



OPEN The effects of amendments on cd and pb under different fertilizer application conditions

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Fertilizer application is a common agricultural practice that enhances soil fertility but can also increase heavy metal mobility in contaminated soils. This study used a pot experiment with four vegetables (water spinach, Chinese cabbage, lettuce, and garland chrysanthemum) to evaluate the impact of BC, ZE, and their combination (CO) on Cd and Pb levels under different fertilization schemes. Results showed that CO treatment significantly enhanced enzyme activities, increasing urease by 2.6–31.6% and catalase by 1.37–14.24% under varying fertilizer conditions. However, sucrase activity increased only with compound fertilizers. The use of compound fertilizers alone raised Cd and Pb levels in vegetable shoots by 0.65 mg·kg⁻¹ and 12.76 mg·kg⁻¹, respectively, while the CO amendment effectively mitigated these increases. BCR sequential extraction indicated that BC and CO shifted Cd and Pb into more stable soil fractions, reducing their bioavailability. Specifically, CO reduced Pb accumulation in shoots by 24.8–49.7%, with BC showing particular efficacy in reducing Cd levels. These findings highlight that BC and ZE, particularly in combination, offer an effective strategy for remediating heavy metal-contaminated soils in agricultural systems, especially when chemical fertilizers are used.

Keywords Soil, Heavy metals, Amendments, Fertilizer, Vegetables

Abbreviations

SOM	soil organic matter
CEC	cation exchange capacity
SSA	specific surface area
OM	organic matter
TCF	ternary compound fertilizer
NF	no fertilizer
CK	control
BC	biochar
ZE	zeolite
CO	combination of biochar and zeolite
WS	water spinach
CC	Chinese cabbage
LE	lettuce
CH	garland chrysanthemum
BF	enrichment factor
TF	transport factor
AP	available phosphorus
AK	available potassium
AN	available nitrogen
F1	acid extracted form
F2	reducible form
F3	oxidizable form
F4	residual form

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Cd	cadmium
Pb	lead
SD	standard deviation
LOD	limits of detection
LOQ	limits of quantification
QA	quality assurance
QC	quality control
ANOVA	analysis of variance

Soil contamination by heavy metals, such as cadmium (Cd) and lead (Pb), is a significant environmental issue, posing risks to ecosystems and human health¹. In China, it is estimated that over 19% of agricultural lands are contaminated with heavy metals, resulting in approximately 12 million tons of contaminated grain annually^{2,3}. Consuming food crops contaminated with Cd and Pb poses serious health risks⁴. Cd can accumulate in the kidneys, leading to kidney dysfunction and bone damage caused by oxidative stress and apoptosis⁵. Pb exposure, even at low levels, is associated with neurotoxicity and cognitive impairment, particularly in children, as it interferes with enzyme function and induces oxidative damage⁶. Cd and Pb disrupt cellular functions by replacing essential metals, generating reactive oxygen species, and inhibiting key enzymes⁷. This contamination is often exacerbated by industrial activities, particularly in mining regions, where heavy metals can accumulate to hazardous levels in the soil.

Various strategies have been employed to address heavy metal contamination in soils, including physical removal, chemical washing, and immobilization. However, physical methods are resource-intensive, requiring substantial amounts of clean soil, while chemical washing can lead to the depletion of essential soil nutrients and structural components^{8,9}. Among the chemical immobilization techniques, the use of natural minerals like zeolite and organic materials such as biochar has gained attention due to their potential to reduce heavy metal bioavailability and transfer to plants^{10–12}.

Zeolite, characterized by its aluminosilicate composition and high cation exchange capacity (CEC), and biochar, known for its ability to improve soil pH and organic carbon content, have been shown to immobilize heavy metals effectively^{13,14}. Despite numerous studies demonstrating the benefits of these amendments individually, there remains a lack of comprehensive research exploring the combined effects of biochar and zeolite under different fertilization regimes. Furthermore, the influence of these amendments on soil physicochemical properties, enzyme activities, and heavy metal uptake by crops in contaminated soils has not been fully elucidated, especially under varying fertilization conditions.

Currently, soil heavy metal pollution poses a significant threat to ecosystems and human health. Although various strategies have been implemented to immobilize heavy metals in soil and reduce their mobility and plant uptake, there is still a lack of systematic research on the specific effects of different fertilizer application schemes on these strategies. In particular, the interaction effects of different soil amendments (such as biochar and zeolite) under different fertilizer applications in Cd and Pb contaminated soils have not been fully explored. This research gap limits our comprehensive understanding of the effectiveness of soil heavy metal remediation technologies in actual agricultural production.

Compared to other commonly used soil amendments, such as organic composts or synthetic stabilizers, the combination of biochar and zeolite offers several advantages. Biochar, with its high surface area and porous structure, effectively adsorbs heavy metal ions, reducing their bioavailability in the soil¹⁴. Zeolite, on the other hand, enhances soil nutrient availability and ion-exchange capacity, further immobilizing heavy metals through its unique molecular structure. Together, these amendments offer a synergistic effect that not only stabilizes metals but also improves soil properties in a more sustainable manner compared to organic composts, which may carry trace amounts of heavy metals, or synthetic stabilizers¹⁵. Therefore, the objectives of this study were as follows: (1) To evaluate the impact of biochar and zeolite amendments, individually and combined, on soil physicochemical properties (such as pH, soil organic matter, and cation exchange capacity) and nutrient availability (N, P, and K). (2) To assess the effects of these amendments on soil enzyme activities (urease, catalase, and sucrase) and the bioavailability and chemical forms of Cd and Pb in the soil. (3) To quantify the accumulation of Cd and Pb in the roots and shoots of vegetables grown in amended soils under varying fertilization regimes. (4) To provide practical guidelines for the combined use of biochar and zeolite in remediating heavy metal-contaminated soils, especially in agricultural systems that apply chemical fertilizers. By conducting a pot experiment with these amendments and evaluating their impact on soil properties, nutrient availability, enzyme activity, and metal accumulation in vegetables, we seek to provide a more comprehensive understanding of their potential benefits and limitations. This research is crucial for developing effective soil remediation strategies, particularly in agricultural systems where chemical fertilizers are commonly used, and for minimizing the risks associated with heavy metal contamination in food crops.

