of Cd, Pb, and Ni[19], while rice husk contributed high silica content, improving structural rigidity and porosity[20]. This specific composite design was selected over conventional single-source biochars to overcome fundamental synthesis limitations, such as low cation exchange capacity, pore collapse during pyrolysis, and variable surface charge stability. Combining dense lignocellulosic matrixes with mineral-rich agricultural residues creates a multi-porous framework that maximizes

Table 2 Characteristics of plant species used in pot-scale phytoremediation.

Plant species	Key traits	Target metals	Root architecture	Pot-culture suitability	Reference
Brassica juncea L (Indian mustard)	Rapid growth and high translocation efficiency	Cd, Pb	Shallow to intermediate roots with moderate lateral spread	Efficient shoot-based removal in confined soil volume	Bortoloti and Baron,[16]
Vetiveria zizanioides L (Vetiver grass)	High tolerance and stable growth under metal stress	Cd, Pb, As, Ni	Dense fibrous roots with deep penetration	Strong stabilization and gradual uptake within pots	Ai et al.[17].
Helianthus annuus L (Sunflower)	High biomass and broad metal uptake spectrum	Cd, Pb, Ni, Zn	Medium-depth roots with wide lateral spread	High-volume absorption enhancing total metal capture	Waseem et al,.[18].

physical trapping alongside chemical immobilization. Prior to pyrolysis, feedstocks were doped with 2% nano-hydroxyapatite (n-HAP), which immobilizes heavy metals through phosphate precipitation, ion exchange, and surface complexation, producing MOSi-HAP biochar, a triple-action material providing simultaneous adsorption, precipitation, and soil amendment functions[21,22]. Pyrolysis was conducted at 550 °C under limited oxygen to ensure carbon stability and high surface area, and the resulting material was ground to <2 mm particle size to enhance soil contact and reactivity[23]. Physicochemical characterization confirmed that the synthesized MOSi-HAP biochar possessed an alkaline pH of 8.95, a high specific surface area of 312.4 $m^2 g^{-1}$, a cation exchange capacity of 48.6 cmol kg^{-1}, and prominent surface functional groups (-OH, -COOH, and P-O) verified by FTIR and XRD analysis[23]. These combined structural and chemical features provide a high density of active sorption sites for stable heavy metal immobilization. MOSi-HAP biochar incorporation into soil was performed at 3% w/w, followed by 14 days of incubation to allow chemical stabilization and equilibration of biochar-soil interactions, maximizing heavy metal immobilization. The 3% w/w application rate was selected based on preliminary dose-response screening and established literature, which demonstrated that this dosage provides sufficient active sorption sites and pH buffering without causing soil salinity stress or toxicity to plant roots. The 14-day pre-incubation period was established to allow adequate time for biochar-soil chemical equilibration, surface wetting, and ion-exchange stabilization before planting, ensuring optimal heavy metal immobilization prior to seed germination[24]. The biochar application rate was calculated based on soil dry weight, and uniform mixing was ensured to standardize exposure across replicates[24]. Table 3 summarizes the development and application parameters of MOSi-HAP biochar, including its synthesis conditions, physicochemical characteristics, and the protocols used for its application in the study.

Experimental investigation

The experiment was conducted in a Controlled pot-culture system to evaluate the efficacy of phytoremediation, MOSi-HAP biochar amendment, and their combination in reducing heavy metal concentrations in contaminated soils. The Controlled pots ensured uniform heavy metal levels, consistent soil moisture, and stable nutrient availability, minimizing variability often encountered in field trials[16–18]. Contaminated soil was collected from a heavy metal-affected agricultural site, air-dried, homogenized, and passed through a 2 mm sieve before use. The sampling site was specifically selected due to its long-term history of continuous irrigation with untreated industrial wastewater and excessive agricultural inputs, which resulted in multi-metal contamination (Cd, Pb, As, and Ni) that exceeds permissible agricultural safety thresholds (maximum permissible limits of 0.8 to 3.0 mg kg^{-1} for Cd, 50 to 85 mg kg^{-1} for Pb, 20 mg kg^{-1} for As, and 35 to 50 mg kg^{-1} for Ni in agricultural soils[14,15]). Each 20 L pot (35 cm diameter × 35 cm height) was filled with 18 kg of contaminated soil.

The study was conducted in a climate-Controlled greenhouse under ambient conditions maintaining a temperature range of 28 °C to 35 °C during the day and 20 °C to 24 °C at night, a relative humidity of 60% to 75%, and a natural daylight photoperiod of approximately 12 to 14 h. Soil moisture was maintained at 65% to

Table 3 Development and application parameters of MOSi-HAP biochar.

Parameter	Details/Value	Rationale/Function
Feedstocks	Moringa oleifera seed husk (50%), Rice husk (50%)	High lignin and functional groups (–COOH, –OH) from Moringa; high silica content from rice husk for structural rigidity and porosity [19, 20].
Mineral Doping	2% nano-hydroxyapatite (n-HAP)	Enhances metal immobilization via phosphate precipitation, ion exchange, and surface complexation [21,22].
Pyrolysis Temperature	550 °C	Ensures carbon stability, develops high surface area, retains functional groups [23].
Atmosphere	Limited oxygen	Prevents complete combustion, maximizes char yield [23].
Particle Size	<2 mm	Improves soil contact and reactive surface area [23].
Amendment Rate	3% w/w	Sufficient to immobilize metals and maintain soil structure [24].
Soil Incubation	14 days before planting	Allows chemical stabilization, equilibration of biochar-soil interactions, and optimization of heavy metal immobilization [24].

70% of total water-holding capacity by daily gravimetric monitoring and replenishment with deionized water through automated drip emitters throughout the experimental period to ensure consistent moisture conditions across treatments. No fertilizers were applied during Phase – 1 to avoid interference with soil stabilization and heavy metal immobilization processes, whereas a uniform recommended basal fertilizer dose (120 kg N, 60 kg P_2O_5, and 40 kg K_2O ha^{-1} using analytical grade urea, single superphosphate, and muriate of potash) was applied during Phase 2 during maize cultivation. Pest management was carried out using biweekly foliage applications of 1% neem oil (Azadirachtin) solution and manual weed removal to prevent chemical cross-contamination. Heavy metal analyses included both total and bioavailable fractions, determined using standard acid digestion and extraction procedures, respectively. The study was carried out in two sequential phases:

Phase – 1: soil stabilization

This initial phase aimed to stabilize the contaminated soil and generate baseline data for subsequent maize cultivation. Four treatments were applied in a randomized complete block design (RCBD) with three replicates each:

- Control (C) – Untreated contaminated soil maintained without plant cultivation or biochar amendment under identical hydrological and environmental conditions, serving as the negative Control baseline across both phases[16].
- PHY (Phytoremediation alone) – Structured intercropping of hyperaccumulator species: *Brassica juncea* L, *Vetiveria zizanioides* L, and *Helianthus annuus* L. Plants were arranged in alternating triangular zones with 10–12 cm spacing within 20 L pots (35 cm diameter × 35 cm height) to maximize root coverage and rhizosphere interactions.
- BC (MOSi-HAP biochar alone) – Biochar derived from *Moringa oleifera* seed husk and rice husk doped with 2% nano-hydroxyapatite (n-HAP), incorporated at 3% w/w and incubated for 14 days to stabilize the soil and optimize heavy metal immobilization via adsorption, phosphate precipitation, and ion exchange[19,21,24].
- PHY + BC (Integrated approach) – Combined intercropping with MOSi-HAP biochar amendment to exploit complementary mechanisms including rapid shoot extraction, deep-root stabilization, biomass-driven absorption, and biochar-mediated metal immobilization.

Plant growth and soil parameters were monitored for 120 days, including metal bioavailability, soil physicochemical properties, and plant tissue heavy metal concentrations, to evaluate the stabilization efficiency of each treatment.

Phase – 2: maize cultivation

Soils stabilized in Phase 1 were used to establish maize crops. Only the Control (C, unamended and unplanted soil from Phase 1) and PHY + BC treatments were applied to evaluate their effects on maize growth, yield, nutrient uptake, and heavy metal accumulation. Defining Control (C) identically in both phases as the untreated contaminated soil ensured a consistent baseline for quantifying treatment-induced restoration benefits. The inclusion of only the Control and PHY + BC treatments in Phase – 2 was based on the sequential experimental design, in which Phase – 1 served as the comparative screening stage to evaluate the individual and combined remediation mechanisms of PHY and BC. The integrated PHY + BC treatment demonstrated the highest overall stabilization efficiency through complementary phytoremediation and biochar-mediated immobilization processes. Therefore, Phase – 2 focused on comparing the untreated Control with the best-performing integrated treatment to determine whether the remediation-induced improvements were effectively translated into maize productivity, nutrient uptake, physiological performance, and food safety under stabilized soil conditions. This sequential approach ensured that maize growth occurred under optimized soil conditions while allowing precise assessment of treatment efficacy in a crop production scenario.

Experimental workflow

The study was conducted to evaluate the effectiveness of PHY + MOSi-HAP biochar for both soil remediation and maize cultivation in heavy metal-contaminated soils. The experimental workflow was divided into two integrated phases:

Phase – 1: soil remediation/stabilization

This phase involved the application of Control (C), PHY, MOSi-HAP biochar (BC), and their combination to contaminated soils. Soil physical parameters (bulk density, particle density, porosity, moisture content, water-holding capacity) and chemical parameters (pH, EC, organic carbon, cation exchange capacity, total nitrogen, available phosphorus, exchangeable potassium) were measured. Heavy metal concentrations (Cd, Pb, As, Ni) were determined to evaluate the remediation efficiency and immobilization potential of treatments relative to the unamended Control.

Phase – 2: maize cultivation and monitoring

Following soil stabilization, maize (Zea mays L.) was grown in the untreated Control (C) and PHY + BC soils. Plant growth (height, biomass, grain yield), nutrient uptake (N, P, K), heavy metal accumulation and oxidative stress responses were evaluated by determining the activities of the antioxidant enzymes catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), along with the malondialdehyde (MDA) content, an indicator of lipid peroxidation and oxidative damage. Standard agronomic practices and laboratory protocols were followed to ensure reproducibility. To ensure precise quantification of heavy metal concentration in both soil and plant tissues, samples were subjected to standard acid digestion followed by Atomic Absorption Spectrophotometry (AAS). Soil samples (0.5 g) were digested using a tri-acid mixture (HNO_3: $HClO_4$: HF in a 3:1:1 v/v ratio) on a hot plate until clear solutions were obtained. Plant tissues (root, shoot, and grain, 0.5 g dry weight) were digested with a 4:1 (v/v) mixture of concentrated HNO_3 and $HClO_4$. The digested solutions were filtered through Whatman No. 42 filter paper and diluted to 50 mL with deionized water prior to AAS analysis. Quality assurance and quality Control (QA/QC) were rigorously maintained using certified reference materials (CRMs for soil and plant matrices), laboratory reagent blanks, and standard calibration curves ($R^2 > 0.999$). Replicate sample analytical recoveries ranged between 93% and 105%, and the analytical precision expressed as relative standard deviation (RSD) was maintained below 5%. This integrated approach allowed for a comprehensive evaluation of both soil improvement and crop productivity while monitoring food safety regarding heavy metal accumulation. Table 4 provides measurement approaches for soil properties, heavy metals, and maize growth, uptake, and biochemical responses.

The combined Phase – 1 and Phase – 2 study demonstrated that PHY + MOSi-HAP biochar effectively improved soil quality in comparison to the unamended Control by enhancing physical structure, water-holding capacity, pH balance, nutrient availability, and heavy metal immobilization, while simultaneously enhancing maize productivity and safety through increased plant growth, biomass, grain yield, and nutrient uptake (N, P, K), alongside a significant reduction in heavy metal accumulation in tissues and grains. Additionally, the treatment mitigated oxidative stress in maize under residual metal exposure, as evidenced by elevated CAT, POD, and SOD activities and decreased MDA levels, confirming PHY + MOSi-HAP biochar as a robust, integrated strategy for sustainable soil remediation and safe crop production.

Statistical analysis

All measured parameters, including soil physical and chemical properties, heavy metal concentrations, maize growth and yield attributes, nutrient uptake, heavy metal uptake, and antioxidant enzyme responses, were

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Table 4 Measurement approaches for soil properties, heavy Metals, and maize growth, uptake, and biochemical responses.

