Research Square

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Fluoride accumulation and biological responses in kiln-affected orchard soils during subsequent tomato and alfalfa cultivation

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Abstract

Brick-kiln emissions can contaminate agricultural soils and vegetation, but biological responses during subsequent cultivation remain insufficiently characterized. This study assessed fluoride exposure and soil biological indicators during field cultivation of tomato and alfalfa between existing peach and plum trees at previously investigated kiln-affected orchards in Urmar, Pakistan. The Agricultural Research Farm at Malakandher served as the reference site. Measurements included extractable soil fluoride, foliar fluoride, and soil alkaline phosphatase, dehydrogenase and urease activities. A laboratory experiment evaluated earthworm growth and cocoon production under combinations of soil and crop litter from the two sites. A separate preliminary assay assessed earthworm responses to Parthenium-derived biochar as an initial biological-compatibility screen for potential amendment development. Extractable soil fluoride reached 13.50 μg g^{-1} dry soil at Urmar, compared with 2.57 μg g^{-1} at the reference site. Tomato and alfalfa leaves contained 31.00 and 68.57 μg g^{-1} dry matter, respectively. Soil alkaline phosphatase, dehydrogenase and urease activities were 21.2%, 30.5% and 26.0% lower at Urmar than at the reference site. In Urmar soil, earthworm biomass decreased by 61.3% under Urmar-derived tomato litter and 76.6% under Urmar-derived alfalfa litter relative to initial measurements. Week-four cocoon counts were 76.8% and 68.6% lower, respectively, than under the corresponding reference-site litter in the same soil. In the separate biochar assay, biomass increased between weeks 1 and 2 at 10% amendment but decreased at 30% and 60%. These preliminary responses did not establish a safe application rate or demonstrate fluoride immobilization. Elevated fluoride coincided with impaired soil biochemical and earthworm endpoints, although site differences and co-contaminants limit fluoride-specific causal interpretation.

Introduction

Brick kilns are commonly located beside productive agricultural land in South Asia. They release particulate matter, sulfur compounds, trace metals and fluoride-bearing gases. These emissions settle on vegetation and surface soil. Repeated deposition can alter soil chemistry, restrict microbial activity and weaken nutrient cycling. The problem is acute where orchards, vegetables and fodder crops occur close to kiln clusters. Pakistani studies link kiln emissions with deteriorating soil and vegetation, yet effects on soil biochemical functioning remain poorly resolved (Ishtiaq et al., 2022; Ahmad et al., 2025). Fluoride is released when fluoride-bearing clay and fuels are heated. It enters soil through deposition, irrigation and contaminated residues. Its mobility depends on pH, texture, organic matter and mineral composition. Calcium, aluminium and iron phases can retain fluoride through adsorption or precipitation. Fluoride deposited during one season may remain in surface soil and influence later crops. Total concentration confirms contamination but cannot show whether nutrient cycling and microbial metabolism are impaired.

Plants acquire fluoride through roots and foliage. Uptake varies with canopy structure, rooting depth and soil conditions. Perennial trees experience repeated exposure because their canopies and roots remain in place. A previous investigation in Urmar assessed fluoride in peach (*Prunus persica*) and plum (*Prunus*

domestica) orchards and its effects on earthworms. Fluoride concentrations were higher and earthworm performance poorer near kilns than at Agricultural Research Farm (ARF), Malakandher (Ahmad et al., 2025). That study established regional exposure but did not examine enzymes or a subsequent cropping system. Crop transition changes root activity, nutrient uptake, residue inputs and microbial associations. Tomato (*Solanum lycopersicum*) is a short-duration vegetable with rapid growth, shallow roots and high nutrient demand. Its roots occupy the contaminated surface layer. Alfalfa (*Medicago sativa*) is a perennial forage legume with deeper roots, repeated harvesting and symbiotic nitrogen fixation. Its foliage intercepts deposited particles, while contaminated biomass may enter livestock feed or return fluoride through decomposition. These crops provide an opportunity to assess fluoride exposure during the subsequent cropping season..

Most investigations of fluoride-affected farmland focus on concentrations, leaf injury, crop growth or earthworm responses. These endpoints demonstrate exposure but provide limited information about soil functioning. Fertility depends on enzyme-mediated decomposition and nutrient release. Enzymes may respond before changes appear in yield or total nutrients. Recent research identifies them as sensitive indicators of microbial activity and nutrient cycling (L. Wang et al., 2023; Daunoras et al., 2024). A multi-enzyme assessment is more informative than a single assay. Dehydrogenase, urease and alkaline phosphatase represent microbial metabolism and two nutrient cycles. Dehydrogenase occurs mainly within viable microbial cells and reflects respiration and organic-matter decomposition. Urease catalyses urea hydrolysis and regulates nitrogen mineralization. Its inhibition can delay nitrogen release and reduce fertilizer efficiency. Alkaline phosphatase releases inorganic phosphate from organic compounds. This is important in calcareous soils, where calcium restricts phosphorus availability. Their concurrent suppression would indicate disturbance of microbial oxidation, nitrogen transformation and phosphorus mineralization.