Materials and methods

Soil, amendments, and fertilizer

The soil sample was collected from the surface layer (0–20 cm) of farmland adjacent to the tungsten mining area in Ganzhou, Jiangxi province, China (114.3191°E, 25.4647°N). The soil was passed through a 2 mm sieve after air-drying for pot experiments and property analysis. The soil is a typical red clay contaminated with cadmium and lead, with concentrations of 2.32 mg·kg⁻¹ and 105.60 mg·kg⁻¹, respectively. Both concentrations exceed the risk thresholds specified in the Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land (GB 15618–2018), which are 0.3 mg·kg⁻¹ for cadmium and 90 mg·kg⁻¹ for lead¹⁶. Soil pH was 5.52, measured by a pH meter at a soil/water of 1:2.5. The soil organic matter (SOM) was detected by bulk

density method, and the cation exchange capacity (CEC) was detected by ammonium acetate exchange method¹. The SOM was 38.06 g·kg⁻¹ and The CEC was 9.75 cmol·kg⁻¹.

Zeolite was sourced from Yusong Environmental Equipment Factory in Gongyi City, China, subsequently crushed and sieved through a #60 mesh. Biochar, derived from rice husk, was produced via pyrolysis at 400 °C in an oxygen-free environment for 4 h, then similarly crushed and sieved through a #60 mesh. Following our previous research¹⁷, zeolite and biochar were combined at a 1:1 mass ratio. The characteristics and elemental compositions of the amendments are detailed in Table 1 and S1, respectively. Fig S1 and S2 show the SEM, XRD, and FTIR spectra of biochar and zeolite. The characterization analysis of the amendment can be found in Supplementary Material S1.

Ternary compound fertilizer (TCF), a type of chemical fertilizer, was purchased from Youyu Fertilizer Company in Guizhou, China, which is a commonly used compound fertilizer in the local area for agriculture production. The basic properties of fertilizer are listed in Table S2, and the mass percentages of N, P and K in fertilizer were 15, 15, and 15%, respectively. In our study, we used four commonly cultivated local vegetable species: water spinach (WS), Chinese cabbage (CC), lettuce (LE), and garland chrysanthemum (CH). The vegetable seeds were all purchased from the official flagship store of Wotian Agriculture.

Treatment

To assess the impact of amendments on Cd and Pb in varying fertilizer application scenarios, we conducted a pot experiment. Each plastic pot, measuring 20 cm in diameter and 11 cm in height, was filled with 2 kg of soil. The application of ternary compound fertilizer was categorized into two levels: 0 g·kg⁻¹ (no fertilizer) and 1 g·kg⁻¹ (ternary compound fertilizer), resulting in two distinct groups based on the fertilizer regime. Amendments were incorporated at a 5% mass ratio (amendment to soil)¹⁷, with a control group receiving no amendments for comparison. Consequently, four treatments were established: CK (control), BC (biochar), ZE (zeolite), and CO (combination of biochar and zeolite), each treatment was conducted under two fertilizer conditions: no fertilizer (NF) and ternary compound fertilizer (TCF). Furthermore, four commonly cultivated local vegetables were selected for the experiment: WS, CC, LE, and CH. Following sterilization with 3% H₂O₂, seeds of these vegetables were sown and thinned to five plants per pot, with each treatment replicated thrice. The plants were grown in a greenhouse maintained at 20% soil moisture and 25 °C, with 10 h of daily light exposure. After two months, all plants were harvested, and growth parameters including plant height, root length, fresh weight, and dry weight were meticulously recorded.

Sample analysis

The plants were collected and separated into shoot and root parts for determination. 0.2 g air-dried plant was digested in a microwave-accelerated digestion system with 8 mL HNO₃ and 1 mL H₂O₂. The digestion progress was listed in Table S3, and the Cd and Pb contents in the digestion solution were determined by inductively coupled plasma mass spectrometry (ICP-MS). Soils were air-dried for analysis after plant harvest. The activities of soil enzymes (sucrase, urease, and catalase) in air-dried soil samples were measured using commercial test kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The measurements were conducted based on ultraviolet-visible spectrophotometry¹⁸. Determination of soil available phosphorus content using sodium bicarbonate dissolution molybdenum blue colorimetric method, the ammonium acetate extraction method was used for available potassium content¹⁹, and the alkaline diffusion method was used for available nitrogen content²⁰. The soil was digested in a microwave-accelerated digestion system, and the digestion progress is listed in Table S4. The contents of Cd and Pb in the digestion solution were determined by ICP-MS. The chemical forms of heavy metals in soil samples were analyzed using the modified European Community Bureau of Reference (BCR) sequential extraction method (see Supplementary Material S2). According to this method, soil heavy metals are divided into four forms: acid extracted (F1), reducible (F2), oxidizable (F3), and residual (F4) forms¹⁷. Using HF-HClO₄-HNO₃ to digest the residual (F4) state of the soil samples (see Supplementary Material S3). In this study, inductively coupled plasma mass spectrometry (ICP-MS) was used to determine Cd and Pb in plant and soil samples. The limits of detection (LOD) and limits of quantification (LOQ) for Cd were 0.01 µg·L⁻¹ and 0.05 µg·L⁻¹, respectively, while those for Pb were 0.02 µg·L⁻¹ and 0.1 µg·L⁻¹. Additionally, the calibration of ICP-

Properties	BC	ZE	CO
pH	7.43	8.05	7.98
Total N (%)	0.79	-	-
Total C (%)	45.15	-	-
Moisture (%)	0.5	0.2%	0.35
SSA (m ² ·g ⁻¹)	89.06	178.24	112.66
OM (g·kg ⁻¹)	436.59	0.76	298.01
CEC (cmol·kg ⁻¹)	87.65	137.68	112.32
Cd (mg·kg ⁻¹)	0.05	0.02	0.03
Pb (mg·kg ⁻¹)	0.78	1.12	1.01

Table 1. Properties of amendments. SSA, specific surface area; OM, Organic matter; CEC, cation exchange capacity.

MS was performed using a series of standard solutions to construct a working curve, with correlation coefficients (R^2) all exceeding 0.999, ensuring the accuracy and repeatability of the measurement data.