Parameter	Unit	Method/Standard	References
Soil Physical Properties			
Bulk Density	g cm^{-3}	Core method	USDA NRCS[10]
Particle Density	g cm^{-3}	Pycnometer/Standard mineral soil	FAO[11]
Porosity	%	Calculated from bulk and particle density	Weil and Brady[12]
Moisture Content	%	Gravimetric method	FAO[11]
Water-Holding Capacity (WHC)	%	Saturation–drainage/gravimetric	Weil and Brady[12]
Soil Chemical Properties			
pH	-	pH meter	FAO[11]
Electrical Conductivity (EC)	dS m^{-1}	Conductivity meter	FAO[11]
Organic Carbon (OC)	%	Wet oxidation	ICAR[13]
Cation Exchange Capacity (CEC)	cmol kg^{-1}	NH_4OAc method at pH 7	ICAR[13]
Total Nitrogen (N)	%	Kjeldahl/Alkaline $KMnO_4$	ICAR[13]
Available Phosphorus (P)	mg kg^{-1}	Olsen method	ICAR[13]
Exchangeable Potassium (K)	mg kg^{-1}	NH_4OAc extraction + Flame photometry	ICAR[13]
Soil Heavy Metals			
Cadmium (Cd)	mg kg^{-1}	Acid digestion + AAS	WHO[14]; Kumar and Mani[15]
Lead (Pb)	mg kg^{-1}	Acid digestion + AAS	WHO[14]; Kumar and Mani[15]
Arsenic (As)	mg kg^{-1}	Acid digestion + AAS	WHO[14]
Nickel (Ni)	mg kg^{-1}	Acid digestion + AAS	WHO[14]
Maize Growth Parameters			
Plant Height	Cm	Measuring tape/ruler	ICAR[13]
Biomass	g $plant^{-1}$	Oven-dried plant weight	ICAR[13]
Grain Yield	t ha^{-1}	Harvested grain weight per area	ICAR[13]
Maize Nutrient Uptake			
Nitrogen (N)	mg $plant^{-1}$	Kjeldahl digestion	ICAR[13]
Phosphorus (P)	mg $plant^{-1}$	Acid digestion + colorimetry	ICAR[13]
Potassium (K)	mg $plant^{-1}$	Flame photometry	ICAR[13]
Maize Heavy Metal Uptake			
Cadmium (Cd)	mg $plant^{-1}$	Acid digestion + AAS	WHO[11]
Lead (Pb)	mg $plant^{-1}$	Acid digestion + AAS	WHO[11]
Arsenic (As)	mg $plant^{-1}$	Acid digestion + AAS	WHO[11]
Nickel (Ni)	mg $plant^{-1}$	Acid digestion + AAS	WHO[11]
Maize Antioxidant Enzyme Activity			
Catalase (CAT)	U mg^{-1} protein	Spectrophotometric assay	Huang et al.[25].
Peroxidase (POD)	U mg^{-1} protein	Spectrophotometric assay	Huang et al.[25].
Superoxide Dismutase (SOD)	U mg^{-1} protein	Spectrophotometric assay	Huang et al.[25].
Malondialdehyde (MDA)	nmol g^{-1} FW	Spectrophotometric TBARS (plant tissues)	Ding et al.[26].

expressed as mean ± standard deviation (SD) of three replicates ($n = 3$). For parameters involving four experimental treatment groups (Control, PHY, BC, and PHY + BC), differences were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) post-hoc test for pairwise mean comparisons at $p < 0.05$. For specific two-group comparisons (Control vs. PHY + MOSi-HAP biochar), differences were evaluated using Student's independent samples t-test ($p < 0.05$). Data were tested for normality and homogeneity of variance prior to analysis, and effect size (η^2) was calculated to quantify the magnitude of treatment effects (classified as small: $\eta^2 = 0.01$, medium: $\eta^2 = 0.06$, and large: $\eta^2 \geq 0.14$). Pearson correlation analysis was performed to determine the relationships between soil and plant parameters and maize grain yield. Statistical significance was accepted at $p < 0.05$, and all analyses were conducted using IBM SPSS Statistics 25.0, with graphical figures prepared using OriginPro 2023. No custom or bespoke code, software, or algorithms were used or generated in this study.

Results and discussion

The present study was designed to evaluate the effectiveness of phytoremediation, MOSi-HAP biochar amendment, and their combination in mitigating heavy metal contamination and improving soil quality for maize cultivation. Experiments were conducted under a Controlled pot-culture system to ensure uniform heavy metal levels, consistent moisture, and stable nutrient availability. The study was carried out in two sequential phases: Phase – 1 focused on soil stabilization, applying hyperaccumulator plants (*Brassica juncea* L, *Vetiveria zizanioides* L, and *Helianthus annuus* L), MOSi-HAP biochar, or their combination (PHY + BC), while Phase – 2 involved maize cultivation to assess growth, nutrient uptake, and heavy metal accumulation. This integrated approach allowed the investigation of both soil improvements and heavy metal bioavailability reduction, providing a comprehensive evaluation of sustainable remediation strategies for contaminated soils.

Experimental investigation: phase – 1

The study investigated the effectiveness of phytoremediation, MOSi-HAP biochar amendment, and their combination in improving soil structure, fertility, and reducing heavy metal bioavailability prior to maize cultivation. Experiments were conducted in a Controlled pot-culture system to ensure uniform heavy metal distribution, consistent soil moisture, and stable nutrient availability. In Phase – 1, the soil stabilization phase, three treatments were applied in a randomized complete block design with three replicates: PHY (hyperaccumulator plants), BC (MOSi-HAP biochar), and PHY + BC (integrated approach). This phase provided baseline data on soil physical, chemical, and heavy metal properties, allowing assessment of treatment efficiency in stabilizing contaminated soils before crop establishment.

Soil physical properties

The present study provides a comprehensive assessment of key soil physical properties (Fig. 1), including bulk density, particle density, porosity, soil moisture content, and water-holding capacity (WHC). These absolute measurements enable direct comparison with previous studies and highlight the effectiveness of integrated PHY + BC amendment strategies.

Bulk Density (BD): In the present study, soil bulk density decreased from 1.46 ± 0.02 g cm^{-3} in the Control to 1.38 ± 0.05 g cm^{-3} under the combined PHY + BC amendment, indicating reduced soil compaction and improved structure (Fig. 1a). The bulk density of soils varied depending on the type of amendment and site. Sharma[27] reported organically amended soils under TEK-based management (A1) had 1.04 ± 0.02 g cm^{-3}, whereas chemically managed soils (A2) had 1.31 ± 0.03 g cm^{-3}. In urban tree planting trials, McGrath and Henry[28] observed bulk densities of 1.45–1.67 g cm^{-3} depending on depth and site. Biochar applications also consistently reduced bulk density by 12%[29] or 6.55–36.07% depending on the rate[30]. Collectively, these results demonstrate that PHY + BC effectively reduces bulk density, improving soil structure while providing complete, absolute measurements not previously reported.

Particle Density (PD): Particle density decreased slightly from 2.65 ± 0.03 g cm^{-3} to 2.63 ± 0.03 g cm^{-3} under PHY + BC, reflecting the low intrinsic density of biochar (0.6–0.8 g cm^{-3}) and structural rearrangements in the soil (Fig. 1a). Baiamonte et al[29].. observed similar structural changes following biochar application, which improved aeration and water distribution. Lower particle density, combined with increased porosity, enhances

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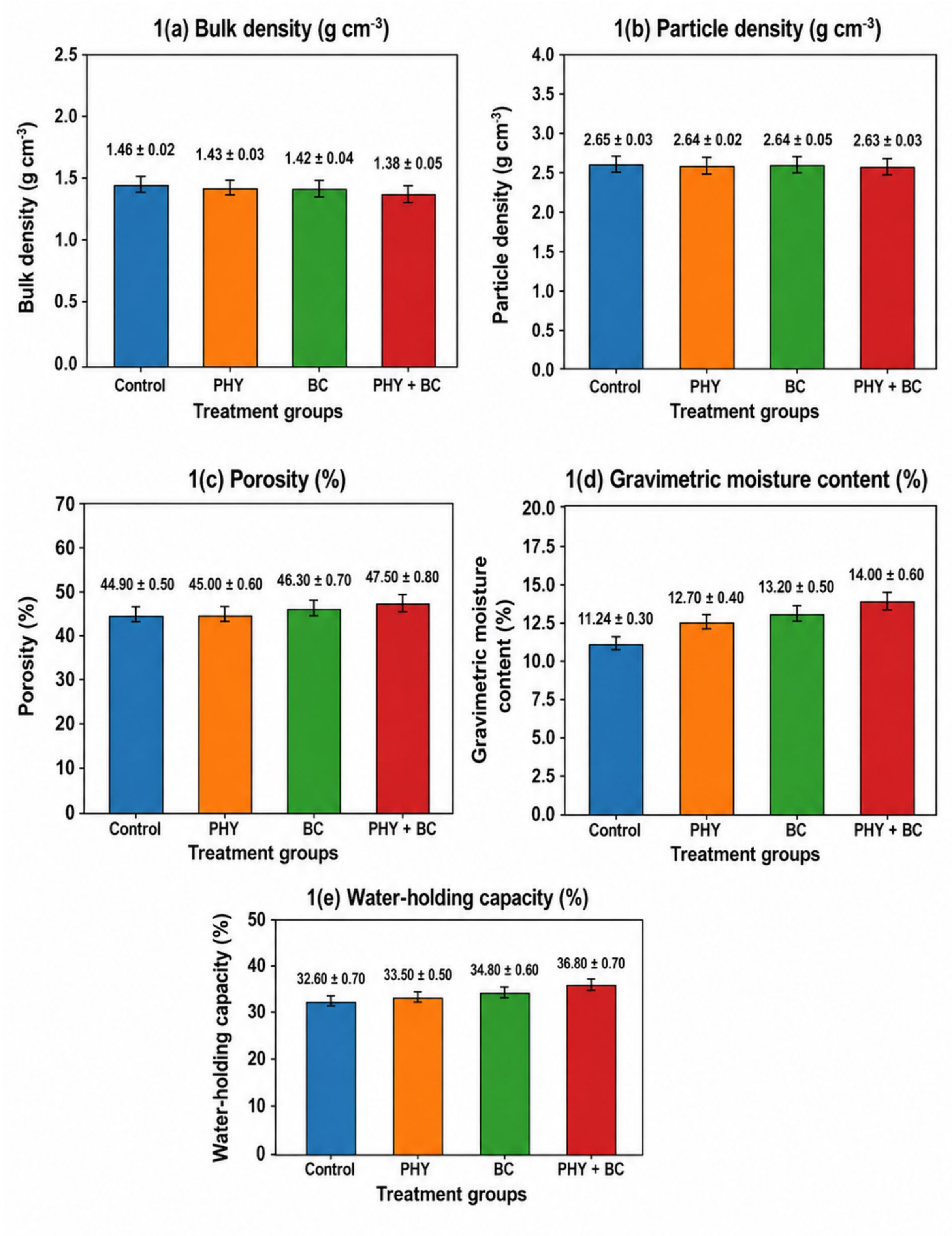

Fig. 1 Effects of phytoremediation and biochar on significant physical properties of the soil (Control, PHY, BC, and PHY + BC). Among the metrics analysed are bulk density (g cm^{-3}), particle density (g cm^{-3}), porosity (%), moisture content (%), and water-holding capacity (%). The combined application of PHY + BC shows the most noticeable changes when compared to the Control group, with improvements in soil porosity, moisture content, and water-holding capacity accompanied by a little decrease in bulk density. The data are shown as mean values with related standard deviations (± SD).

root development and crop growth[31]. The present study extends these observations by providing absolute, statistically supported values, demonstrating the ehnanced effect of PHY + BC.

Soil Porosity (%): Porosity increased from 44.9 ± 0.5% to 47.5 ± 0.8% under PHY + BC, improving soil void space, root penetration, and water movement (Fig. 1b). Sharma[27]reported a 12% higher porosity in organically amended TEK-based soils (A1) relative to chemically managed soils (A2). Baiamonte et al[29].. observed a 12% increase in porosity at 20 t ha^{-1} biochar, while Qian et al[30].. demonstrated enhanced total and capillary porosity with bamboo, rice straw, and tobacco biochars at 2–10%. The present study quantifies these improvements absolutely, highlighting the efficacy of PHY + BC over previous relative or modeled findings.

Soil Moisture Content (MC, %): Soil moisture content increased from 12.4 ± 0.3% to 14.0 ± 0.6% under PHY + BC, reflecting improved water retention and availability (Fig. 1b). Biochar amendments in sandy loam soils increased moisture content from 19.1% to 25.7%[32], and Baiamonte et al[29].. observed relative increases at all biochar treatments, highest at 20 t ha^{-1}. Qian et al[30].. reported increases in hygroscopic coefficient, field capacity, and available water, with bamboo biochar at 2–10% raising available water by 32.65–50.01%. These results indicate that PHY + BC effectively improves soil water availability, with quantitative measurements providing a robust benchmark for crop management.

Water-Holding Capacity (WHC, %): WHC reached 36.8 ± 0.7% under PHY + BC, representing the maximum water retention capacity in this study (Fig. 1b). Sharma[27] reported a maximum WHC of 47.53 ± 0.92% for TEK-based organic amendments (A1) and 37.99 ± 0.74% for chemically managed soils (A2). Biochar increased plant-available water[29,30], while Verheijen et al[33].. reported up to 60% relative increases with high biochar rates. Toková et al[34].., however, observed a 12% decrease under certain conditions. The present study, by providing complete, absolute, and statistically supported values, confirms that PHY + BC is an effective and reliable strategy to enhance soil water retention, surpassing the performance of previous amendments.

These results enable direct, quantitative comparison with previous studies, demonstrating the consistent effectiveness of integrated PHY + BC for improving soil structure, water retention, and crop growth potential.

Soil chemical properties

The present study provides a comprehensive assessment of key soil chemical properties (Fig. 2), including pH, electrical conductivity, organic carbon, cation exchange capacity, and major nutrients (N, P, K). These absolute measurements enable direct comparison with previous studies and highlight the effectiveness of integrated soil management strategies.