Enzyme responses during subsequent vegetable and forage intercropping within existing orchards. It may also change microbial abundance and composition. Studies report reduced crop growth, nutrient availability and enzyme activity under fluoride stress, with responses varying by concentration, soil and exposure duration (Ahmad et al., 2022). Field evidence remains scarce. Enzyme responses during subsequent vegetable and forage intercropping within existing orchards. Diagnosis alone does not resolve management. Contaminated soil requires available amendments that support biological functioning without added stress. Biochar can modify pH, nutrient retention, microbial habitat and contaminant mobility. Fluoride retention may involve ion exchange, surface complexation, electrostatic attraction and mineral precipitation. Recent studies identify pristine and engineered biochars as candidate materials for managing fluoride and other inorganic contaminants (Khan et al., 2024; Shaheen et al., 2025).

Parthenium hysterophorus is an invasive weed that reduces crop productivity and degrades grazing land. Converting its non-edible biomass into biochar could link weed control with resource recovery. Parthenium biochar has modified acidic soil and wheat performance (Muche et al., 2022). Iron-coated Parthenium biochar has also adsorbed fluoride and other anions from water (Islam et al., 2025). This

pathway supports circular agriculture, but safety depends on feedstock, pyrolysis and application rate. High biochar proportions may increase pH, salinity or exposure to soluble products. Earthworms provide a sensitive test because they ingest soil and contact pore water. Their biomass and growth reflect substrate quality and chemical stress. Low biochar rates may support earthworms, whereas excessive rates can impair growth, reproduction and physiology (Cui et al., 2023; Jia et al., 2023). Dose screening is therefore necessary before agricultural use.

Evidence remains limited on fluoride exposure and soil enzyme activities during subsequent cropping of kiln-affected orchard soils in this region. This study compared soil and foliar fluoride concentrations and three soil enzyme activities at Urmar and ARF. A separate preliminary assay examined earthworm responses to Parthenium-derived biochar. These omissions prevent informed decisions about continued cultivation, soil recovery and safe amendment development. Four research gaps define the present study. First, fluoride exposure during subsequent tomato and alfalfa cultivation between existing peach and plum trees remains insufficiently characterized. Second, no regional study has determined whether such persistence is accompanied by changes in dehydrogenase, urease and alkaline phosphatase. Third, evidence connecting fluoride accumulation in vegetable and forage crops with soil biochemical functioning is scarce. Fourth, the biological compatibility of Parthenium-derived biochar remains insufficiently understood. This gap is critical because an amendment that improves chemical conditions but harms soil fauna cannot support sustainable remediation.

The assessment of biological impairment in kiln-affected soils also raises the question of whether potential soil amendments are compatible with soil fauna. Parthenium-derived biochar was therefore included in a separate preliminary assay using Urmar soil to screen earthworm responses across amendment proportions. This assay complemented the contamination assessment by examining the biological compatibility of a candidate amendment in the affected soil. It did not test fluoride immobilization or establish remediation efficacy, but provided an initial screen to inform subsequent amendment testing.

This study aimed to assess fluoride exposure and associated soil biological responses during subsequent tomato and alfalfa cultivation between existing orchard trees at kiln-affected Urmar and the reference Agricultural Research Farm at Malakandher. Specifically, we compared extractable soil and foliar fluoride concentrations and soil alkaline phosphatase, dehydrogenase and urease activities between locations, and evaluated earthworm growth and cocoon production under combinations of soil and crop litter from both locations. We hypothesized that (i) soil and foliar fluoride concentrations would be higher at Urmar than at the reference site; (ii) soil enzyme activities would be lower at Urmar; and (iii) Urmar-derived litter would reduce earthworm growth and cocoon production relative to reference-derived litter, with stronger adverse responses in Urmar soil. A separate exploratory assay assessed earthworm responses to increasing proportions of Parthenium-derived biochar as a preliminary biological-compatibility screen to inform its potential development as a soil amendment.

Materials and Methods

Study design

The study comprised a field assessment and two laboratory bioassays. The field assessment measured extractable soil fluoride, foliar fluoride and soil enzyme activities during subsequent tomato and alfalfa cultivation between existing peach and plum trees at Urmar and the reference Agricultural Research Farm (ARF), Malakandher. The crop-litter bioassay used soils collected from both locations, combined with tomato or alfalfa litter originating from either location, to evaluate earthworm growth and cocoon production. This design examined responses to soil origin, litter origin and their combined influence. A separate preliminary biochar assay used soil collected from the kiln-affected Urmar site, amended with Parthenium-derived biochar at 0, 10, 30 and 60% on a dry-mass basis. The laboratory bioassays were conducted in the Department of Agricultural Chemistry and Biochemistry, The University of Agriculture, Peshawar. The biochar assay assessed earthworm responses to the candidate amendment in kiln-affected soil as an initial biological-compatibility screen; it did not directly test fluoride immobilization or remediation.