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean±standard deviation (SD). The enrichment factor of heavy metals in vegetables (BF) can express the metals uptake capacity of the plants from the soil. Transport factor (TF) is used to evaluate the mobility of heavy metals from soil to vegetables. The calculated methods of BF and TF as shown in Table 2. Statistical significance was determined by one-way analysis of variance (ANOVA) using R4.0.0. Conduct correlation analysis using SPSS 26. During the analysis, various quality assurance/quality control (QA/QC) methods were employed, which involved the establishment of duplicate, blank, and standard samples. The QA/QC analysis was conducted on soil-certified reference materials (GBW07430), with a recovery rate ranging from 93.6 to 105.2%. All figures were created using Origin 2022.

Results and discussion

The effects of amendments on soil nutritive elements

The impact of amendments on soil nutritional elements (AP, AK, and AN) is depicted in Fig. S3. The application of BC and CO has significantly increased AP. As shown in Fig S3a, under NF conditions, compared with CK, BC and CO increased AP by 25.0–46.2% and 16.7–42.3%, respectively. This growth is consistent with the findings of Hartley et al.²¹, who observed that the high affinity of biochar for aluminum and iron ions promotes the release of dissolved phosphorus, thereby increasing AP levels in the soil. In addition, the ability of biochar to affect soil AP is consistent with similar studies emphasizing the role of biochar in enhancing nutrient retention and availability²². The research results also indicate that although ZE does not contain nutrients such as nitrogen, phosphorus, and potassium, due to its soil regulating properties, it can indirectly affect AP under specific crop conditions²³. This effect is particularly evident in the LE planting scenario, where ZE supports root development and nutrient absorption.

Compared with NF, TCF significantly increased the levels of AP, AK, and AN in the soil, attributed to the composition of the fertilizer (refer to Table S2). As shown in Fig S3b, under TCF conditions, the effects of BC and CO treatment are more significant, with an average increase of 43.1% in AP compared to NF. Previous studies have shown that the combined use of amendments and fertilizers produces a synergistic effect, which is more effective in improving nutrient utilization efficiency than using a single amendment alone²⁴. This synergistic effect may be due to the physical and chemical properties of biochar (refer to Table 1), which improves soil structure and nutrient retention capacity²⁵. Overall, the combined application of amendments and fertilizers has had a significant effect on the optimization of soil nutrients.

The effect of amendments on soil physical and chemical properties

Soil pH

In the NF condition, Fig. 1a illustrates the influence of amendments on soil pH. Relative to the CK, the incorporation of BC, ZE, and CO led to an average soil pH increase of 0.30, 0.14, and 0.21 units for four plant types, respectively. The alkaline properties of biochar play an important role in neutralizing soil acidity, while its porous structure helps improve soil aeration and enhance microbial activity, thereby contributing to increased pH stability²⁶. Zeolite, as an alkaline hydrated aluminosilicate, has negatively charged sites that can exchange cations with soil solution, thereby increasing soil pH and improving nutrient retention, consistent with the findings of Cui et al.¹³. and Wu et al.¹⁴. Moreover, the combined use of biochar and zeolite showed a synergistic effect in increasing soil pH, likely due to their complementary mechanisms in altering soil chemical properties. This is in agreement with the study by Ibrahim et al.²⁷, which indicated that the combined application of biochar and zeolite can effectively improve soil quality by enhancing pH stability. The extent of pH change was also influenced by the type of vegetable planted. The study found that soils planted with WS and CC had a higher increment in pH compared to LE and CH, particularly under treatments such as BC + WS, CO + WS, BC + CC, and CO + CC. This finding suggests that soils planted with LE and CH have better acid-base buffering capacity. This observation merits further exploration in future research to fully understand the interaction between crop types and soil amendment effects.

Under TCF conditions, the incorporation of BC, ZE, and CO led to pH increases of 0.18, 0.07, and 0.11 units, respectively, as shown in Fig. 1b. The most pronounced increase occurred in the BC + CC treatment, where pH surged by 0.29 units. Notably, the soil pH under TCF conditions was lower than that under NF conditions. Compared to CK in NF, the CK pH under TCF decreased by 0.76 units after fertilization. Chemical fertilizers are commonly used in agricultural production to address soil degradation and enhance crop yields; however, long-term use is associated with soil acidification, which can lower soil pH²⁸. In contrast, the range of pH changes under TCF conditions was less pronounced than that under NF. It is noteworthy that the soil pH

Factor	Calculation
Enrichment factor in shoot (BF ₁)	BF ₁ = f/s
Enrichment factor in root (BF ₂)	BF ₁ = u/s
Transport factor (TF)	BF ₁ = f/u

Table 2. Metals enrichment factor and transport factor in plants. f, metal content in shoot (mg·kg⁻¹); s, metal content in soil (mg·kg⁻¹); u, metal content in root (mg·kg⁻¹).