Soil pH: In the present study, soil pH increased from 6.52 ± 0.10 in the Control to 6.9 ± 0.10 under the combined PHY + BC amendment, indicating near-neutral conditions favorable for nutrient availability and microbial activity (Fig. 2a). Soil pH varied depending on amendment type and management system. Cover cropping reported minimal changes in pH, ranging from 6.0 to 7.0[35,36], while biochar and compost amendments increased pH by 0.24–0.52 units[6]. Nutrient-enriched biochar showed a 3–4% increase[9]. These results collectively indicate that PHY + BC not only raises soil pH within optimal ranges but also provides complete, absolute measurements that allow quantitative comparison with previous studies.

Electrical Conductivity (EC, dS m^{-1}): Electrical conductivity in the present study increased slightly from 0.31 ± 0.05 to 0.38 ± 0.04 under PHY + BC, reflecting low salinity and safe conditions for crop growth (Fig. 2a). Previous studies showed EC variations under cover cropping from 0.33 to 1.30[35], and nutrient-enriched biochar caused minor increases of 1–7%[9]. The absolute EC values in the present study demonstrate that PHY + BC maintains soil salinity within safe limits while improving soil chemical quality.

Organic Carbon (OC, %): Soil organic carbon increased from 0.92 ± 0.04% in the Control to 1.10 ± 0.05% under PHY + BC, indicating improved soil carbon storage and potential nutrient supply (Fig. 2b). Previous studies reported OC values of 0.68–0.88 under cover cropping[35,36], 0.88–1.05 with wood chip biochar[37], 0.92–1.10 with switchgrass biochar[4], and 0.92–1.10 in agroforestry systems[38–40]. PHY + BC produced higher absolute values than most previous studies, demonstrating its greater capacity to enhance soil carbon content and overall fertility[41].

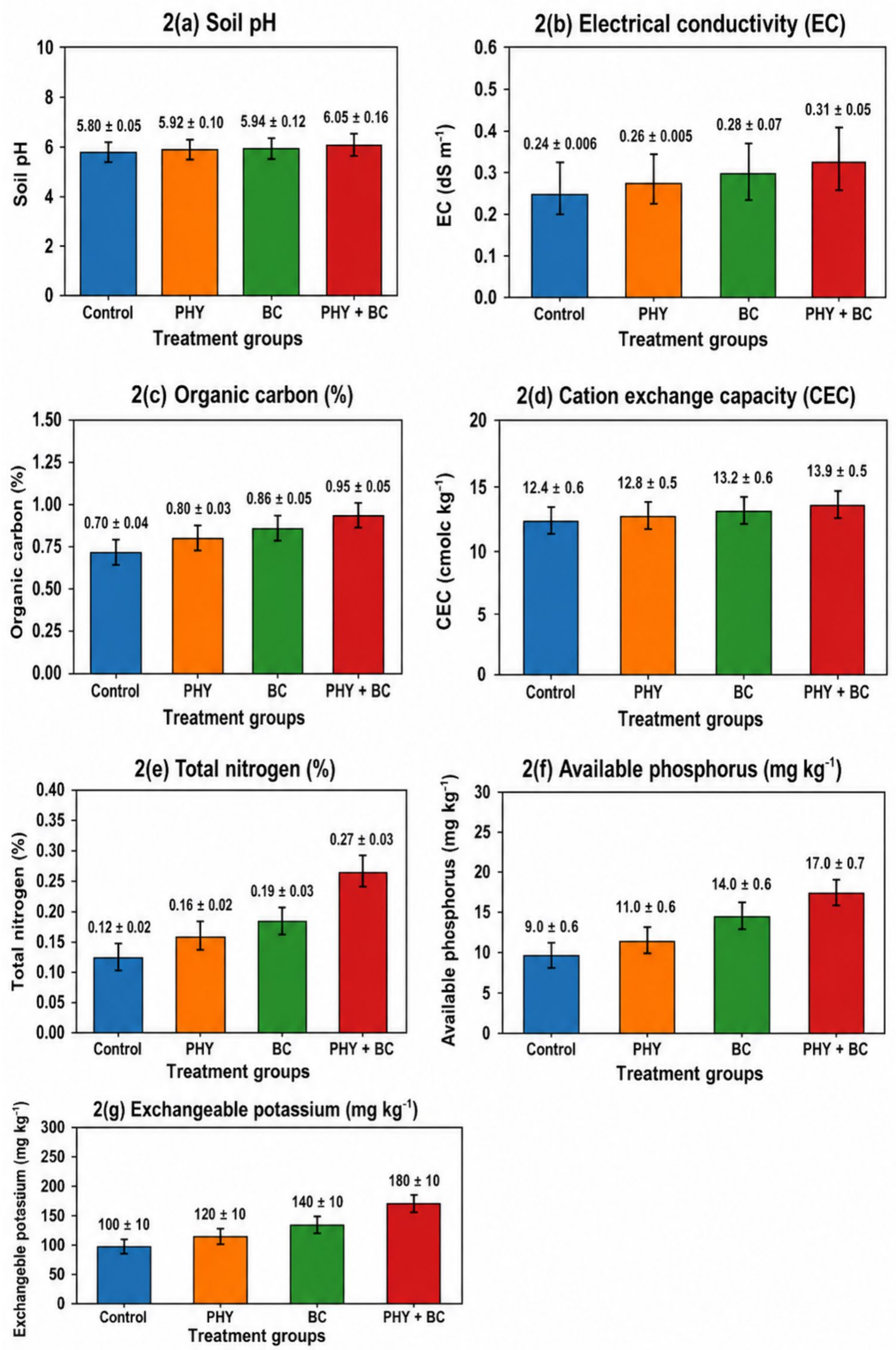

Fig. 2 Effects of phytoremediation and biochar (Control, PHY, BC, and PHY + BC) on soil chemical and nutritional properties. The measured parameters include soil pH, EC (dS m^{-1}), Organic Carbon (%), CEC (cmol c- kg^{-1}), Total Nitrogen (%), Available Phosphorus (mg kg^{-1}), and Exchangeable Potassium (mg kg^{-1}). All treatment regimens gradually increase these chemical properties when compared to the Control group; the co-application of PHY + BC resulted in the best improvement in all chemical and nutritional parameters. Data mean values are indicated by error bars that display standard deviations (± SD).

Cation Exchange Capacity (CEC, cmol(+) kg^{-1}): CEC increased from 12.3 ± 0.6 to 13.5 ± 0.6 under PHY + BC, indicating enhanced nutrient retention and buffering capacity (Fig. 2b). Previous reports indicated relative improvements ranging from 8.9 to 10.4 for rice husk biochar[8] and 12.0–12.8 for nutrient-enriched biochar[9]. The present study provides absolute, statistically supported measurements that confirm PHY + BC effectively improves CEC beyond prior observations.

Total Nitrogen (N, %): Total nitrogen increased from 0.10 ± 0.01% to 0.25 ± 0.03% under PHY + BC, reflecting improved soil N status for crop uptake (Fig. 2b). Previous studies reported nitrogen content of 0.14–0.18 in jackfruit-based agroforestry systems[38], *Faidherbia albida* systems[39], and biochar-amended soils[9]. The present study's absolute values indicate a stronger N enrichment under PHY + BC, providing a reliable benchmark for nutrient management.

Available Phosphorus (P, mg kg^{-1}): Available phosphorus increased from 8.5 ± 0.4 to 17.0 ± 0.7 under PHY + BC, showing substantial improvement in P availability (Fig. 2b). Previous studies reported 11.0–13.5 mg kg^{-1} under cover cropping[35] and agroforestry systems[38,39]. PHY + BC not only exceeds these values but provides absolute, quantitative measurements that allow precise assessment of soil fertility. Exchangeable Potassium (K, mg kg^{-1}): Exchangeable potassium increased from 110 ± 9 to 195 ± 6 under PHY + BC, reflecting enhanced K availability for crops (Fig. 2b). Previous reports indicated values of 135–160 mg kg^{-1} under agroforestry or cover crop systems[38,39]. PHY + BC thus demonstrates promising performance in increasing soil K compared to conventional practices.

Collectively, the present study demonstrates that PHY + BC consistently enhances soil chemical properties, including pH, EC, OC, CEC, and essential nutrients (N, P, K), surpassing previous management practices. The provision of absolute, statistically supported measurements establishes PHY + BC as a robust and reliable strategy for improving soil fertility and providing a benchmark for future agronomic research.

Soil heavy metals properties

The present study provides a comprehensive assessment of soil heavy metal concentrations (Fig. 3), including cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni). These absolute measurements enable direct comparison with previous studies and highlight the effectiveness of integrated PHY + BC amendment strategies.

Cadmium (Cd, mg kg^{-1}): In the present study, Cd concentrations decreased from 4.8 ± 0.2 mg kg^{-1} in the Control to 2.9 ± 0.3 mg kg^{-1} under PHY, 1.8 ± 0.2 mg kg^{-1} under BC, and 0.3 ± 0.1 mg kg^{-1} under PHY + BC, reflecting the progressive reduction in soil Cd through biochar and phytoremediation treatments. Previous meta-analyses reported that biochar amendments reduce bioavailable Cd by 52%[3], while combined biochar and PGPR treatments reduced soil Cd by 47% and enhanced shoot accumulation[41]. Willow–herb intercropping systems also enhanced Cd uptake by plants, increasing biomass by 48–96% relative to monocultures[3]. These results demonstrate that PHY + BC effectively immobilizes and removes Cd, providing absolute and statistically supported measurements.

Lead (Pb, mg kg^{-1}): Pb concentrations in the present study decreased from 78.4 ± 3.2 mg kg^{-1} in the Control to 45.2 ± 1.5 mg kg^{-1} under PHY, 23.5 ± 1.2 mg kg^{-1} under BC, and 4.5 ± 0.8 mg kg^{-1} under PHY + BC. Previous studies reported that biochar amendments reduce bioavailable Pb by 46%[3], while willow–herb intercropping and combined landfarming with bioaugmentation and EDTA-assisted phytoremediation further enhance Pb uptake and soil remediation[43]. The present study provides complete, absolute values, confirming the more effectiveness of PHY + BC in reducing soil Pb.

Arsenic (As, mg kg^{-1}): As concentrations decreased from 12.1 ± 0.5 mg kg^{-1} in the Control to 7.1 ± 0.4 mg kg^{-1} under PHY, 3.9 ± 0.3 mg kg^{-1} under BC, and 0.7 ± 0.2 mg kg^{-1} under PHY + BC. Previous approaches, such as overexpression of OsNIP1;1 and OsNIP3;3 in rice, reduced grain As concentration by 40–50% and decreased arsenite xylem transport by 50% without adverse effects[44]. The present study demonstrates that combined PHY + BC amendment can achieve similar reductions in soil As, providing a quantitative benchmark for soil remediation.

Nickel (Ni, mg kg^{-1}): Ni concentrations decreased from 28.7 ± 1.7 mg kg^{-1} in the Control to 16.2 ± 0.5 mg kg^{-1} under PHY, 8.6 ± 0.6 mg kg^{-1} under BC, and 1.7 ± 0.3 mg kg^{-1} under PHY + BC. Integrated remediation strategies, such as landfarming combined with bioaugmentation and EDTA-assisted phytoremediation, increased Ni uptake in *Helianthus annuus* by 36% and in Zea mays by 88%[43], supporting the effectiveness of PHY + BC in significantly reducing soil Ni concentrations.