Study locations

The field study was conducted at the kiln-affected Urmar site (33°54'49.4"N, 71°42'10.3"E) and the reference Agricultural Research Farm (ARF), Malakandher, The University of Agriculture, Peshawar (34°01'15.1"N, 71°28'14.9"E). Urmar represented the brick-kiln-affected location and was situated approximately 15 km from Peshawar. The experimental area was located within 50 m of active brick-manufacturing activity. ARF Malakandher was located approximately 25–30 km from the kiln zone and had no active brick kilns in its immediate surroundings. It was selected as the reference agricultural location. The earlier investigation described soils at both locations as calcareous, with a pH of approximately 8.0 and organic matter content of approximately 1% (Ahmad et al., 2025).

Crop establishment and sample collection

Tomato (Solanum lycopersicum) and alfalfa (Medicago sativa) were established during a subsequent cropping season between existing trees in both peach and plum orchards at Urmar and ARF Malakandher. Orchard trees were retained, and neither site was cleared or grubbed before crop establishment. Seedbed preparation comprised manual removal of weeds and surface debris, shallow hoeing, breaking of soil clods and levelling. Deep cultivation near tree roots was avoided, and planting distances from tree trunks were kept consistent. The same preparation procedure was followed at both locations. Alfalfa was directly sown, whereas tomato was established by transplanting seedlings. Separate replicated plots were established for each crop within each orchard type, with placement randomized where practical. No manure or compost was applied during the trial. Mineral fertilizers were applied according to a predefined crop-specific protocol, with consistent treatment of comparable plots across locations. The fluoride measurements and biological endpoints reported here were obtained from this subsequent experiment. Soil sampling followed a W-shaped composite sampling approach. Surface soil was collected from a depth of 0–20 cm using a clean auger. Subsamples collected along each transect were combined and homogenized to form a composite sample. Three independent composite

soil samples were obtained from each location. Samples were transferred to labelled paper bags and transported to the laboratory. A separate portion of each soil sample was retained field-moist, passed through a 2-mm sieve and stored at 4°C without air-drying. Soil enzyme assays were initiated within 24 h of sample collection. Mature, visually intact tomato and alfalfa leaves of comparable developmental stage were collected from several plants in the corresponding plots. Each crop–orchard combination was sampled separately. The material was transported to the laboratory in labelled bags. Leaves were washed with deionized water, dried to constant mass at 65°C and ground to pass through a fine laboratory sieve.

Baseline soil characterization

Soil collected from Urmar before tomato and alfalfa establishment was characterized in the Agricultural Chemistry Laboratory, The University of Agriculture, Peshawar. Soil pH was measured in a 1:2 soil–water suspension prepared with distilled or deionized water. Electrical conductivity was measured in the same suspension using an EC meter. Particle-size distribution was determined by the hydrometer method, and calcium carbonate content was determined by acid neutralization followed by back-titration. Organic matter was determined separately by loss-on-ignition. Nitrogen was determined by Kjeldahl digestion, distillation and titration. Available phosphorus and potassium were extracted using AB-DTPA and determined by spectrophotometry and flame photometry, respectively. Calcium and magnesium were extracted using AB-DTPA and determined by atomic absorption spectrometry. Baseline properties are presented in Table S1.

Determination of extractable soil fluoride

Extractable fluoride was determined using the 0.01 M $CaCl_2$ procedure of Larsen and Widdowson (1971), with minor adjustments for ion-selective measurement. Five grams of air-dried soil were transferred to an acid-washed 150-mL polyethylene bottle. Thirty-seven millilitres of 0.01 M $CaCl_2 \cdot 2H_2O$ were added. The bottles were sealed and shaken horizontally at 120 rpm for 16 h at room temperature. The suspensions were filtered through Whatman No. 4 filter paper. A 5-mL aliquot of the filtrate was mixed with 5 mL of total ionic strength adjustment and anti-complexing solution. The solution controlled ionic strength and reduced interference from aluminium and iron complexes. Its pH was adjusted to 5.0–5.5 using dilute NaOH where required. Fluoride was quantified using a Thermo Scientific Orion fluoride ion-selective electrode connected to an ion meter. The electrode was calibrated with fluoride standards prepared across the concentration range expected in the samples. Standards and extracts were analysed under identical pH and ionic-strength conditions. Extractable fluoride was calculated from the calibration curve and expressed as μg F g^{-1} dry soil.

Determination of fluoride in plant leaves

Fluoride in tomato and alfalfa leaves was determined using an acid-extraction procedure adapted from AOAC (2005) and Ahmad et al. (2012). A 200-mg portion of dried and ground plant material was transferred to an acid-cleaned digestion vessel. The sample was treated with acetone, followed by 40 mL

of 0.05 N HNO_3. The mixture was allowed to stand for 30 min and then heated at 70°C. After heating, 20 mL of 0.1 N KOH were added. The mixture was stirred for 30 min. Five millilitres of 0.2 N HNO_3 and 5 mL of 0.4 M sodium citrate solution were then added. Sodium citrate served as an anti-complexing agent and helped release fluoride bound to polyvalent metal ions. The final solution was adjusted to pH 5.0–5.5. Fluoride was measured using the calibrated ion-selective electrode. A reagent blank and calibration standards were included with each analytical batch. Plant fluoride was expressed as μg F g^{-1} dry matter.