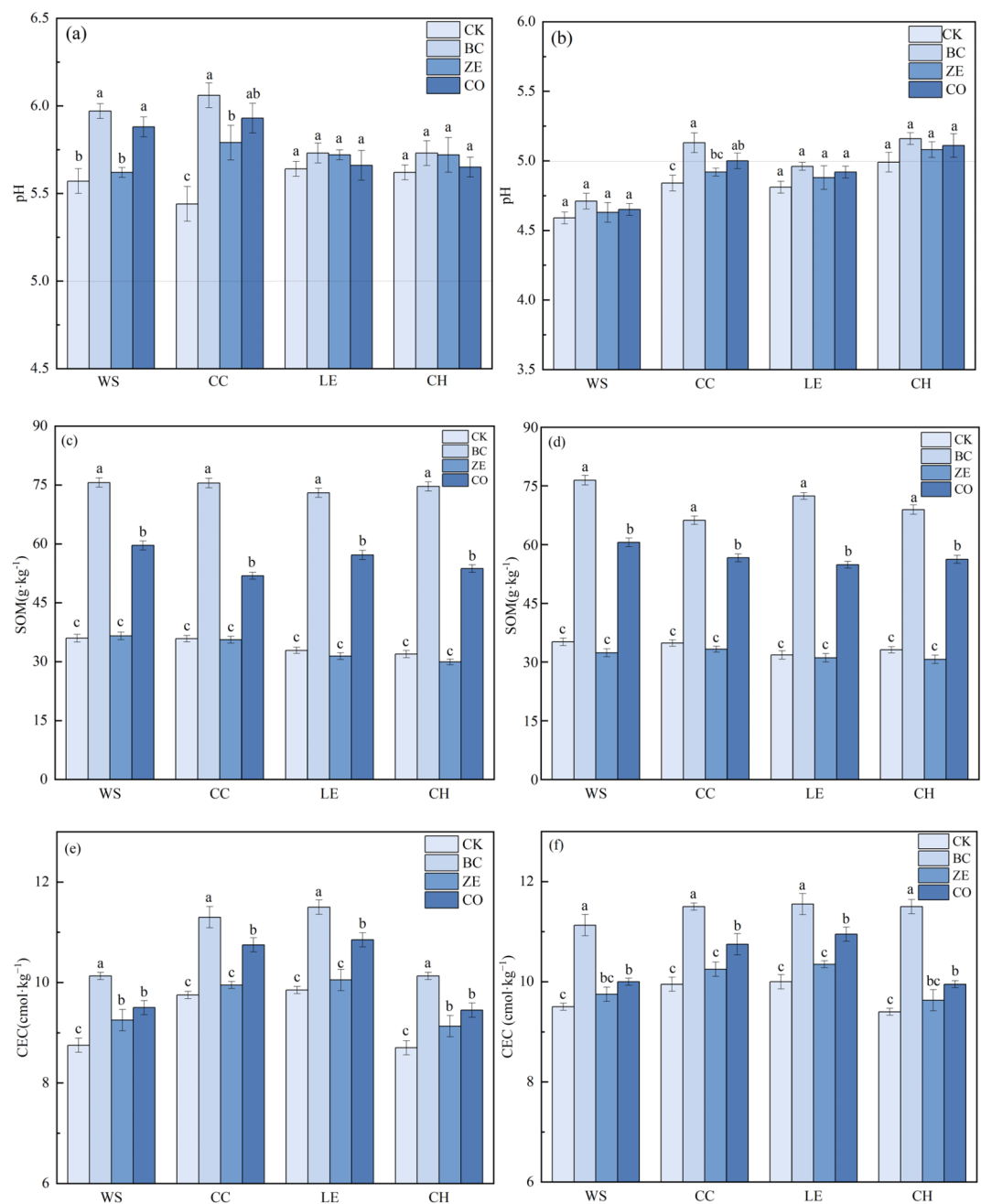


Fig. 1. The change of soil pH, SOM, and CEC. (a), (c), and (e) represented NF condition; (b), (d), and (f) represented TCF condition. Note: Data were presented as the mean $\pm$ SD ($n=3$). $p < 0.05$ was considered significant, and groups of the same plant with different superscript letters show significant differences.

for WS cultivation significantly decreased under both fertilization conditions ($p > 0.05$), indicating that WS may have a different impact on soil acidification dynamics compared to other crops. The above results indicate that BC and ZE, especially when combined, effectively increase soil pH, helping to counteract soil acidification from fertilizer use.

Soil SOM

Soil organic matter (SOM) is a pivotal physical and chemical attribute of soil, with its variations depicted in Fig. 1c and d. Our results showed that the application of TCF did not significantly impact SOM levels compared to treatments under NF conditions, which is likely due to the low organic matter content in TCF (refer to Table S2). However, under both NF and TCF conditions, BC and CO treatments resulted in substantial increases in SOM, ranging from 52.42 to 57.22% and 30.93–42.51%, respectively, compared to their controls. In contrast, ZE had minimal effects on SOM. This indicates that the increase in SOM in CO treatments is primarily attributed to biochar rather than zeolite. Previous studies have shown that biochar can enhance SOM through mechanisms

such as stabilization and reduced mineralization. For instance, Glab et al.²⁹ and Peng et al.³⁰ demonstrated biochar's efficacy in increasing SOM through stabilization of organic-mineral complexes and by improving soil aggregate stability. Our findings align with these studies, showing that biochar's high organic matter content directly boosts SOM and reinforces soil stability. Furthermore, although plants are usually a significant SOM source through root exudates and litter, the short experiment duration and complete plant harvesting limited these contributions, which is consistent with Ruf et al.³¹ findings on the importance of longer-term plant interactions for SOM accrual. These findings suggest that BC, due to its high organic content, substantially raises SOM levels, with combined applications further enhancing soil stability. These findings suggest that BC, due to its high organic content, substantially raises SOM levels, with combined applications further enhancing soil stability.

Soil CEC

Cation exchange capacity (CEC) is a critical characteristic for nutrient retention in agricultural soils. The variation in soil CEC is depicted in Fig. 1e and f. Our data showed that BC, ZE, and CO treatments enhanced CEC by 13.6–14.6%, 2.0–5.4%, and 7.9–9.3%, respectively, under NF conditions, with similar increases under TCF conditions. Notably, biochar and zeolite's contributions to CEC are supported by their structural properties. For example, Atkinson et al.³² highlighted that biochar's porous structure and functional groups facilitates ion exchange, while Akbar et al.³³ explained that zeolite's aluminosilicate framework provides high CEC due to its negatively charged tetrahedral structures. Post-amendment application, a comparative increase of approximately 4.6% in CEC was observed against the control, with the order being BC > CO > ZE > CK. Under NF conditions, BC, ZE, and CO contributed to average CEC enhancements of 13.9%, 3.5%, and 8.6%, respectively, reflecting the intrinsic high CEC values of biochar and zeolite (refer to Table 1). Remediation materials, to a certain degree, may serve as alternatives to fertilizers in augmenting soil CEC effects. BC and ZE jointly improve soil CEC, enhancing nutrient retention and providing a supportive environment for plant growth.