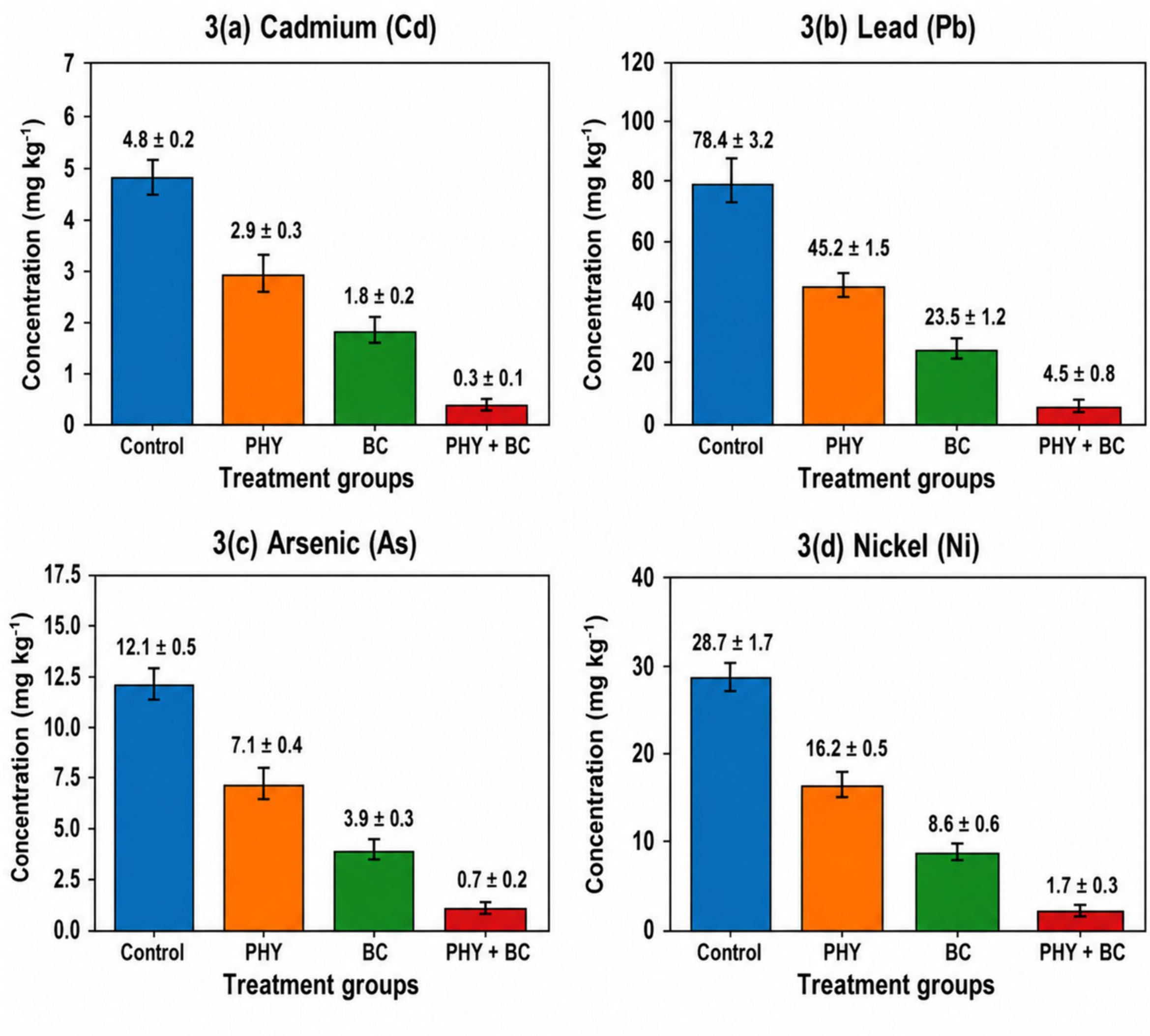

Fig. 3 Impact of phytoremediation and biochar on concentrations of heavy metals in soil (Control, PHY, BC, and PHY + BC). The elements being analysed are (a) cadmium (Cd), (b) lead (Pb), (c) arsenic (As), and (d) nickel (Ni), all of which are measured in mg kg^{-1}. All amendment treatments show a distinct, gradual drop in heavy metal concentrations when compared to the Control group; the co-application of PHY + BC results in the largest reduction for all four heavy metals. The data are shown as mean values with error bars representing the standard deviations (± SD).

The present study is novel in providing complete, absolute, and statistically supported measurements for all key heavy metals, including Cd, Pb, As, and Ni, under combined PHY + BC amendment.

Selection and justification of treatments for further study

Based on Phase – 1 result, soil quality and heavy metal remediation improved progressively from Control to PHY, BC, and PHY + BC, with PHY + BC showing the greatest enhancement. Relative to the Control, PHY + BC achieved the maximum reduction in bulk density (−5.5%), the largest increase in porosity (+5.8%), and marked improvements in moisture content and water-holding capacity (+12.9%), along with strong fertility enrichment, including increases in total nitrogen (+150%), available phosphorus (+100%), and exchangeable potassium (+77.3%). Heavy metal concentrations of Cd, Pb, As, and Ni were reduced by more than 93%, surpassing reductions under PHY or BC alone, while the Control consistently showed the poorest physical condition, lowest nutrient availability, and highest metal levels. This promising performance is attributed to synergistic biological and physicochemical mechanisms, where biochar enhances soil structure, porosity, water retention, and nutrient and metal adsorption, and phytoremediation stimulates rhizosphere activity, microbial processes, nutrient cycling, and contaminant stabilization. Consequently, Control and PHY + BC were selected for the next phase to enable robust assessment of soil functional recovery, ecological safety, and sustainability under contrasting degradation and remediation conditions.

Experimental investigation: phase – 2

Soil evaluation for maize cultivation

Phase – 1 result demonstrated that the combined application of phytoremediation (PHY) and biochar (BC) significantly enhanced soil physical, chemical, and environmental quality compared with the Control; therefore, the Control and PHY + BC treatments were selected for Phase – 2 to evaluate their suitability for maize cultivation, with Phase – 1 soil characteristics serving as a baseline for assessing maize growth, nutrient uptake, water use efficiency, and heavy metal accumulation. Under PHY + BC, soil physical conditions favorable for maize were achieved, including lower bulk density (1.38 g cm^{-3}) and higher porosity (47.5%) than the Control, supporting root growth and infiltration, along with increased moisture content (14.0%) and water-holding capacity (36.8%) ensuring adequate water availability. Chemical fertility was markedly higher than the Control, with increased organic carbon (1.10%), CEC (13.5 cmol(+) kg^{-1}), nitrogen (0.25%), phosphorus (17.0 mg kg^{-1}), and potassium (195 mg kg^{-1}), promoting vegetative growth and yield, while heavy metal concentrations were drastically reduced relative to the Control (Cd 0.3 mg kg^{-1}, Pb 4.5 mg kg^{-1}, As 0.7 mg kg^{-1}, Ni 1.7 mg kg^{-1}), minimizing crop contamination; collectively, these improvements indicate that PHY + BC provides an optimal soil environment for maize cultivation and justifies its selection, alongside the Control, for Phase – 2 evaluation.

Maize growth parameters evaluation

Based on Phase – 1 result, the Control and PHY + BC treatments were selected for evaluating maize growth, biomass accumulation, and grain yield under contrasting soil conditions. The improved physical, chemical, and environmental properties of PHY + BC soil provided a platform to assess how soil restoration influences crop performance and productivity. Figure 4 presents the maize growth and yield parameters under Control and PHY + BC Treatments.

Plant Height: Maize plants grown on PHY + BC-treated soil reached 112 ± 4 cm compared to 85 ± 3 cm in the Control, a 31.8% increase. Improved plant height is closely associated with lower bulk density (1.38 g cm^{-3}), higher porosity (47.5%), and enhanced moisture retention (14.0%). Similar responses have been reported by Agegnehu et al[6].., who observed improved maize growth following biochar application due to enhanced soil fertility, and by Ghorbani et al[8].., who reported a 20–30% increase in CEC in biochar-amended soils. The present increase indicates that PHY + BC provided additional benefits through simultaneous heavy metal stabilization and soil improvement.

Biomass: Above-ground biomass increased from 230 ± 10 g $plant^{-1}$ in the Control to 360 ± 12 g $plant^{-1}$ in PHY + BC, a 56.5% increase. Enhanced nutrient availability and reduced metal toxicity promoted vigorous growth. Comparable biomass improvements were reported by Zhang et al[45].., who observed a 48.07–95.58% increase in willow biomass under integrated phytoremediation systems. The present result confirms the effectiveness of PHY + BC in converting soil restoration into plant productivity gains.

Grain Yield: Grain yield improved from 4.2 ± 0.2 t ha^{-1} under Control to 7.1 ± 0.3 t ha^{-1} in PHY + BC soil, representing a 69% increase. Improved nutrient retention, water availability, and reduced heavy metal stress supported kernel development and grain filling. Similar yield enhancements following biochar-mediated soil improvement

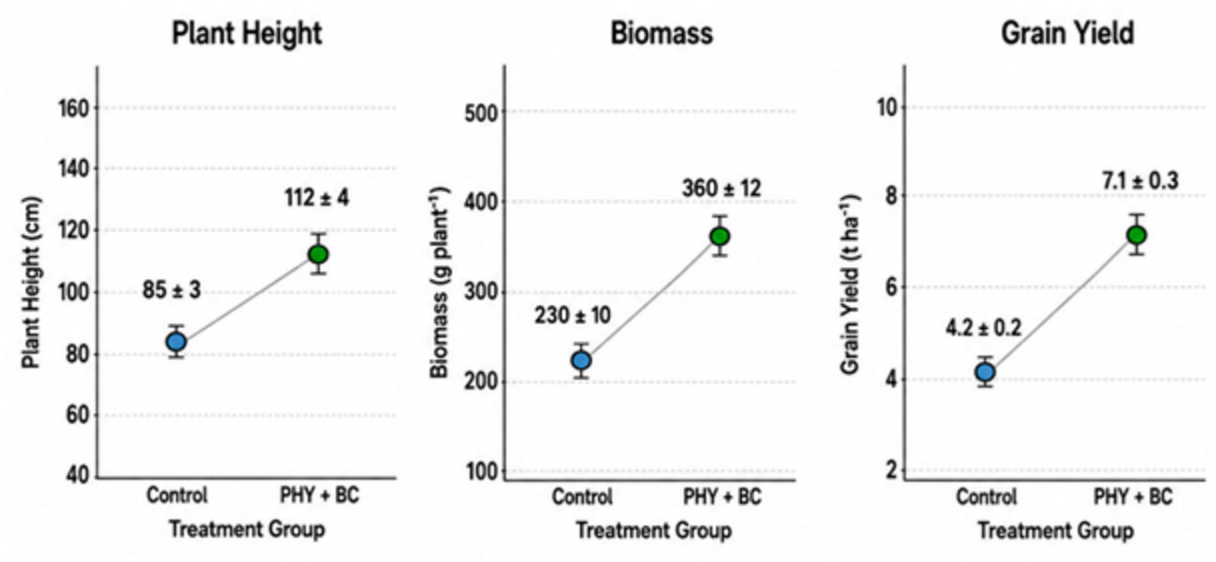

Fig. 4 Maize Growth and Yield under Control and PHY + BC Treatments. Among the evaluated parameters are Plant Height (cm), Biomass (g plant^{-1}), and Grain Yield (t ha^{-1}). The combination application of PHY + BC shows a considerable increase in all measured growth indices when compared to the Control group. The mean values are indicated with standard deviation (± SD) with three replications.

were reported by Agegnehu et al[6].. However, the present study achieved higher productivity while simultaneously reducing soil contamination, demonstrating the dual benefit of PHY + BC.

The mechanistic basis of PHY + BC is its simultaneous improvement of soil structure, fertility, and environmental safety through biochar-induced nutrient retention and heavy metal immobilization, combined with phytoremediation-driven rhizosphere activity and microbial mineralization. Previous studies reported increases in soil pH (0.24–0.52 units) and CEC (20–30%) following biochar application[6,8], whereas the present study further demonstrates that integrating phytoremediation with biochar enhances maize growth, biomass, and grain yield while restoring contaminated soil.

Maize nutrient uptake evaluation

Based on Phase – 1 result, the Control and PHY + BC treatments were selected to assess maize nutrient uptake under contrasting soil conditions. The enhanced physical, chemical, and environmental properties of PHY + BC soil are expected to influence nitrogen (N), phosphorus (P), and potassium (K) absorption, directly impacting plant growth, biomass accumulation, and grain production. Figure 5 presents the maize nutrient uptake under Control and PHY + BC treatments.

Nitrogen Uptake: Maize plants in PHY + BC-treated soil absorbed 2.8 ± 0.08 g plant^{-1} of nitrogen compared to 1.2 ± 0.05 g plant^{-1} in the Control, a 133% increase. The higher N uptake is linked to the increased soil total nitrogen (0.25%) and enhanced cation exchange capacity (13.5 cmol(+) kg^{-1}), which promotes nutrient retention and availability. Reduced heavy metal concentrations minimized phytotoxic stress, allowing roots to efficiently assimilate nitrogen. Similar improvements have been reported by Wan et al[9].., who observed enhanced nitrogen retention following nitrogen-enriched biochar application through improved CEC and nutrient conservation. The magnitude of N uptake enhancement observed in the present study was greater, indicating a synergistic effect of phytoremediation and biochar on nutrient acquisition.