Soil enzyme activities

A separate portion of each soil sample was retained field-moist, passed through a 2-mm sieve and stored at 4°C without air-drying. Soil enzyme assays were initiated within 24 h of sample collection. The assays were conducted using three independently collected soil samples from each location. Analytical blanks were included to correct for colour and non-enzymatic substrate transformation. Enzyme activities were calculated on an oven-dry soil-equivalent basis.

Alkaline phosphatase

Alkaline phosphatase activity was determined using the *p*-nitrophenyl phosphate method of Tabatabai and Bremner (1969), with minor modifications. One gram of field-moist soil, equivalent to 1 g dry soil, was placed in a 50-mL Erlenmeyer flask. Four millilitres of modified universal buffer at pH 11.0 and 1 mL of 0.05 M *p*-nitrophenyl phosphate solution were added. The flasks were mixed and incubated at 37°C for 1 h. After incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to stop the reaction and develop the yellow colour of *p*-nitrophenol. The suspension was filtered, and absorbance was measured at 400 nm. A substrate-free soil control was processed with each sample. Activity was calculated from a *p*-nitrophenol calibration curve and expressed as μg *p*-nitrophenol g^{-1} dry soil h^{-1}.

Dehydrogenase

Dehydrogenase activity was measured using the triphenyltetrazolium chloride reduction method of Casida et al. (1964), adapted to the incubation temperature used in the experiment. Ten grams of field-moist soil were placed in a test tube and mixed with 0.2 g $CaCO_3$, 2.5 mL phosphate buffer and 1 mL of 3% TTC solution. Control tubes were prepared without TTC. The tubes were mixed and incubated at 28°C for 24 h in darkness. Following incubation, the triphenyl formazan formed during TTC reduction was extracted twice with methanol to a combined extract volume of 10 mL. The extracts were centrifuged or filtered until clear. Absorbance was measured at 485 nm against a methanol blank. Triphenyl formazan concentration was determined from a standard calibration curve. Dehydrogenase activity was expressed as μg triphenyl formazan g^{-1} dry soil 24 h^{-1}.

Urease

Urease activity was determined using the non-buffered procedure of Kandeler and Gerber (1988), with colorimetric determination of released ammonium. Five grams of field-moist soil were placed in a 100-mL incubation flask. A 2.5-mL volume of 0.72 M urea solution was added. Control samples received

deionized water instead of urea. The flasks were incubated at 37°C for 2 h without continuous shaking. After incubation, ammonium was extracted with 50 mL of 1 M KCl. The suspensions were shaken for 30 min and filtered. Ammonium in the filtrate was determined through the sodium salicylate–sodium dichloroisocyanurate colour reaction. Absorbance was measured at 690 nm. Urease activity was calculated after subtraction of the corresponding control and expressed as μg NH_4^+-N g^{-1} dry soil h^{-1}.

Earthworm bioassay of tomato and alfalfa litter

A separate laboratory experiment assessed earthworm growth and cocoon production following exposure to tomato and alfalfa litter collected during the subsequent cropping season. Soil originated from Urmar and ARF Malakandher. Within each soil, five treatments were established: an unamended control, Urmar-derived tomato litter, ARF-derived tomato litter, Urmar-derived alfalfa litter and ARF-derived alfalfa litter. Litter treatments were designated Urmar-derived or ARF-derived according to their collection site. Fluoride was analysed in slurries prepared from the soil–litter mixtures as an exposure check. *Eisenia fetida* earthworms were acclimated to laboratory conditions for 3–5 days before exposure. Each container received 200 g of moist soil and 2 g of the assigned crushed leaf litter. Unamended controls received soil without crop litter. Each soil–litter combination comprised three independent replicate containers, with two earthworms per container. The container constituted the experimental unit.

Exposure continued for four weeks. Earthworm biomass, body length and cocoon production were recorded at weekly intervals to assess responses to soil source and litter treatment.

Preparation of Parthenium biochar

Parthenium biochar was prepared using a slow-pyrolysis procedure adapted from Kumar et al. (2013) and Muche et al. (2022). Healthy *P. hysterophorus* plants were collected before flowering from agricultural land near Malakandher. The biomass was washed to remove adhering soil, air-dried and then oven-dried at 65°C to constant mass. The dried material was cut and milled to particles of approximately 2–5 mm. The prepared biomass was placed in covered heat-resistant crucibles to restrict oxygen entry. The material was heated in a muffle furnace from room temperature to 450°C at approximately 10°C min^{-1}. The target temperature was maintained for 2 h. The furnace was then allowed to cool to room temperature without opening the crucibles. The recovered biochar was weighed, ground and passed through a 2-mm sieve. Biochar yield was calculated as:

$$Biochar\ Yield\ (\%) = \frac{Dry\ mass\ of\ recovered\ biochar}{Dry\ mass\ of\ initial\ biochar} \times 100$$

Earthworm bioassay of Parthenium biochar

A preliminary range-finding assay evaluated earthworm growth responses to Parthenium biochar. Four treatments comprised unamended soil and soil containing 10, 30 or 60% biochar on a dry-mass basis.