The effects of amendments on soil enzyme

Urease enzyme activity

Ammonia, produced by the enzymatic reaction of urease, serves as an effective nitrogen source for plants. Moreover, urease activity acts as an indicator of soil nutritional status and nitrogen fertilizer efficiency³⁴. Figure 2a illustrates that urease activity experienced an increment of 0.013 mg-g⁻¹ and 0.005 mg-g⁻¹ following BC and CO treatments, respectively. This enhancement aligns with the findings of Zhao et al.³⁵, where biochar increased SOM, thereby enhancing nitrogen availability in the soil. The stability of the organic-mineral complexes provided by BC and CO may support increased urease activity, consistent with the SOM correlation discussed in Chen et al.³⁶ studies. In contrast, ZE treatment had no significant effect on urease activity and even showed an inhibitory effect in soils planted with WS and LE, as shown in Fig. 2b. This is consistent with the findings of Tzanakakis et al.³⁷, who reported that zeolite effectively inhibited soil urease activity. Figure 2b demonstrates that soils under TCF exhibited a lower average urease activity (0.061 mg-g⁻¹) compared to NF (0.104 mg-g⁻¹), which can be attributed to soil acidification and the mobilization of heavy metals induced by fertilizer application, potentially mitigated by amendments³⁸. The above results indicate that CO applications enhance urease activity, supporting nitrogen availability and cycling in the soil.

Catalase enzyme activity

Soil catalase activity is a crucial indicator of soil's oxidation-reduction potential and significantly influences the impact of toxic elements on soil health¹³. As shown in Fig. 2c and d, under NF conditions, the application of CO led to an increase in catalase activity by 1.37–14.24% across four different vegetable types, suggesting that CO can play a significant role in enhancing soil quality. This increase indicates that CO can significantly support soil health by promoting microbial stability and reducing oxidative stress. Previous studies, such as those by Xu et al.³⁹ and Chang et al.⁴⁰, have shown that organic amendments, including compost and biochar, can enhance soil catalase activity by providing essential nutrients that facilitate microbial growth and function, aligning well with our results. In the case of solitary applications, BC and ZE showed varied impacts on catalase activity depending on the vegetable type. For instance, during CH cultivation, ZE application did not lead to a significant increase in catalase activity, which may be attributed to unique soil-microbe interactions influenced by the crop type. Conversely, BC application in CC cultivation improved catalase activity by 1.37%. TCF treatments led to an average increase in catalase activity of 23.31%, attributed to the addition of nutritive elements such as AP, AK, and AN⁴¹. Combined treatments involving fertilizers with BC, ZE, and CO resulted in increases in soil catalase activity by 1.88 to 7.52%, 2.45 to 15.52%, and 2.59 to 10.47% respectively, underscoring the crucial role of fertilizers in catalase activity enhancement. The enhanced catalase activity resulting from the combined application of amendments and fertilizers suggests a potential reduction in soil oxidative stress, which may mitigate the adverse effects of toxic elements on soil health. This finding highlights the effectiveness of BC and ZE in synergistically boosting catalase activity, thereby potentially reducing oxidative damage in contaminated soils.

Sucrase enzyme activity

Soil sucrase catalyzes the hydrolysis of sucrose into glucose and fructose, serving as an important indicator of soil fertility and nutrient availability⁴². Figure 2e and f illustrate that under NF conditions, CO application did not significantly affect sucrase activity, indicating that the synergistic effect may not be sufficient in the absence of fertilizers. Notably, ZE application enhanced sucrase activity in soils planted with CC and CH, with increases of 8.4% and 22.8%, respectively, compared to CK. This enhancement can be attributed to ZE's ability to improve soil structure and microbial habitat, facilitating greater microbial activity and enzyme production, as