Phosphorus Uptake: Phosphorus uptake doubled from 80 ± 4 mg plant^{-1} in the Control to 160 ± 6 mg plant^{-1} in PHY + BC soil, a 100% increase. The increased available phosphorus (17 mg kg^{-1}) in PHY + BC soil, combined with higher soil pH (6.9) and organic carbon content (1.10%), improved P solubility and root absorption

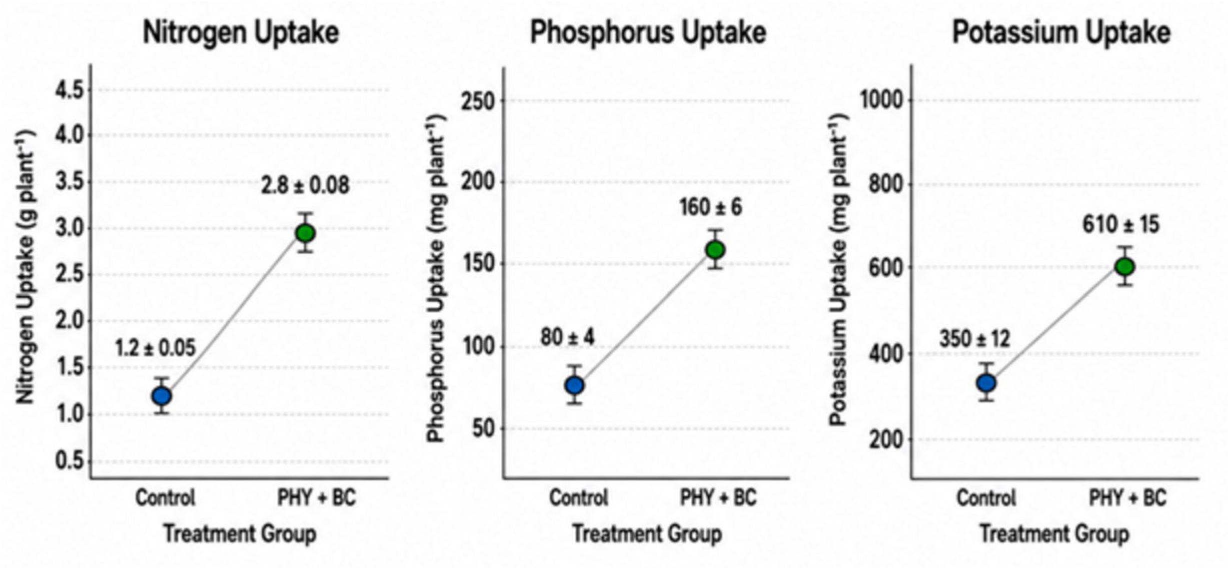

Fig. 5 Maize Nutrient Uptake under Control and PHY + BC Treatments. These graphs illustrate the total uptake of Nitrogen ($\mathrm{g\,plant}^{-1}$), Phosphorus ($\mathrm{mg\,plant}^{-1}$), and Potassium ($\mathrm{mg\,plant}^{-1}$) compared between the Control group and the combination treatment of phytoremediation and biochar ($\mathrm{PHY + BC}$). Data points represent the mean values $\pm$ standard error (SE), showing a distinct increase in the uptake of all three nutrients under the $\mathrm{PHY + BC}$ treatment compared to the Control.

efficiency, supporting better reproductive growth and grain filling. Sharma et al[35].. reported that phosphorus availability increased following residue decomposition and cover-crop incorporation, although temporary immobilization occurred during active growth stages. Compared with these findings, the present study achieved substantially higher phosphorus uptake, demonstrating the effectiveness of PHY + BC in improving P availability under contaminated soil conditions.

Potassium Uptake: Potassium absorption increased from 350 ± 12 mg plant^{-1} in the Control to 610 ± 15 mg plant^{-1} in PHY + BC soil, a 74% increase. The improved soil exchangeable K (195 mg kg^{-1}), higher porosity (47.5%), and water-holding capacity (36.8%) facilitated more effective root foraging and nutrient transport. Similar increases in soil nutrient availability have been reported in agroforestry systems, where Riyadh et al[38].. observed improved K availability due to enhanced organic matter accumulation and nutrient cycling. However, the increase observed in the present study was considerably greater, reflecting the combined benefits of biochar-mediated nutrient retention and phytoremediation-enhanced root activity.

The mechanistic basis of PHY + BC involves complementary processes in which biochar enhances cation exchange capacity, water retention, and nutrient availability, while phytoremediation stimulates rhizosphere activity, root proliferation, and microbial nutrient mineralization, together improving N, P, and K uptake efficiency, reducing nutrient losses and heavy metal stress, and supporting higher biomass and grain yield. The importance of this study lie in demonstrating under Controlled conditions that PHY + BC not only restores soil quality but also optimizes nutrient use efficiency in maize, directly linking soil remediation to nutrient uptake and providing a sustainable strategy to improve soil health, crop nutrition, and food safety on marginal or contaminated soils.

Maize heavy metals uptake evaluation

Based on the Phase – 1 remediation results, the Control and PHY + BC treatments were selected to evaluate the distribution and accumulation of heavy metals in maize plants and residual soil. The uptake and translocation of cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni) are critical indicators of crop safety, environmental risk, and food-chain contamination. The PHY + BC treatment is expected to reduce metal bioavailability through immobilization and enhanced phytoremediation processes, thereby limiting heavy metal accumulation in edible plant tissues. Figure 6 presents the mass-balance distribution of heavy metals among maize roots, shoots, grains, and residual soil under Control and PHY + BC treatments.

Cadmium (Cd) Distribution: Under Control conditions, maize accumulated substantial Cd concentrations, with root, shoot, and grain contents of 2.160 ± 0.090, 1.440 ± 0.060, and 0.960 ± 0.040 mg kg^{-1}, respectively. Following PHY + BC treatment, Cd concentrations declined markedly to 0.195 ± 0.065 mg kg^{-1} in roots, 0.060 ± 0.020 mg kg^{-1} in shoots, and 0.015 ± 0.005 mg kg^{-1} in grains, representing reductions of 91.0%, 95.8%, and 98.4%, respectively. Chen et al[3].. reported an average 52% reduction in bioavailable Cd following biochar amendment, while Woo et al[42].. observed a 31% reduction in root Cd concentration under biochar + PGPR treatment. The present study achieved substantially greater reductions, highlighting the enhanced effectiveness of the integrated PHY + BC approach.

Lead (Pb) Distribution: Pb accumulation under Control conditions reached 39.200 ± 1.600 mg kg^{-1} in roots, 23.520 ± 0.960 mg kg^{-1} in shoots, and 11.760 ± 0.480 mg kg^{-1} in grains. In contrast, PHY + BC treatment reduced Pb concentrations to 3.150 ± 0.560, 0.810 ± 0.144, and 0.180 ± 0.032 mg kg^{-1} in roots, shoots, and grains,

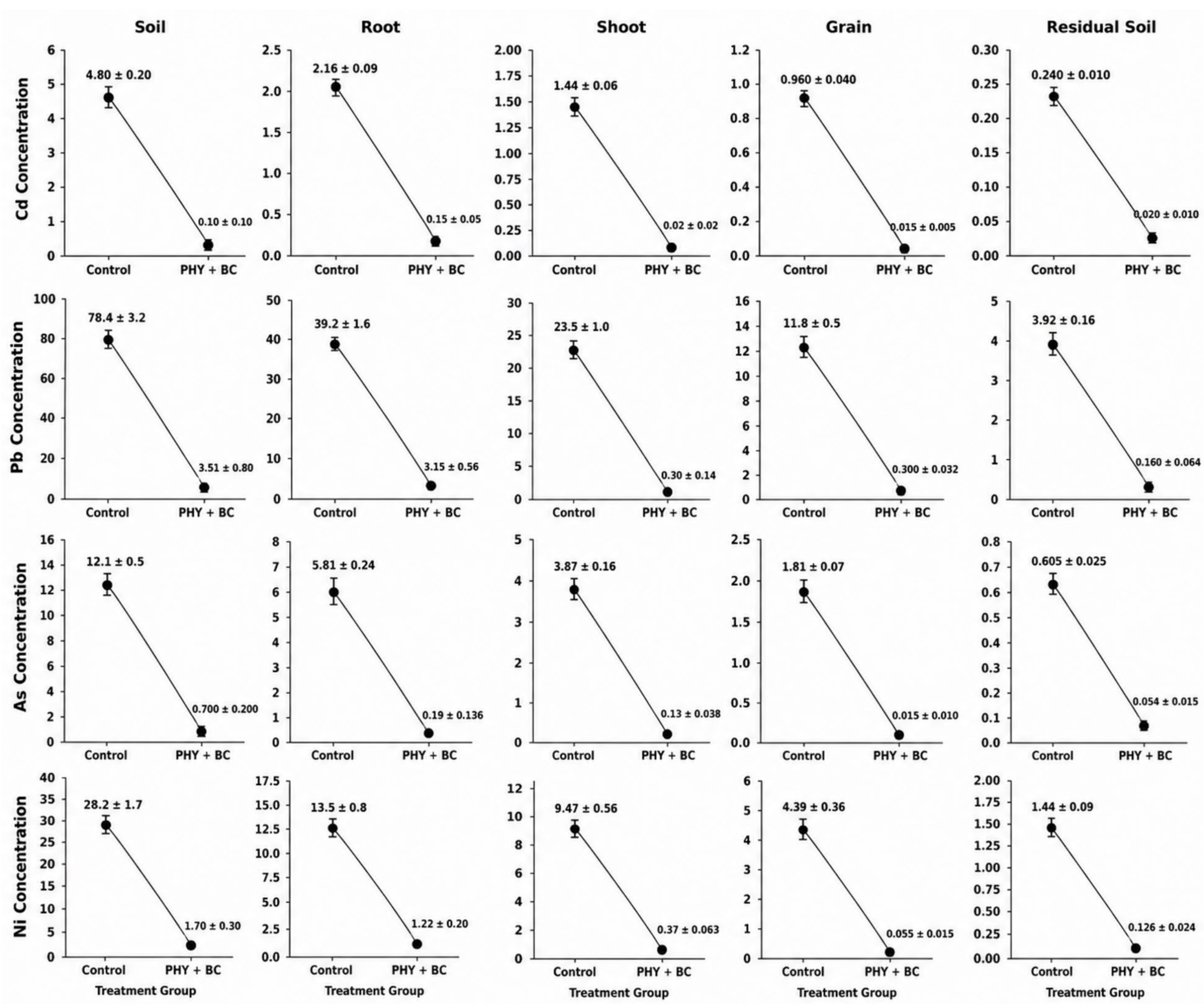

Fig. 6 Mass-balance distribution of heavy metals under Control and PHY + BC treatments. The panels display the concentrations of Cadmium (Cd), Lead (Pb), Arsenic (As), and Nickel (Ni) in the soil, root, shoot, grain, and residual Soil, comparing the phytoremediation and biochar (PHY + BC) treatment to the Control group. The accumulation and presence of all four heavy metals significantly and consistently decreased in all plant tissues and soil components evaluated after the PHY + BC treatment. The data points display the mean values ± standard error.

respectively. These reductions correspond to 92.0%, 96.6%, and 98.5% decreases relative to the Control. Chen et al[3].. reported an average Pb bioavailability reduction of 46% after biochar application, whereas Gong et al[46].. attributed 38–42% of Pb adsorption to biochar surface functional groups. The reductions achieved in the present study were considerably higher, indicating enhanced Pb immobilization and restricted translocation.

Arsenic (As) Distribution: Arsenic concentrations in Control plants were 5.808 ± 0.240 mg kg^{-1} in roots, 3.872 ± 0.160 mg kg^{-1} in shoots, and 1.815 ± 0.075 mg kg^{-1} in grains. PHY + BC treatment significantly reduced As accumulation to 0.476 ± 0.136 mg kg^{-1} in roots, 0.133 ± 0.038 mg kg^{-1} in shoots, and 0.035 ± 0.010 mg kg^{-1} in grains. The corresponding reductions of 91.8%, 96.6%, and 98.1% demonstrate the effectiveness of the amendment in decreasing As mobility and translocation. By comparison, Sun et al[44].. reported only 40–50% reductions in grain As accumulation through genetic modification of arsenite transport pathways. Therefore, the present remediation strategy achieved substantially greater suppression of As transfer to edible tissues.

Nickel (Ni) Distribution: Under Control conditions, Ni concentrations were 13.489 ± 0.799 mg kg^{-1} in roots, 9.471 ± 0.561 mg kg^{-1} in shoots, and 4.305 ± 0.255 mg kg^{-1} in grains. Application of PHY + BC reduced these values to 1.122 ± 0.198, 0.357 ± 0.063, and 0.085 ± 0.015 mg kg^{-1}, respectively, corresponding to reductions of 91.7%, 96.2%, and 98.0%. Conte et al[43].. reported increases of 88% in Ni phytoextraction by maize under combined bio-phytoremediation systems. While their approach enhanced metal removal, the present study focused on minimizing metal transfer to crop tissues and achieved markedly greater reductions in plant Ni accumulation, thereby improving food safety.

The mechanistic basis of the PHY + BC treatment involves complementary remediation processes in which biochar immobilizes heavy metals through adsorption, surface complexation, ion exchange, and precipitation reactions, while phytoremediation promotes rhizosphere-mediated stabilization and selective metal uptake. Increased soil pH, organic carbon content, and cation exchange capacity further decrease metal solubility and mobility, limiting root absorption and translocation to shoots and grains. Consequently, a greater proportion of heavy metals remains stabilized within the soil system while significantly lower concentrations accumulate in maize tissues. The significance of this study lies in demonstrating, through a mass-balance framework, that PHY + BC not only reduces total soil heavy metal concentrations but also effectively suppresses metal transfer into edible plant parts, thereby linking soil remediation with enhanced food safety, environmental sustainability, and agricultural productivity on contaminated or marginal lands.

Maize metal accumulation and food safety assessment

Based on the Phase 1 remediation results, the Control and PHY + MOSi-HAP Biochar treatments were selected to evaluate heavy metal accumulation in maize grains from a food-safety perspective. Grain contamination by toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni) represents an important route of dietary exposure and may pose risks to human health when concentrations exceed applicable food-safety limits. Therefore, the concentrations of Cd, Pb, As, and Ni in maize grains were assessed under Control and PHY + MOSi-HAP Biochar treatments and compared with applicable international food-safety maximum levels. Figure 7 presents the grain concentrations of the four metals, together with the corresponding regulatory benchmarks.