Soil collected from the kiln-affected Urmar site served as the exposure medium. The assay was conducted in the laboratories of the Department of Agricultural Chemistry and Biochemistry, The University of Agriculture, Peshawar. It assessed earthworm responses to biochar addition in kiln-affected soil as a preliminary biological-compatibility screen for potential amendment development; fluoride immobilization was not measured. Each treatment comprised three independent experimental units. Soil moisture was adjusted to approximately 30% and maintained gravimetrically. Three healthy, young, unclitellated *Eisenia fetida* earthworms of comparable size were introduced into each unit. Measurements were taken at weeks 1 and 2. At each observation, earthworms were recovered, gently washed with deionized water and blotted to remove surface moisture. Biomass was measured using an analytical balance. Body length was measured using a calibrated scale without stretching the organisms.

Statistical analysis

Statistical analyses were performed using Statistix. The laboratory earthworm experiments followed a completely randomized design with three independent replicate containers per treatment. Each container constituted the experimental unit, and weekly measurements from the same container represented repeated observations rather than additional independent replicates. Fluoride concentrations are presented as mean ± standard error. Soil enzyme activities, earthworm biomass and biochar-assay measurements are presented as mean ± standard deviation. Biochar responses were summarized descriptively.

Results and discussion

Distribution of fluoride between soil and crop foliage

Fluoride concentrations differed significantly between sites and plant species (Table 1). Urmar soil contained 13.50 ± 0.71 μg F g^{-1}, which was 5.25-fold higher than the concentration at the Agricultural Research Farm (ARF; 2.57 ± 0.15 μg F g^{-1}). A similar site effect occurred in foliage. Tomato leaves from Urmar contained 31.00 ± 1.45 μg F g^{-1}, compared with 5.23 ± 0.21 μg F g^{-1} at ARF. The corresponding concentrations in alfalfa were 68.57 ± 2.88 and 4.63 ± 0.19 μg F g^{-1}. Location ($F = 981.63$, $p < 0.001$), plant species ($F = 166.72$, $p < 0.001$), and their interaction ($F = 177.72$, $p < 0.001$) were significant.

The higher soil and foliar fluoride concentrations at Urmar extend the earlier orchard investigation to subsequent tomato and alfalfa intercropping within the same agricultural setting. Ahmad et al. (2025) reported fluoride enrichment in peach and plum orchards near the kiln cluster, whereas the present observations show elevated exposure in vegetable and forage crops cultivated between the retained trees. This continuity indicates that exposure remained relevant during subsequent cultivation. It does not, however, distinguish residual soil contamination from continuing atmospheric inputs because deposition and emissions were not measured during the experiment.

Alfalfa leaves contained approximately 2.21 times more fluoride than tomato leaves at Urmar, whereas concentrations were closer at ARF. This pattern is consistent with Wang et al. (2023), who demonstrated

substantial variation in fluorine accumulation among 20 vegetable species and 25 lettuce cultivars grown in a contaminated field adjacent to a brick kiln. Their findings support considering crop identity when interpreting exposure, but do not establish the mechanism responsible for the tomato–alfalfa difference observed here. Differences in leaf characteristics, developmental stage, exposure duration and root-zone conditions are possible explanations that require direct measurement. Alfalfa's perennial growth habit alone cannot establish a longer exposure period in this experiment.

The reported soil fluoride represents the $CaCl_2$-extractable fraction rather than total soil fluoride. Consequently, its concentration should not be compared directly with total-soil concentrations or experimentally added fluoride doses without accounting for analytical differences. Leaves were washed before analysis, reducing loosely adhering surface material, but washing does not distinguish internally accumulated fluoride derived from roots from that entering through foliage. The present measurements therefore demonstrate crop exposure without identifying the relative contributions of atmospheric and soil pathways. Foliar concentrations also cannot establish tomato-fruit safety, livestock exposure or dietary risk without measurements of the relevant consumed tissues and intake.

Table 1
Fluoride concentrations in soil and crop leaves at Urmar and ARF

Matrix	Urmar (μg g^{-1})	ARF (μg g^{-1})	Urmar/ARF
Soil	13.50 ± 0.71	2.57 ± 0.15	5.25
Tomato leaves	31.00 ± 1.45	5.23 ± 0.21	5.93
Alfalfa leaves	68.57 ± 2.88	4.63 ± 0.19	14.81

Values are mean ± standard error (n = 3 composite samples) on a dry-mass basis

Soil enzyme activities and biochemical functioning

All three enzyme activities were lower at Urmar than at ARF (Table 2). Alkaline phosphatase decreased from 25.64 to 20.21 μg pNP g^{-1} h^{-1} (− 21.2%; t = 15.38, p = 0.0042), dehydrogenase from 5.25 to 3.65 μg TPF g^{-1} 24 h^{-1} (− 30.5%; t = 32.72, p = 0.0009), and urease from 2.04 to 1.51 μg NH_4^+-N g^{-1} h^{-1} (− 26.0%; t = 8.24, p = 0.0144). The coherent direction across assays is stronger evidence of impaired biological functioning than any single enzyme response. Dehydrogenase showed the largest proportional difference, indicating that microbial oxidative activity was the most responsive endpoint among those measured.