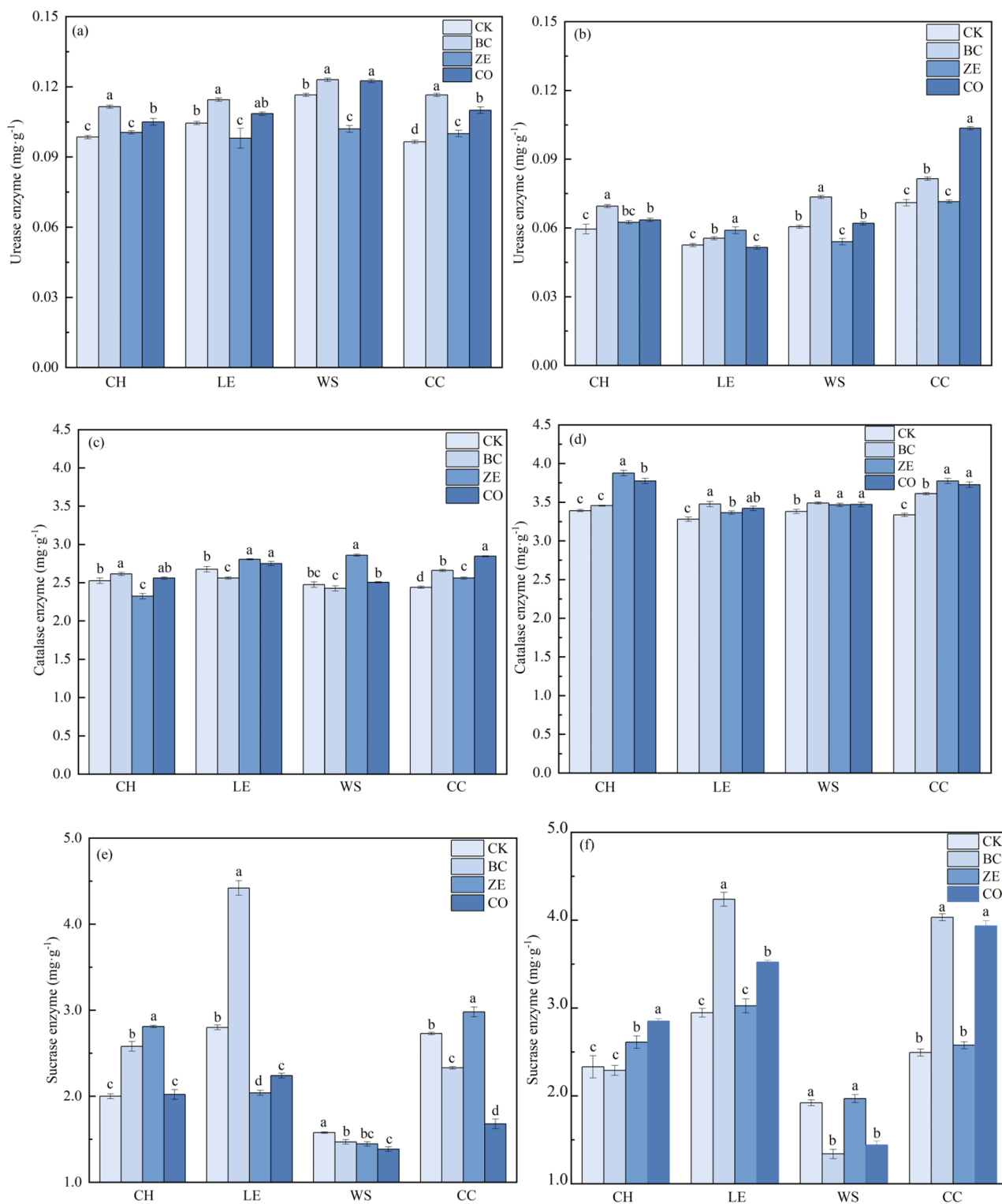


Fig. 2. The change of enzyme activity. (a), (c), and (e) represented NF condition; (b), (d), and (f) represented TCF condition. Note: Data were presented as the mean $\pm$ SD ($n=3$). $p < 0.05$ was considered significant, and groups of the same plant with different superscript letters show significant differences.

highlighted in studies by Mondal et al.⁴³. BC treatment boosted sucrase activity in soils planted with LE and CH by 36.7% and 22.5%, respectively, but had an adverse effect on soils planted with WS and CC. Furthermore, when comparing TCF with NF conditions, there was an average increase of 6.06% in sucrase activity with fertilizer application, likely due to the added nutrients (AP, AK, and AN) in the fertilizer³⁶. These results suggest that BC

and ZE, particularly under fertilized conditions, can effectively enhance sucrase activity, thereby supporting soil microbial health and promoting carbon cycling, which is essential for soil fertility.

The effects on morphology of heavy metals in soil

Following a two-month period of potted plant experiments, we measured the cadmium and lead concentrations in various treatment groups (CK, BC, ZE, CO) of unfertilized water spinach soil, as presented in Table S5. The different treatments significantly impacted the concentrations of Cd and Pb, with the CO group showing the most effective reduction. This finding suggests that the combination of biochar and zeolite has substantial potential for remediating heavy metal-contaminated soil. According to the Soil Environmental Quality—Risk Control Standard for Soil Contamination of Agricultural Land (GB 15618—2018), although the Cd concentration in each treatment group exceeded the standard, the Pb concentration was below the risk screening value¹⁶.

The form of heavy metals significantly impacts their bioavailability, migration, and phytotoxicity. The acid-extractable (F1) and reducible (F2) forms are more accessible to plants, in contrast to the oxidizable (F3) and residual (F4) forms, which are more stable and exhibit lower bioavailability⁴⁴. The forms of Cd and Pb in the soil were analyzed using the BCR sequential extraction procedure, as illustrated in Fig. 3.

Figure 3a illustrates that in the CK group, untreated, Cd predominantly occurs in the F1 form, characterized by high mobility and potential environmental harm. Upon the addition of BC, ZE, and CO, there was a noticeable reduction in the F1 fraction, decreasing to 45%, 46%, and 35%, respectively, with CO exhibiting the most pronounced effect. This result is consistent with previous research, where BC, due to its high surface area, can increase soil pH and adsorb heavy metals, thereby reducing their mobility⁴⁵. Meanwhile, ZE, with its high cation exchange capacity, immobilizes heavy metals through ion exchange, converting them into forms with lower bioavailability⁴⁶. The combination of BC and ZE enhances these immobilization mechanisms, resulting in a more stable distribution of heavy metal forms and reducing bioavailability in soil¹⁷. The addition of BC, ZE, and CO significantly increased the F4 fraction from 12 to 30%, 26%, and 29%, respectively, with CO's impact surpassing that of ZE alone. BC specifically influenced the morphology of F2, resulting in a 13% decrease compared to the CK group, while ZE significantly affected F3, reducing it by 4%. The modifications in Cd's morphology due to BC and CO can be primarily attributed to biochar's ability to transform soluble metals into insoluble forms that bind with organic matter^{47,48}.

Figure 3b demonstrates that in the control group, the F1 form of Pb concentration was 7.65 mg/kg, constituting 6% of the total Pb. Upon application of BC, ZE, and CO, the F1 fraction of Pb decreased to 4%, 3%, and 2%, respectively. Notably, the application of ZE resulted in a significant reduction of the F2 fraction, primarily transforming it into the more stable F4 fraction (increasing from 15 to 37%), consistent with the findings of Ye et al.⁴⁹, which demonstrated that ZE application effectively reduces the bioavailability of Pb in soil. Similarly, BC treatment contributed to an increase in the F4 fraction from 15 to 20%, aligning with previous research that shows biochar enhances heavy metal immobilization through its large adsorption surface area and negative charge content⁵⁰. Thus, the introduction of these amendments effectively lowered the bioavailable fractions (F1 and F2) while increasing the proportion of heavy metals in the more stable F4 fraction, a similar pattern observed in related studies⁵¹. Among the BC and CO treatments, the CO group exhibited a higher percentage of F4, likely due to the elevated soil pH in the CO group facilitating the formation of $\text{Ca}_2\text{Pb}_8(\text{PO}_4)_6(\text{OH})_2$ and $\text{Pb}_5(\text{PO}_4)_3\text{OH}$. The findings emphasize that applying CO can transform the bioavailable forms of Cd and Pb into more stable, less mobile forms, thereby reducing environmental risks.