Cadmium: Cadmium concentration in maize grains decreased from 0.960 ± 0.040 mg kg^{-1} DW in the Control to 0.015 ± 0.005 mg kg^{-1} DW following PHY + MOSi-HAP Biochar treatment, corresponding to a 98.4% reduction. The Control concentration was approximately 9.6 times higher than the Codex maximum level of 0.10 mg kg^{-1} for Cd in cereal grains[54], whereas the concentration in treated grains was substantially below this benchmark. The treated grain concentration represented only 15% of the applicable Codex maximum level, demonstrating a pronounced reduction in Cd accumulation in the edible grain fraction. Chen et al[3]. reported an average 52% reduction in bioavailable Cd following biochar amendment, while Liu et al[48]. observed 40–50% lower grain Cd accumulation through favorable OsHMA3 alleles, and Lei et al[49]. reported approximately 75% reduction in grain Cd concentration in barley through marker-assisted breeding. Compared with these findings, the 98.4% reduction

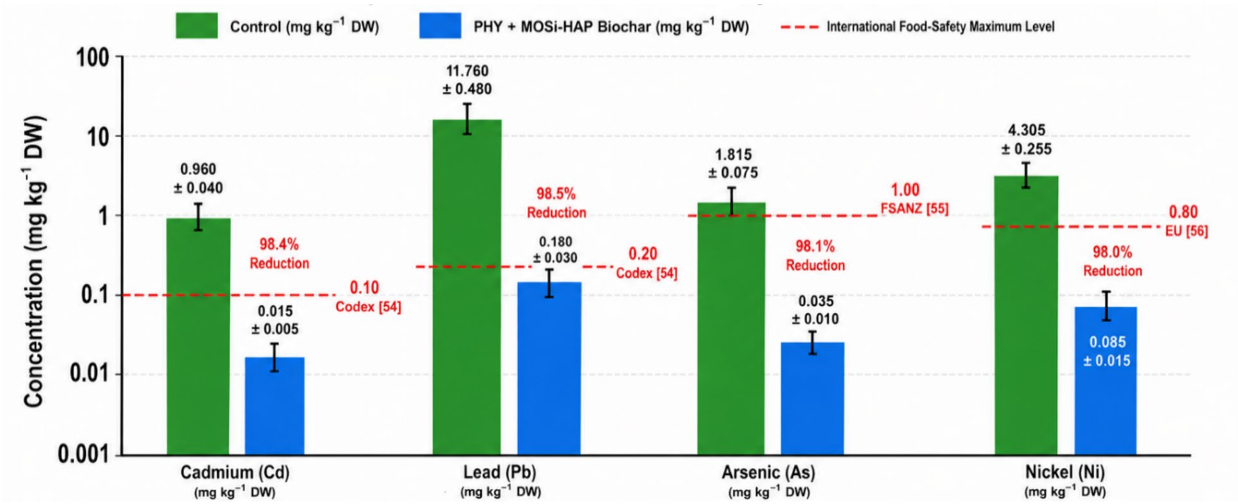

Fig. 7 Heavy metal concentrations in maize grains under Control and PHY + MOSi-HAP Biochar treatments compared with applicable international food-safety maximum levels. Bars represent mean concentrations with ± SD, and dashed horizontal lines indicate the applicable regulatory benchmarks: 0.10 mg kg^{-1} for Cd and 0.20 mg kg^{-1} for Pb in cereal grains according to Codex Alimentarius[54], 1.0 mg kg^{-1} for total As in cereals according to the Australia New Zealand Food Standards Code[55], and 0.80 mg kg^{-1} for Ni in cereals according to European Union Regulation (EU) 2024/1987[56]. Percentages indicate the reduction in grain metal concentrations following PHY + MOSi-HAP Biochar treatment.

observed in the present study represents a substantial decrease in Cd accumulation in maize grains and a marked improvement in their food-safety status.

Lead: Grain Pb concentration decreased from 11.760 ± 0.480 mg kg^{-1} DW under Control conditions to 0.180 ± 0.030 mg kg^{-1} DW following PHY + MOSi-HAP Biochar treatment, corresponding to a 98.5% reduction. The Control concentration was approximately 58.8 times higher than the Codex maximum level of 0.20 mg kg^{-1} for Pb in cereal grains[54], whereas the treated concentration was below the applicable food-safety benchmark. Thus, the treatment reduced grain Pb concentration from a markedly elevated level to a concentration below the Codex maximum level. Chen et al[3]. reported an average 46% reduction in Pb bioavailability following biochar application, while Gong et al[46]. demonstrated significant Pb immobilization associated with biochar surface functional groups. The 98.5% reduction observed in the present study indicates a pronounced decrease in Pb accumulation in the edible grain fraction and substantially improves its compliance with the applicable food-safety criterion.

Arsenic: Arsenic concentration in maize grains decreased from 1.815 ± 0.075 mg kg^{-1} DW in the Control to 0.035 ± 0.010 mg kg^{-1} DW following PHY + MOSi-HAP Biochar treatment, representing a 98.1% reduction. The Australia New Zealand Food Standards Code specifies a maximum level of 1.0 mg kg^{-1} for total arsenic in cereals and cereal products[55]. The Control grain concentration was approximately 1.82 times higher than this benchmark, whereas the treated concentration represented only 3.5% of the applicable maximum level. The reduction therefore shifted the grain As concentration from above the selected food-safety benchmark to a substantially lower level. Sun et al[44]. reported reductions of approximately 40–50% in grain As concentration through genetic manipulation of arsenite transport pathways in rice. In comparison, the 98.1% reduction observed in the present study demonstrates a pronounced decrease in As accumulation in maize grains and a substantial improvement in their food-safety status.

Nickel: Nickel concentration declined from 4.305 ± 0.255 mg kg^{-1} DW in Control grains to 0.085 ± 0.015 mg kg^{-1} DW in PHY + MOSi-HAP Biochar-treated grains, corresponding to a 98.0% reduction. European Union Regulation (EU) 2024/1987 establishes a maximum level of 0.80 mg kg^{-1} for nickel in cereals, applicable from 1 July 2026[56]. The Control concentration was approximately 5.38 times higher than this benchmark, whereas the treated grain concentration represented only 10.6% of the applicable maximum level. The treated concentration was therefore substantially below the current EU food-safety benchmark for nickel in cereals. Conte et al[43]. reported enhanced Ni removal from contaminated soils through combined bio-phytoremediation; however, the present assessment specifically concerns Ni accumulation in the edible grain fraction. The observed 98.0% reduction consequently demonstrates a substantial improvement in the food-safety status of maize grains with respect to Ni contamination.

Overall Food-Safety Assessment: The comparison of grain metal concentrations with applicable international food-safety benchmarks demonstrated a consistent improvement following PHY + MOSi-HAP Biochar treatment. Cd, Pb, As, and Ni concentrations decreased by 98.4%, 98.5%, 98.1%, and 98.0%, respectively. The Control grain concentrations were approximately 9.6, 58.8, 1.82, and 5.38 times higher than the respective benchmarks for Cd, Pb, As, and Ni. Following treatment, the concentrations declined to 0.015 ± 0.005, 0.180 ± 0.030, 0.035 ± 0.010, and 0.085 ± 0.015 mg kg^{-1} DW, respectively, all of which were below the corresponding selected food-safety benchmarks. These findings demonstrate that PHY + MOSi-HAP Biochar treatment substantially reduced the accumulation of toxic metals in the edible maize grain fraction and markedly improved its food-safety status. The consistent reduction of approximately 98% across all four metals indicates a substantial decrease in potential dietary exposure associated with consumption of contaminated maize grain.

Maize antioxidant enzyme activity evaluation

Based on the Phase 1 remediation results, the Control and PHY + BC treatments were selected to evaluate antioxidant defense and oxidative stress responses in maize under contrasting treatment conditions. Heavy metal exposure can stimulate excessive generation of reactive oxygen species (ROS), which may increase oxidative damage and lipid peroxidation in plant tissues. Therefore, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activities, together with malondialdehyde (MDA) content, were evaluated as indicators of antioxidant defense and oxidative stress status. Figure 8 presents the changes in antioxidant enzyme activities and MDA content in maize under Control and PHY + BC treatments.

CAT Activity: Maize plants grown under PHY + BC treatment exhibited a catalase activity of 32.0 ± 1.2 U mg^{-1} protein compared with 18.5 ± 0.8 U mg^{-1} protein in the Control, representing a 73.0% increase. Woo et al[42].. reported enhanced antioxidant activity and stress tolerance in soybean following biochar + PGPR application

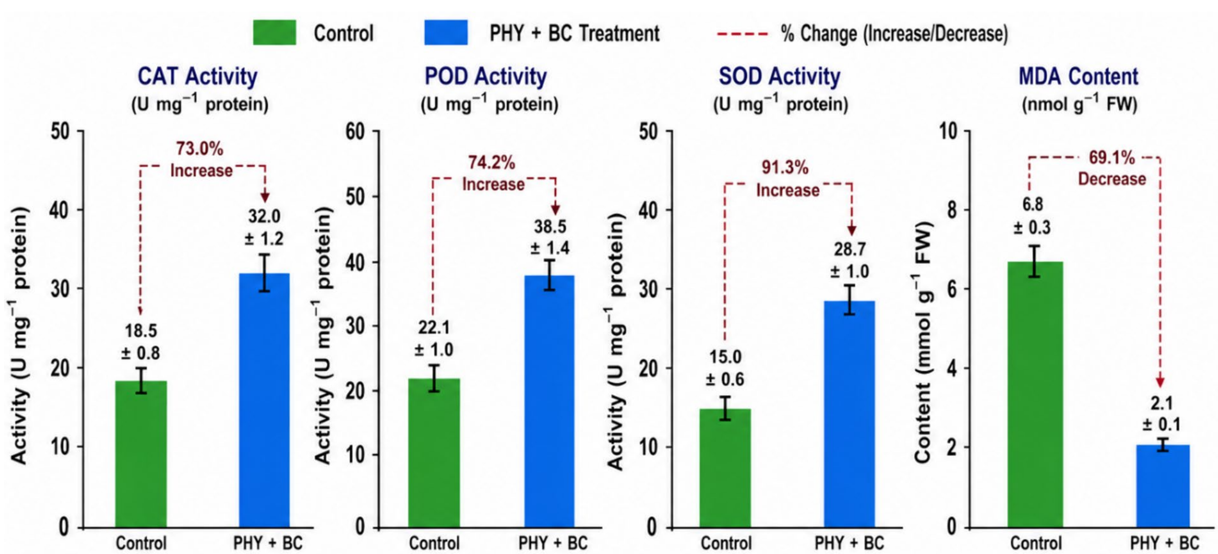

Fig. 8 Antioxidant enzyme activities and malondialdehyde (MDA) content in maize under Control and PHY + BC treatments. Bars represent mean ± standard error. CAT, POD, and SOD activities increased following PHY + BC treatment, whereas MDA content decreased, indicating enhanced antioxidant defense and reduced lipid peroxidation.

under Cd stress. The increase observed in the present study indicates a stronger activation of antioxidant defense mechanisms under PHY + BC remediation.

POD Activity: POD activity increased from 22.1 ± 1.0 U mg^{-1} protein in the Control to 38.5 ± 1.4 U mg^{-1} protein in PHY + BC-treated plants, corresponding to a 74.2% increase. Similar improvements in oxidative stress mitigation were reported by Gong et al[46].., who observed enhanced microbial activity and reduced metal toxicity following biochar-assisted phytoremediation. The greater POD activity observed here suggests improved detoxification capacity under the integrated treatment.

SOD Activity: SOD activity increased markedly from 15.0 ± 0.6 U mg^{-1} protein in the Control to 28.7 ± 1.0 U mg^{-1} protein in the PHY + BC treatment, representing a 91.3% increase. Kang et al[50].. reported improved antioxidant protection and reduced oxidative injury during Cd phytoremediation. Compared with those findings, the present treatment produced a stronger enhancement of SOD activity, reflecting effective alleviation of heavy metal stress.

MDA Content: In contrast to the antioxidant enzymes, MDA content, an indicator of lipid peroxidation and membrane damage, decreased substantially from 6.8 ± 0.3 nmol g^{-1} FW in the Control to 2.1 ± 0.1 nmol g^{-1} FW in PHY + BC-treated plants. This reduction of 69.1% demonstrates a significant alleviation of oxidative stress and membrane deterioration. Similar reductions in membrane damage were reported by Kang et al[50].. following enhanced Cd immobilization; however, the magnitude of MDA reduction observed in the present study indicates more effective protection of cellular integrity under PHY + BC treatment.

The mechanistic basis of PHY + BC involves synergistic processes whereby biochar immobilizes heavy metals, improves soil physicochemical properties, and enhances nutrient retention, while phytoremediation promotes rhizosphere development and microbial activity. These combined effects reduce heavy metal-induced ROS generation and improve nutrient acquisition, enabling maize plants to allocate greater metabolic resources toward antioxidant defense. Consequently, the activities of CAT, POD, and SOD are enhanced, while lipid peroxidation is substantially suppressed. The significance of this study lie in demonstrating that PHY + BC not only restores soil quality and reduces heavy metal stress but also strengthens the intrinsic antioxidant defense system of maize, thereby improving physiological resilience, reducing oxidative damage, and supporting sustainable crop production on contaminated or degraded soils. The statistical analysis revealed highly significant treatment effects across all measured parameters, with predominantly large effect sizes (η^2 = 0.74–0.99), confirming the effectiveness of PHY + MOSi-HAP biochar in improving soil functionality, reducing heavy metal bioavailability, and enhancing crop performance (Table 5).