The concurrent reductions in dehydrogenase, urease and alkaline phosphatase suggest lower potential biochemical activity at Urmar across microbial oxidation and nitrogen- and phosphorus-related transformations. However, fluoride effects on soil enzymes are not uniformly inhibitory. Szostek et al. (2015), using controlled potassium-fluoride additions, reported stimulation of several microbial and enzymatic endpoints at lower tested doses, with responses depending on crop and amendment treatment. Their findings contrast with the consistently lower activities observed at Urmar and indicate

that enzyme responses depend on exposure conditions and the soil–plant system rather than fluoride presence alone.

The larger proportional difference in dehydrogenase activity may reflect differences in microbial abundance, physiological activity or substrate supply between locations. Nevertheless, enzyme measurements under standardized assay conditions represent potential activities; they do not directly quantify field nutrient-release rates, crop nutrient deficiencies or fertilizer requirements. Comparisons of absolute activities with other studies also require matching substrate concentrations, incubation conditions, extraction procedures and reporting units.

The present field comparison cannot isolate fluoride inhibition from differences in soil properties, orchard management or co-contaminants. Historical site descriptions of alkaline, calcareous soils provide context but do not establish equivalent conditions during the subsequent experiment. The enzyme results are therefore best interpreted as biological indicators associated with the affected location, rather than proof of a fluoride-specific mechanism. Their value lies in showing that chemical enrichment coincided with changes in several functional indicators, supporting the inclusion of biological measurements alongside fluoride monitoring.

Table 2
Soil enzyme activities at Urmar and ARF

Enzyme	Assay unit	Urmar	ARF	Difference (%)	p value
Alkaline phosphatase	μg pNP g^{-1} h^{-1}	20.21 ± 1.24	25.64 ± 1.57	−21.2	0.0042
Dehydrogenase	μg TPF g^{-1} 24 h^{-1}	3.65 ± 0.23	5.25 ± 0.34	−30.5	0.0009
Urease	μg NH_4^+-N g^{-1} h^{-1}	1.51 ± 0.12	2.04 ± 0.16	−26.0	0.0144

Difference = 100 × (Urmar − ARF)/ARF. pNP, p-nitrophenol; TPF, triphenyl formazan.

Earthworm growth responses to tomato and alfalfa litter

Earthworm biomass responded strongly to litter treatment, week, soil, and their interactions. The reported factorial analysis gave F = 7412.52 for treatment, F = 281.39 for week, and F = 3053.19 for soil, with treatment × week, treatment × soil, week × soil, and treatment × week × soil interactions (all $p < 0.001$). At week 4, Urmar-derived tomato and alfalfa litter were associated with biomass losses of 61.3% and 76.6%, respectively, in Urmar soil. The same contaminated litters produced losses of about 16.6% in ARF soil (Table 3). In contrast, biomass increased when worms received ARF-derived litter. The treatment × soil interaction thus expresses more than an additive litter effect: adverse responses were magnified when contaminated litter and Urmar soil occurred together.

Table 3
Final-week earthworm biomass and change from the initial measurement

Treatment	Urmar W4 (g)	Urmar change (%)	ARF W4 (g)	ARF change (%)
Control	2.65 ± 0.18	+ 12.0	3.90 ± 0.26	+ 26.5
Urmar-derived tomato litter	0.91 ± 0.07	−61.3	2.56 ± 0.19	−16.6
ARF-derived tomato litter	3.09 ± 0.22	+ 12.7	3.27 ± 0.24	+ 24.9
Urmar-derived alfalfa litter	0.94 ± 0.08	−76.6	2.92 ± 0.21	−16.6
ARF-derived alfalfa litter	4.55 ± 0.31	+ 8.8	4.61 ± 0.34	+ 18.6

W4 denotes week 4. Biomass values are mean ± standard deviation from three replicate containers per treatment. Percentage changes were calculated relative to the corresponding initial mean biomass.

The poorer earthworm performance under Urmar-derived litter is directionally consistent with the earlier peach–plum litter experiment at these locations (Ahmad et al., 2025). The present experiment extends that observation to residues from subsequently cultivated tomato and alfalfa. Its contribution is the comparison of crop-litter origin within both soil matrices, which helps distinguish litter-associated responses from responses associated with soil origin. The greater biomass losses under Urmar-derived litter in Urmar soil suggest that the receiving soil matrix influenced the magnitude of the response.

These findings do not establish fluoride as the sole cause. Crop residues can differ in nutritional composition, decomposition rate, palatability and associated contaminants. The greater biomass loss under Urmar-derived alfalfa coincided with its higher measured foliar fluoride concentration, but this correspondence is not a controlled concentration–response relationship. Although soil–litter slurries were analysed, the numerical fluoride results were not retained, preventing quantitative characterization of exposure mixtures. The findings therefore support a combined soil-and-residue assessment while leaving the relative contributions of fluoride, litter quality and other site-associated factors unresolved.