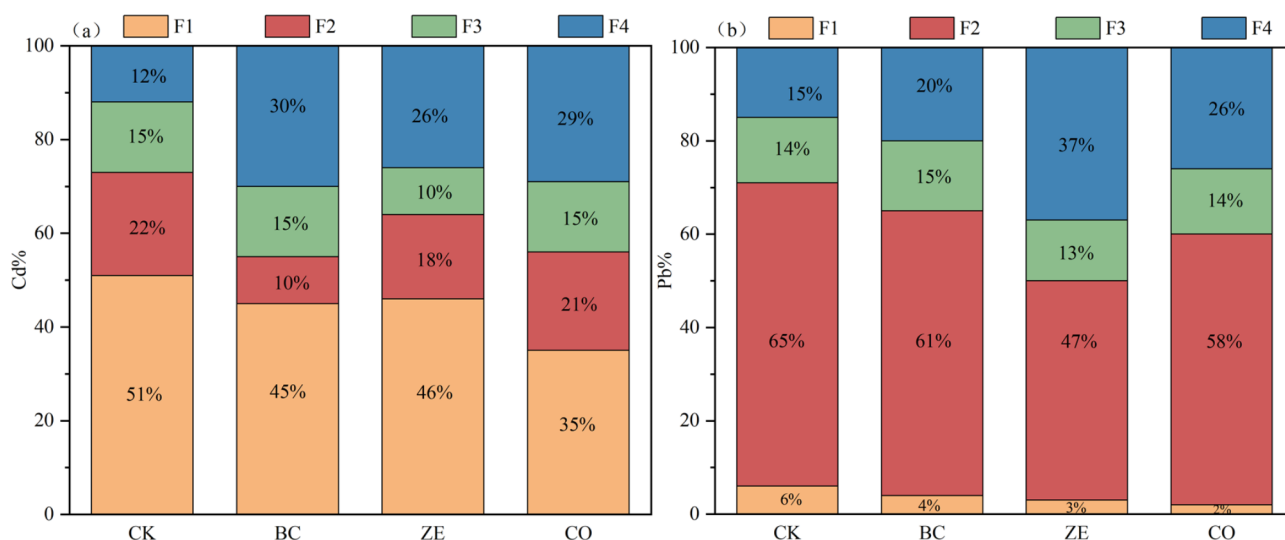


Fig. 3. Percentage of heavy metal morphology. (a) represented Cd Percentage of each morphology; (b) represented Pb Percentage of each morphology.

Heavy metals in vegetable plants

Cd accumulation

The absorption of Cd by plants is governed by several factors, including Cd's mobility and the plant species. Our prior research indicates that biochar, zeolite, and their combination can effectively reduce Cd's mobility in soil¹⁷. Our results are consistent with previous studies, showing that both BC and ZE treatments significantly reduced cadmium mobility, leading to decreased cadmium concentrations in roots and stems under NF and TCF conditions. Except in specific cases such as NF + LE and NF + CC, BC and CO treatments were particularly effective in reducing cadmium content. The study by Kim et al.⁵² also indicated that biochar effectively immobilized heavy metals and reduced plant uptake. In contrast, ZE treatment also reduced cadmium levels, but was less effective than BC and CO treatments. The effectiveness ranking was as follows: BC > CO > ZE. Cd accumulation was predominantly higher in the roots than in the shoots across all vegetable types, except for LE. Compared to NF, TCF usage resulted in a mean increase of 0.65 mg·kg⁻¹ in shoot Cd content, attributed to soil acidification due to fertilization⁵³. This is further corroborated by significant Cd increases in WS and CC alongside notable pH rises (Fig. 4a and b).

For the NF condition, the Cd accumulation capacities of four vegetables were ranked as follows: LE > CH > WS > CC. In the CK group, the mean Cd concentrations in the roots and shoots were 0.75 and 0.64 mg·kg⁻¹, respectively. These concentrations exceeded the Chinese Food Safety Standard (GB 2762–2017), which sets the maximum allowable limit for Cd in leafy vegetables at 0.2 mg·kg⁻¹, indicating a potential health risk⁵⁴. Following treatments with BC, ZE, and CO, Cd accumulation decreased significantly ($p < 0.05$). Relative to CK, the reductions in shoot Cd concentration were 40.59%, 14.53%, and 31.19% for BC, ZE, and CO treatments, respectively, while in the roots, decreases of 30.53%, 7.91%, and 20.63% were observed. However, despite these reductions, the Cd levels in all treatments still approached or exceeded the food safety threshold, highlighting the persistent contamination issues and the need for more effective or prolonged treatments. The application of fertilizers led to an increased risk of Cd accumulation in the edible parts of four types of local vegetables, a finding consistent with previous studies⁵⁵. Under TCF conditions, Cd concentrations in the roots and shoots increased by 32.0% and 62.5%, respectively, compared to the NF condition, due to soil acidification caused by fertilization. Although the amendments reduced Cd accumulation significantly, the levels remained above the safety standard.

The bioconcentration factor (BF) and translocation factor (TF) of Cd in vegetables under NF conditions are presented in Table 3. The results indicate that the amendments significantly reduce the BF for all treatments, indicating their effectiveness in limiting Cd uptake from soil to the roots, potentially leading to cleaner production practices. Specifically, BC and ZE treatments increased the TF for CC and CH, respectively. Conversely, CO decreased the TF for all vegetables, highlighting the promising application potential of combined amendments. This aligns with Paul et al.⁵⁶, who suggested that combined amendments could offer a more balanced approach to immobilizing heavy metals. This study suggests that CO application is the most effective strategy for reducing Cd accumulation in vegetables.

Pb accumulation

Under NF conditions, the mean Pb accumulations in the roots and shoots without any amendment application (CK) were 4.26 and 8.98 mg·kg⁻¹, respectively, as shown in Fig. 5a. These concentrations significantly exceed the Chinese Food Safety Standard (GB 2762–2017), which sets the maximum allowable limit for Pb in leafy vegetables at 0.3 mg·kg⁻¹¹⁵⁴. The application of amendments reduced Pb accumulation in plants, with the greatest reduction in shoots observed in the CO group at 32.4%, followed by BC at 31.6%, and ZE at 16.2%. These results