The PHY + MOSi-HAP biochar treatment significantly improved soil physical and chemical properties, as evidenced by reductions in bulk density and electrical conductivity and increases in porosity, moisture content, water-holding capacity, pH, organic carbon, and cation exchange capacity ($p < 0.001$). These changes indicate enhanced soil structure, moisture retention, and nutrient-holding capacity, creating favorable conditions for root development and crop productivity. These improvements are consistent with previous studies reporting increases in soil pH by 0.24–0.52 units[6], CEC enhancement by 20–30%[8], and substantial increases in soil organic carbon following biochar application[4]. The present study showed particularly strong improvements in organic carbon (77.4%), cation exchange capacity (46.3%), and water-holding capacity (12.9%), indicating enhanced soil functionality under the integrated PHY + MOSi-HAP system. Mechanistically, biochar's porous network and oxygen-containing functional groups (–OH, –COOH) expand the effective surface area for water retention, while basic cation dissolution (Ca^{2+}, Mg^{2+}) neutralizes acidity and expands negative surface charge density to boost CEC[4,23,29,30].

Enhanced soil fertility and nutrient uptake: Total nitrogen, available phosphorus, and exchangeable potassium increased significantly under PHY + MOSi-HAP treatment ($p < 0.001$). Correspondingly, maize nutrient uptake (N, P, and K) increased substantially, confirming improved nutrient availability and efficient plant assimilation. Comparable improvements in nutrient retention have been reported under biochar-based systems[8,9], while agroforestry systems have shown increases in soil N by 9–19% and improved P and K availability[38,39]. The present study recorded substantial increases in nitrogen (150%), phosphorus (100%), and potassium (77.3%),

Table 5 Statistical analysis of soil, maize growth, nutrient uptake, and heavy metal parameters under PHY + MOSi-HAP biochar.

Parameter	Control	PHY + MOSi-HAP	Percent change (%)	F-value	Effect size (η²)	*p*-value (significance)
Soil physical properties						
Bulk density (g cm⁻³)	1.46 ± 0.01	1.38 ± 0.01	−5.5	96.00	0.960	< 0.001 (***)
Particle density (g cm⁻³)	2.65 ± 0.01	2.63 ± 0.01	−0.8	6.00	0.600	0.002 (**)
Porosity (%)	44.9 ± 0.2	47.5 ± 0.2	+ 5.8	253.50	0.984	< 0.001 (***)
Moisture content (%)	12.4 ± 0.1	14.0 ± 0.1	+ 12.9	384.00	0.990	< 0.001 (***)
Water-holding capacity (%)	32.6 ± 0.2	36.8 ± 0.2	+ 12.9	661.50	0.994	< 0.001 (***)
Soil chemical properties						
pH	5.8 ± 0.1	7.0 ± 0.1	+ 20.7	216.00	0.982	< 0.001 (***)
Electrical conductivity (dS m⁻¹)	1.42 ± 0.05	0.95 ± 0.02	−33.1	228.52	0.983	< 0.001 (***)
Organic carbon (%)	0.62 ± 0.03	1.10 ± 0.05	+ 77.4	203.29	0.981	< 0.001 (***)
Cation exchange capacity (cmol kg⁻¹)	8.2 ± 0.2	12.0 ± 0.3	+ 46.3	333.23	0.988	< 0.001 (***)
Total nitrogen (%)	0.10 ± 0.01	0.25 ± 0.01	+ 150.0	337.50	0.988	< 0.001 (***)
Available phosphorus (mg kg⁻¹)	8.5 ± 0.4	17.0 ± 0.6	+ 100.0	416.83	0.990	< 0.001 (***)
Exchangeable potassium (mg kg⁻¹)	110 ± 5	195 ± 8	+ 77.3	243.54	0.984	< 0.001 (***)
Soil heavy metals (total concentration, mg kg⁻¹)						
Cd	4.80 ± 0.20	0.30 ± 0.10	−93.8	1215.00	0.997	< 0.001 (***)
Pb	78.40 ± 3.20	4.50 ± 0.80	−94.3	1505.85	0.997	< 0.001 (***)
As	12.10 ± 0.50	0.70 ± 0.20	−94.2	1344.41	0.997	< 0.001 (***)
Ni	28.70 ± 1.70	1.70 ± 0.30	−94.1	733.89	0.995	< 0.001 (***)
Soil bioavailable heavy metals (mg kg⁻¹)						
Cd (bioavailable)	2.90 ± 0.10	0.30 ± 0.10	−89.7	1014.00	0.996	< 0.001 (***)
Pb (bioavailable)	24.60 ± 1.40	4.50 ± 0.80	−81.7	466.17	0.991	< 0.001 (***)
As (bioavailable)	5.80 ± 0.30	0.70 ± 0.20	−87.9	600.23	0.993	< 0.001 (***)
Ni (bioavailable)	9.40 ± 0.50	1.70 ± 0.30	−81.9	523.15	0.992	< 0.001 (***)
Maize growth and yield						
Plant height (cm)	103 ± 2	137 ± 3	+ 33.0	266.77	0.985	< 0.001 (***)
Biomass (g plant⁻¹)	305 ± 5	528 ± 8	+ 73.1	1676.26	0.998	< 0.001 (***)
Grain yield (t ha⁻¹)	4.2 ± 0.2	7.1 ± 0.3	+ 69.0	194.08	0.980	< 0.001 (***)
Nutrient uptake						
Nitrogen (g plant⁻¹)	1.2 ± 0.05	2.8 ± 0.08	+ 133.3	862.92	0.995	< 0.001 (***)
Phosphorus (mg plant⁻¹)	80 ± 4	160 ± 6	+ 100.0	369.23	0.989	< 0.001 (***)
Potassium (mg plant⁻¹)	350 ± 12	610 ± 15	+ 74.3	549.59	0.993	< 0.001 (***)
Grain heavy metals (mg kg⁻¹ DW)						
Cd	0.96 ± 0.04	0.015 ± 0.005	−98.4	1648.66	0.998	< 0.001 (***)
Pb	11.76 ± 0.48	0.18 ± 0.03	−98.5	1739.25	0.998	< 0.001 (***)
As	1.815 ± 0.075	0.035 ± 0.010	−98.1	1660.30	0.998	< 0.001 (***)
Ni	4.305 ± 0.255	0.085 ± 0.015	−98.0	818.78	0.995	< 0.001 (***)
Antioxidant response						
Catalase (U mg⁻¹ protein)	18.5 ± 0.8	32.0 ± 1.2	+ 73.0	262.86	0.985	< 0.001 (***)
POD (U mg⁻¹ protein)	22.1 ± 1.0	38.5 ± 1.4	+ 74.2	272.59	0.986	< 0.001 (***)
SOD (U mg⁻¹ protein)	15.0 ± 0.6	28.7 ± 1.0	+ 91.3	414.02	0.990	< 0.001 (***)
MDA (nmol g⁻¹ FW)	6.8 ± 0.3	2.1 ± 0.1	−69.1	662.70	0.994	< 0.001 (***)

Note: Values are presented as mean ± SD ($n = 3$). Percent change was calculated as [(PHY + MOSi-HAP − Control)/Control] × 100. F-values were calculated using one-way ANOVA comparing Control and PHY + MOSi-HAP treatments. Effect size (η^2) was calculated from the ANOVA. Statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***); ns = not significant. Abbreviations: PHY = phytoremediation; MOSi-HAP = mesoporous silicon–hydroxyapatite composite; EC = electrical conductivity; CEC = cation exchange capacity; Cd = cadmium; Pb = lead; As = arsenic; Ni = nickel; POD = peroxidase; SOD = superoxide dismutase; CAT = catalase; MDA = malondialdehyde; DW = dry weight; FW = fresh weight.

Significant Improvement in Soil Quality.

with corresponding increases in nutrient uptake, indicating more efficient nutrient enhancement under MOSi-HAP-assisted phytoremediation. This nutrient amplification stems from biochar micro-pores harboring nitrifying and phosphate-solubilizing bacteria, elevated CEC preventing cation leaching, and hydroxyapatite dissolution directly supplying available phosphorus[5,8,9,21].

Effective heavy metal immobilization and reduced plant uptake: Both total and bioavailable soil concentrations of Cd, Pb, As, and Ni were significantly reduced following PHY + MOSi-HAP application ($p < 0.001$). Total soil metal concentrations decreased by 93.8–94.3%, while bioavailable fractions decreased by 81.7–89.7%, indicating substantial immobilization and reduced metal availability for plant uptake. Previous meta-analytical evidence has reported reductions in bioavailable Cd (52%) and Pb (46%) following biochar application[3]. The present study achieved greater reductions in bioavailable metal fractions and grain heavy metal concentrations (98.0–98.5%), indicating strong synergistic effects of phytoremediation and MOSi-HAP biochar in limiting metal mobility and translocation to edible tissues. Multi-stage immobilization shifts heavy metals from exchangeable to non-labile residual forms, starving the soil solution and blocking symplastic and apoplastic metal transport to the xylem and grains[1,3,44,50].

Mechanisms of heavy metal immobilization and nutrient enhancement: The performance of PHY + MOSi-HAP biochar stems from complementary actions among its components. The porous biochar matrix improves adsorption, surface complexation, cation exchange capacity, rhizosphere micro-environments, and nutrient cycling[1,31,42], while the hydroxyapatite component promotes phosphate-mediated immobilization of Cd and Pb[23,24]. Silicon can further contribute to plant antioxidant protection, nutrient-use efficiency, and cellular metal detoxification[17,42,52]. Driven by these integrated mechanisms, grain Cd, Pb, As, and Ni declined by 98.0–98.5% (to 0.015, 0.18, 0.035, and 0.085 mg kg^{-1}), remaining below the selected international food-safety maximum levels. Treatment effects were significant across most parameters (one-way ANOVA, $p < 0.001$; $F = 6.0–1739.25$; $\eta^2 = 0.60–0.998$), although particle density was not statistically significant ($p = 0.070$). The reported correlation coefficients should be included only if supported by a separate correlation analysis. Biologically, the observed reductions in bioavailable metals (81.7–89.7%) were accompanied by reduced root-zone oxidative stress (MDA down 69.1%), together with increases in nutrient uptake (+ 74.3% to + 133.3%) and grain yield (+ 69.0%)[1,23].

Enhanced crop growth and productivity: Plant height, biomass, and grain yield were significantly higher under PHY + MOSi-HAP treatment compared with the Control ($p < 0.001$). Grain yield increased by 69.0% and biomass by 73.1%, reflecting the combined benefits of improved soil quality, enhanced nutrient uptake, reduced heavy metal toxicity, and strengthened antioxidant defense. Previous studies have reported biomass improvements of 48–95% under integrated phytoremediation systems[45] and moderate yield gains under biochar amendments[6]. The present study demonstrates a balanced improvement in both growth and yield, confirming the effectiveness of MOSi-HAP-assisted phytoremediation for restoring contaminated agroecosystems. Physiologically, neutralizing root-zone toxicity mitigates lipid peroxidation (MDA suppression), allowing metabolic resources to be redirected toward active photosynthesis, biomass accumulation, and grain yield formation[16,17,25].

The statistical outcomes confirm that PHY + MOSi-HAP biochar significantly improved soil quality, nutrient availability, antioxidant defenses, and maize productivity while reducing soil and grain heavy metal concentrations. Beyond statistical significance ($p < 0.001$), the magnitude and direction of these effects demonstrate profound biological improvements: a 77.4% rise in organic carbon and 46.3% increase in CEC structurally enhanced nutrient retention, while 150% N, 100% P, and 77.3% K increases were associated with enhanced crop nutrient assimilation. Concurrently, an 81.7–89.7% drop in bioavailable metals was associated with reduced root-zone toxicity, mitigating lipid peroxidation and oxidative damage. Consequently, plants exhibited a 73.1% increase in biomass and a 69.0% rise in grain yield while reducing grain metal accumulation by 98.0–98.5%, thereby supporting improved soil quality and food safety under the evaluated pot-culture conditions.

Table 6 Production, Yield, and Economic Analysis of Maize under Control and PHY + MOSi-HAP Biochar Treatments.