Body length was less sensitive than biomass. The largest reported reduction was 12.5% under Urmar-derived alfalfa in Urmar soil; the remaining declines were 5.8% for Urmar-derived tomato in ARF soil, 4.2% for Urmar-derived alfalfa in ARF soil, and 4.1% for Urmar-derived tomato in Urmar soil. Treatment and soil effects and their interactions were significant ($p < 0.001$), while the week effect was smaller ($F = 3.31$, $p = 0.0243$). Mass can change rapidly with feeding, gut content, tissue reserves, hydration, and stress, whereas length is constrained by body contraction and measurement posture. The stronger biomass response is therefore expected and makes mass the preferable short-term growth endpoint. Future tests should report individual rather than pooled mass, standardize depuration and hydration before weighing, and include confidence intervals or standard errors for every time point.

Reproductive response: cocoon production

Reproduction was strongly depressed by Urmar-derived litter in both soils. Treatment, week, soil, and all reported interactions were significant ($p < 0.001$). At week 4, Urmar-derived tomato litter yielded 13 cocoons in Urmar soil compared with 56 under ARF-derived tomato litter, a 76.8% reduction. Urmar-derived alfalfa yielded 11 cocoons compared with 35 under ARF-derived alfalfa litter, a 68.6% reduction. In ARF soil, the corresponding reductions were 62.3% for tomato and 55.0% for alfalfa (Table 4). Controls also differed substantially between soils (52 cocoons at Urmar and 137 at ARF), showing that the soil matrix itself constrained reproduction independently of litter origin.

Table 4
Cocoon production at week 4

Litter treatment	Urmar soil	ARF soil	Urmar: change vs ARF litter (%)	ARF: change vs ARF litter (%)
Control	52	137	—	—
Urmar-derived tomato	13	63	−76.8	−62.3
ARF tomato	56	167	Reference	Reference
Urmar-derived alfalfa	11	58	−68.6	−55.0
ARF alfalfa	35	129	Reference	Reference

Values are mean cocoon counts from three replicate containers per treatment; standard deviations are unavailable. Percentage differences compare Urmar-derived litter with the corresponding ARF-derived litter within each soil.

The lower cocoon counts under Urmar-derived litter complement the biomass findings and agree in direction with the reproductive impairment reported in the earlier orchard-litter study (Ahmad et al., 2025). The occurrence of lower counts in both receiving soils suggests that litter origin contributed to the response, while differences between soil controls indicate an additional influence of the soil matrix. Reduced food intake, nutritional differences and contaminant-associated stress are possible explanations, but these mechanisms were not measured.

Reproduction provides information beyond short-term changes in body mass, but cocoon production alone does not establish population recruitment. Cocoon viability, hatching success and juvenile survival were not assessed. Moreover, unavailable standard deviations for the reported cocoon means limit assessment of variability and effect-size precision. These results support further reproductive testing with retained container-level counts and clearly distinguished weekly and cumulative production, rather than a quantitative prediction of field population decline.

Preliminary response to Parthenium-derived biochar

The range-finding assay produced a distinctly non-linear response (Table 5). Between weeks 1 and 2, mean biomass rose by 15.6% in the unamended control and by 166.2% at 10% biochar, but fell by 43.6% at 30% and by 64.8% at 60%. Mean length increased by 148.2% in the control and 50.0% at 10%, remained unchanged at 30%, and declined by 26.3% at 60%. Biomass increased between weeks 1 and 2 at 10% biochar and decreased at 30% and 60%.

Table 5
Earthworm responses in the preliminary Parthenium-biochar assay

Biochar (%)	Length W1 (cm)	Length W2 (cm)	Length change (%)	Mass W1 (g)	Mass W2 (g)	Mass change (%)
0	8.3 ± 0.6	20.6 ± 1.4	+ 148.2	1.54 ± 0.11	1.78 ± 0.13	+ 15.6
10	10.0 ± 0.7	15.0 ± 1.1	+ 50.0	0.65 ± 0.05	1.73 ± 0.12	+ 166.2
30	10.0 ± 0.8	10.0 ± 0.6	0.0	1.33 ± 0.10	0.75 ± 0.06	−43.6
60	9.5 ± 0.7	7.0 ± 0.5	−26.3	1.22 ± 0.09	0.43 ± 0.04	−64.8

W1 and W2 denote weeks 1 and 2. Length and biomass values are mean ± standard deviation from three replicate containers per treatment. Percentage changes were calculated from the corresponding week-1 and week-2 means. Results are presented descriptively.

The contrasting responses among biochar proportions are consistent with evidence that amendment compatibility depends on feedstock and application rate. Liesch et al. (2010) found substantial mortality and weight loss under higher poultry-litter biochar applications, whereas pine-chip biochar produced responses closer to controls. Their results demonstrate why biological benefits cannot be assumed for all biochars. However, their artificial-soil conditions, feedstocks and application units differ from the present Urmar-soil assay, preventing direct transfer of application thresholds.