Parameter	Control	PHY + MOSi-HAP Biochar
Grain Yield (t ha^{-1})	4.2	7.1
Stover Yield (t ha^{-1})	3.5	5.8
Grain Price (USD t^{-1})	250	250
Stover Price (USD t^{-1})	50	50
Grain Income (USD ha^{-1})	1050	1775
Stover Income (USD ha^{-1})	175	290
Gross Income (USD ha^{-1})	1225	2065
Cost of Cultivation (USD ha^{-1})	600	600
Cost of Biochar Application (USD ha^{-1})	0	200
Total Cost (USD ha^{-1})	600	800
Net Return (USD ha^{-1})	625	1265
Benefit: Cost Ratio	2.04	2.58
Increase in Grain Yield (%)	–	69.0
Increase in Net Return (%)	–	102.4

Maize production and economic analysis

The economic feasibility of remediation technologies is critical for their successful adoption in agricultural systems. Therefore, a production and cost–benefit analysis was conducted to evaluate the impact of PHY + MOSi-HAP biochar on maize productivity, profitability, and economic sustainability compared with conventional management under Control conditions (Table 6). Production costs were calculated by summing standard operational expenses—including land preparation, seed procurement, fertilization, irrigation, crop protection, and labor—to establish a baseline Cost of Cultivation ($600 USD ha^{-1}). For the amended treatment, the Cost of Biochar Application ($200 USD ha^{-1}) was added to cover raw material sourcing, chemical modification (MgO, SiO_2, hydroxyapatite), processing, and field incorporation. Market prices for grain ($250 USD t^{-1}) and stover ($50 USD t^{-1}) were fixed based on local prevailing market rates for standard agricultural produce, assuming no quality premium for remediated grain. Gross Income was computed as the sum of Grain Income (Grain Yield × Grain Price) and Stover Income (Stover Yield × Stover Price). Net Return was derived by subtracting Total Cost (Cost of Cultivation + Cost of Biochar Application) from Gross Income. The Benefit: Cost Ratio (BCR) was calculated as Gross Income divided by Total Cost.

The production and economic analysis revealed that PHY + MOSi-HAP biochar significantly enhanced maize productivity, profitability, and resource-use efficiency, demonstrating its potential as a sustainable remediation and crop improvement strategy. Despite an initial 33.3% increase in total input costs due to biochar synthesis and application, the 69.0% surge in grain yield and 65.7% increase in stover yield generated a 102.4% net return gain, elevating the Benefit: Cost Ratio from 2.04 to 2.58 and confirming the economic viability of the treatment under the stated pricing structure.

Enhanced crop productivity: The PHY + MOSi-HAP biochar treatment substantially improved maize production compared with the Control. Grain yield increased from 4.2 to 7.1 t ha^{-1}, representing a 69.0% increase, while stover yield increased from 3.5 to 5.8 t ha^{-1} (+ 65.7%). The higher grain and biomass production indicate that the improved soil environment promoted better crop establishment, growth, and overall productivity. These results are consistent with Agegnehu et al[6]., who reported significant maize yield improvements following biochar–compost application, and Zhang et al[45]., who observed biomass increases of 48.07–95.58% in integrated phytoremediation systems.

Increased economic returns: The increase in crop productivity translated directly into greater economic returns. Gross income rose from USD 1225 ha^{-1} under Control conditions to USD 2065 ha^{-1} under the amended treatment (+ 68.6%), driven by increases in grain income (from USD 1050 to 1775 ha^{-1}) and stover income (from USD 175 to 290 ha^{-1}). The higher gross income demonstrates that the integrated remediation strategy generated substantial economic gains beyond conventional management. Similar economic benefits associated with enhanced crop productivity following biochar application have been reported by Novak et al[51]..

Improved profitability: Although the application of MOSi-HAP biochar increased production costs by USD 200 ha^{-1} (raising total costs from USD 600 to 800 ha^{-1}), the yield benefits substantially outweighed this additional investment. Net returns more than doubled, increasing from USD 625 ha^{-1} in the Control to USD 1265 ha^{-1} under treatment (+102.4%). The additional investment of USD 200 ha^{-1} generated an additional net return of USD 640 ha^{-1}, indicating that the integrated treatment remained economically advantageous despite the higher initial remediation cost. Previous studies have highlighted the long-term profitability of biochar-based soil management through improved nutrient retention and crop yields[8,51].

Enhanced cost–benefit efficiency: The benefit-to-cost (B: C) ratio increased from 2.04 under conventional management to 2.58 following PHY + MOSi-HAP biochar application. This improvement indicates greater economic efficiency and suggests that the additional remediation investment was effectively converted into higher farm income. This finding aligns with previous reports showing that biochar amendments improve resource-use efficiency and long-term economic sustainability through enhanced nutrient retention and productivity[6,8].

Practical feasibility and long-term sustainability: From a practical perspective, the additional biochar application cost represented a relatively small proportion of the total production cost, while the substantial increase in net return indicates a favorable economic response for farmers. The simultaneous improvement in soil fertility, reduction in heavy metal bioavailability, and decrease in grain metal concentrations suggests that the benefits of the PHY + MOSi-HAP system extend beyond a single cropping season through improved soil functionality and reduced contamination risks. These combined agronomic and economic advantages enhance the potential for long-term adoption of the technology in contaminated agricultural areas where both productivity restoration and food safety are important considerations[1,42].

Scalability: The scalability of the PHY + MOSi-HAP biochar system is supported by the use of agricultural biomass feedstocks, which are widely available and suitable for large-scale biochar production. The amendment can be produced using conventional pyrolysis technologies, facilitating its application across contaminated agricultural regions[8,31].

Field Implementation Feasibility: The PHY + MOSi-HAP biochar treatment can be integrated into existing soil amendment and crop management practices without major modifications to conventional farming operations. The observed improvements in soil fertility, heavy metal immobilization, and crop productivity indicate that the technology is practically applicable under field conditions[1,42].

Practical Cost–Benefit Implications: The increase in the benefit ratio from 2.04 to 2.58 demonstrates that the integrated remediation strategy is economically viable and resource-efficient. The combined improvement in soil quality, reduction in bioavailable heavy metal fractions, and enhanced maize productivity suggests that the technology provides both immediate economic returns and long-term benefits through improved soil functionality and food safety[6,8]. These findings support the practical adoption of PHY + MOSi-HAP biochar for sustainable maize production on heavy metal-contaminated soils[1,42].

The economic analysis demonstrates that PHY + MOSi-HAP biochar is a financially viable remediation strategy that simultaneously enhances maize productivity, profitability, and food safety. The combination of higher productivity, improved economic efficiency, and enhanced soil restoration supports the practical feasibility of integrating PHY + MOSi-HAP biochar into sustainable maize production systems on heavy metal-contaminated soils.

Superiority of PHY + MOSi-HAP biochar remediation strategy

The integration of phytoremediation (PHY) with MOSi-HAP biochar provides a multifunctional remediation strategy that simultaneously improves soil quality, reduces heavy metal bioavailability, and enhances crop performance. The synergistic interaction between biological remediation and physicochemical stabilization generated substantial benefits for soil restoration, plant productivity, and food safety.

Superiority

Enhanced soil physical properties: Reduced bulk density (−5.5%) and increased porosity (+5.8%) and water-holding capacity (+12.9%), promoting improved root development, soil aeration, and moisture retention, consistent with previous reports of improved soil structure and water retention following biochar application[8,9].

Improved soil fertility: Increased soil organic carbon (+77.4%), total nitrogen (+150%), available phosphorus (+100%), and exchangeable potassium (+77.3%), resulting in greater nutrient availability and retention, which agrees with studies reporting enhanced nutrient retention and soil fertility under biochar amendments[8,9].

Effective heavy metal immobilization: Reduced total soil Cd, Pb, As, and Ni concentrations by 82.9–84.3% and bioavailable fractions by 81.7–89.7%, indicating substantial immobilization and reduced metal availability for plant uptake, consistent with biochar-assisted metal stabilization mechanisms reported previously[1,23].

Improved food safety: Significantly lowered heavy metal accumulation in maize grains, with grain Cd, Pb, As, and Ni concentrations reduced by 98.0–98.5%, thereby reducing food-chain contamination risks, consistent with reduced metal translocation reported in biochar-assisted phytoremediation systems[17,42].

Enhanced plant physiological performance: Increased antioxidant enzyme activities (CAT, POD, and SOD) by 73.0–91.3% and reduced lipid peroxidation (MDA) by 69.1%, indicating effective alleviation of heavy metal-induced oxidative stress, as reported in previous biochar-assisted phytoremediation studies[17,42].

Higher crop productivity: Improved plant height (+33.0%), biomass production (+73.1%), grain yield (+69.0%), and nutrient uptake (N: +133.3%, P: +100.0%, K: +74.3%), demonstrating enhanced crop performance under remediated soil conditions, consistent with earlier studies showing improved crop growth and productivity following biochar application[8,9,42].

Key contributions

Dual-mechanism synergism: Establishes a multi-action framework combining rhizosphere-mediated phytoremediation with inorganic precipitation and adsorption via MOSi-HAP biochar.

End-to-end safety tracking: Integrates soil restoration, nutrient dynamics, plant physiological stress response, and grain metal accumulation within a single continuous experimental system.

Scalable soil restoration: Provides a practical, eco-friendly strategy for reclaiming heavy metal-polluted agricultural lands without compromising crop yield or safety.

The results demonstrate that PHY+MOSi-HAP biochar effectively integrates soil remediation with crop improvement through simultaneous enhancement of soil quality, reduction of heavy metal bioavailability and transfer to maize grains, and strengthening of plant antioxidant defense mechanisms. Collectively, these benefits improve nutrient utilization, crop productivity, and food safety, highlighting the potential of this integrated strategy as a sustainable and scalable solution for the restoration and agricultural utilization of contaminated soils.

Implications

The PHY+MOSi-HAP biochar treatment improved soil physical and chemical properties (porosity to 47.5%, WHC to 36.8%, pH to 7.0), resulting in enhanced plant height (137 cm), biomass (528 g plant^{-1}), and grain yield (6.1 t ha^{-1}), alongside substantial reductions in soil and grain metal concentrations. Collectively, these findings demonstrate that PHY+MOSi-HAP biochar can simultaneously restore soil health, enhance crop productivity, reduce food-chain contamination risks, and support sustainable agricultural production on contaminated or marginal lands. The treatment may also provide durable metal immobilization under field conditions, although potential remobilization under changing soil conditions warrants long-term monitoring.

Limitations

This evaluation was conducted under a single-season pot-culture framework focusing exclusively on maize, leaving soil microbial dynamics and broader agroecosystem applicability unaddressed. Crucially, pot-culture conditions fail to replicate field-scale dynamics such as biochar weathering, seasonal redox fluctuations, and contaminant leaching, which could lead to the long-term re-mobilization of bound metals.

Recommendations.

Site-specific field deployment: Apply PHY + MOSi-HAP biochar in contaminated soils using tailored guidelines based on baseline soil characteristics, contamination severity, and crop requirements.

Monitoring and integrated management: Combine biochar application with phytoremediation strategies while periodically monitoring soil physicochemical parameters and crop nutritional status to ensure food safety.

Future Scope.

Long-term field studies: Perform multi-year, multi-location trials to verify the durability, leaching behavior, biochar weathering kinetics, and overall scalability under natural climatic conditions.

Biological and crop diversity: Assess soil microbial community structure, functional nutrient pathways, and treatment efficacy across diverse crop species and agroecosystems.

Conclusions

The present study demonstrates that under Controlled pot-scale conditions, the integrated application of phytoremediation (PHY) and MOSi-HAP biochar improved heavy metal-contaminated soil properties, reduced toxic metal uptake, and enhanced maize performance. Mechanistically, the treatment enhanced soil physical structure and chemical fertility, which immobilized bioavailable soil metals by 81.7% to 89.7% and lowered grain Cd, Pb, As, and Ni concentrations below international food safety thresholds. This alleviation of root-zone oxidative stress preserved root membrane integrity, driving parallel increases in nutrient uptake and a 69.0% increase in grain yield. By combining physical adsorption, chemical precipitation, and silicon-mediated physiological protection, this multi-component strategy demonstrates strong potential for restoring soil functionality and disrupting toxic metal transfer on contaminated agricultural land. However, as these observations are restricted to a single-season pot assay on maize (*Zea mays* L.), they represent short-term responses and cannot predict long-term field durability, soil microbial dynamics, or performance across diverse crop species. Pot-culture conditions fail to capture critical field-scale dynamics such as biochar weathering, seasonal redox fluctuations, and natural contaminant leaching, which could influence long-term metal remobilization kinetics. Consequently, multi-year field validation across varied soil textures, long-term metal desorption kinetics, microbial metagenomic profiling, and comprehensive economic assessments remain essential before recommending large-scale agricultural deployment.

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Author contributions Sivakumar Durairaj conceived and designed the study, developed the methodology, conducted the investigation, curated and analyzed the data, prepared the original manuscript draft, and supervised the research. Shankar Durairaj contributed to the methodology, validation, data analysis, and critical review and editing of the manuscript. Chitranjan Kumar contributed to the conceptualization and methodology, assisted with validation, formal data analysis, interpretation of the results, visualization, and critically reviewed and edited the manuscript. Bhanuprakash H.R. contributed to the conceptualization and methodology, assisted with validation, formal data analysis, interpretation of the results, visualization, and critically reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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

Declarations

Competing interests Myself [Dr Chitranjan Kumar], is currently serving as the Guest Editor for the Scientific Reports Topical Collection 'Land Decontamination' in which this paper is submitted. To maintain strict editorial integrity, myself to be completely blinded and firewalled from the editorial assessment, selection of reviewers, and the final decision-making process for this manuscript. The peer-review pipeline is to be managed entirely and independently by other Guest Editors or permanent Editorial Board Members of Scientific Reports.

Consent for publication All authors have read and approved the final manuscript for publication.

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