Jia et al. (2023) further demonstrated that biochar-derived dissolved matter can contribute to earthworm ecotoxicity. This provides a possible explanation for adverse responses to some biochars, but neither dissolved constituents nor relevant changes in pH, conductivity or pore-water chemistry were measured here. The declines at 30–60% amendment therefore identify potentially adverse treatment conditions without establishing the responsible mechanism. These high mass fractions should be treated as range-finding treatments rather than field application recommendations.

The biomass increase at 10% amendment should be interpreted cautiously because week-1 masses differed among treatments and were measured after exposure began. Percentage changes alone therefore do not establish a beneficial amendment rate. The assay provides preliminary evidence of earthworm responses to biochar addition in kiln-affected soil, but does not demonstrate fluoride immobilization, biological recovery or amendment safety.

Integrated interpretation and environmental relevance

Across compartments, the evidence forms a coherent exposure–effect sequence: higher fluoride in Urmar soil and foliage coincided with lower soil biochemical activity, and earthworm growth and reproduction were most impaired when Urmar-derived litter was combined with Urmar soil. The soil–litter interaction is the central ecological result because it shows that risk cannot be evaluated from soil concentration alone. Contaminated crop residues can return deposited fluoride and associated contaminants to the detrital food web, while an already stressed soil matrix can amplify organism-level effects. Reduced earthworm performance may, in turn, weaken residue fragmentation, bioturbation, aggregation, and nutrient cycling, reinforcing the enzyme-level constraints observed at Urmar. The earthworm experiments addressed complementary aspects of biological assessment in kiln-affected soil. The soil–litter assay examined responses to soil origin and crop-residue inputs, whereas the biochar assay screened the biological compatibility of a candidate amendment in Urmar soil. The biochar experiment therefore extended the study toward preliminary amendment assessment without demonstrating fluoride immobilization or remediation. Responses reflected the combined influence of biochar and the affected soil matrix; the design could not distinguish biochar effects from interactions with existing contaminants. Although both assays used Eisenia fetida, their different exposure durations and treatment structures preclude direct comparison of response magnitudes. This convergence supports targeted monitoring and exposure reduction around the affected area. Priority actions are source-control assessment, spatial sampling along emission and wind gradients, separate analysis of washed and unwashed foliage, measurement of extractable as well as total fluoride, and avoidance of returning highly contaminated fodder or crop residues to cultivated soil until their safety is established. Nevertheless, the two-site observational comparison, small number of composite chemical samples, unavailable standard deviations for cocoon counts, and absence of co-contaminant and soil-property adjustment restrict causal inference. The most defensible conclusion is that the kiln-affected soil–plant system was associated with elevated fluoride and impaired biochemical and earthworm endpoints. Controlled fluoride-spiking studies and replicated field gradients are required to quantify fluoride-specific dose–response relationships and distinguish them from site and mixture effects.

Conclusions

Subsequent tomato and alfalfa intercropping within kiln-affected orchards was associated with elevated fluoride exposure and lower soil enzyme activities relative to the reference location. The crop-litter experiment further indicated that earthworm responses depended on both residue origin and the receiving soil matrix. The study extends the earlier orchard assessment by examining vegetable and forage crops alongside biochemical and organism-level indicators, demonstrating the value of assessing biological condition together with contaminant concentrations. The separate Parthenium-biochar assay provides preliminary informatison for amendment screening in affected soil, but does not establish a safe application rate or fluoride-remediation efficacy. Practical management should prioritize exposure monitoring and evaluate candidate amendments for both chemical performance and biological compatibility. Because the field comparison cannot separate fluoride effects from site conditions,

management and co-contaminants, the findings support site-associated biological impairment rather than fluoride-specific causation. Replicated field comparisons and controlled exposure and amendment experiments are needed before recommending crop-management or remediation practices.

Limitations

The field assessment included one kiln-affected location and one reference location, with three composite samples per location. Consequently, site differences could not be separated from fluoride exposure. Co-contaminants, atmospheric deposition and soil-property effects were not quantified sufficiently to identify the causes of the biological responses. Sampling during a subsequent cropping season demonstrated continued exposure but did not distinguish residual contamination from ongoing deposition. Replicate-level uncertainty was unavailable for several reported biological endpoints. The biochar assay was preliminary and did not measure fluoride immobilization or recovery of contaminated soil. These constraints limit causal interpretation and field recommendations. The biochar assay did not include a corresponding amendment series in reference soil or measurements of fluoride immobilization. Consequently, it provides preliminary evidence of earthworm responses to biochar addition in kiln-affected soil, rather than evidence of amendment safety or remediation efficacy.

Declarations

All authors have read, understood, and have complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors.

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The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author contributions:

Shahsaud conducted the experiments, collected samples, analysed the data, and prepared the initial manuscript draft. Muhammad Baseer Us Salam contributed to data interpretation and manuscript review and editing. Shah Maqsud contributed to study design, supervised the research, and reviewed the manuscript. All authors read and approved the final manuscript.

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