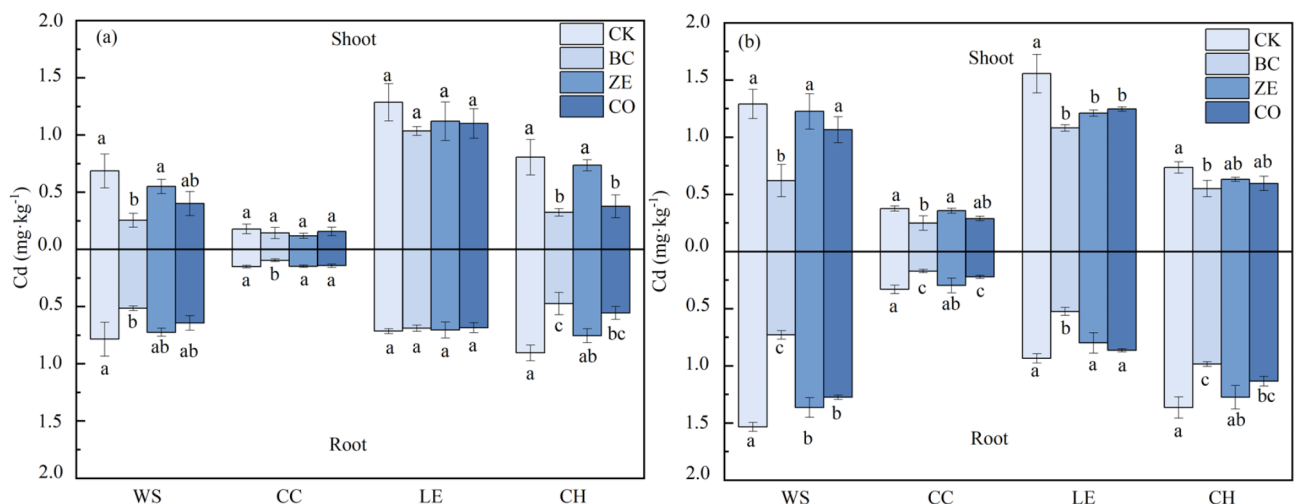


Fig. 4. Cd accumulation in plants. (a) represented NF condition; (b) represented TCF condition. Note: Data were presented as the mean $\pm$ SD ($n = 3$). $p < 0.05$ was considered significant, and groups of the same plant with different superscript letters show significant differences.

Vegetables	Amendments	TF	BF ₁	BF ₂
WS	CK	0.87	0.32	0.37
	BC	0.49	0.13	0.26
	ZE	0.76	0.25	0.33
	CO	0.62	0.20	0.32
CC	CK	1.19	0.08	0.07
	BC	1.48	0.07	0.05
	ZE	0.80	0.05	0.07
	CO	1.09	0.07	0.07
LE	CK	1.80	0.86	0.48
	BC	1.50	0.64	0.42
	ZE	1.59	0.66	0.42
	CO	1.61	0.52	0.32
CH	CK	0.89	0.50	0.56
	BC	0.68	0.20	0.30
	ZE	0.95	0.43	0.46
	CO	0.68	0.21	0.32

Table 3. TF and BF of cd in vegetables eatable part under NF condition.

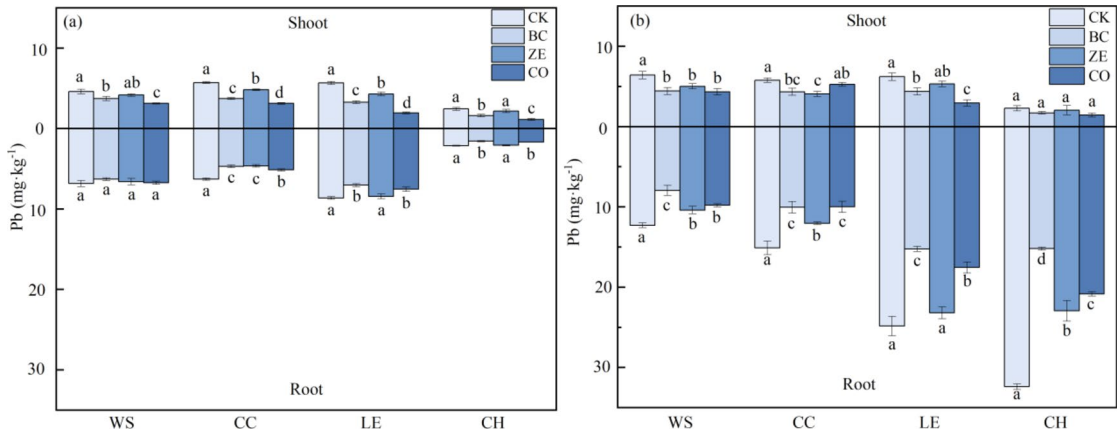


Fig. 5. Pb accumulation in plants. **(a)** represented NF condition; **(b)** represented TCF condition. Note: Data were presented as the mean ± SD (*n* = 3). *p* < 0.05 was considered significant, and groups of the same plant with different superscript letters show significant differences.

suggest that combining amendments is more effective than using a single agent in decreasing Pb levels in plants, a mechanism attributed to the enhanced efficiency of the combination in immobilizing Pb in the soil^{17,28}. Despite these reductions, Pb concentrations remained above the permissible limit, indicating that while the amendments are effective, they are insufficient on their own to bring levels below hazardous thresholds. Except for CC, few changes in Pb accumulation in the roots were observed after the application of amendments. Under TCF conditions, as presented in Fig. 5b, the mean Pb concentrations in the shoots and roots with CK treatment were 5.16 and 21.16 mg·kg⁻¹, respectively, indicating that the Pb concentration in roots was four times higher than in shoots. The application of BC, ZE, and CO significantly reduced Pb levels in plant shoots, with mean reductions of 28.2%, 20.1%, and 32.4%, respectively. Notably, the CO treatment led to the greatest reduction in Pb in the shoots of LE and CH, with decreases of 52.6% and 36.6%, respectively. Among the treatments, BC was the most effective at reducing Pb accumulation in roots, with a mean reduction of 42.8% across all four vegetable types. However, despite these reductions, Pb levels still exceeded the Chinese Food Safety Standard (GB 2762–2017) limits, underscoring the need for further optimization of amendment application. Furthermore, a comparison of Pb accumulation under NF and TCF conditions revealed a noticeable increase in Pb concentration due to fertilizer application, consistent with Cd accumulation patterns, with the largest increase observed in the roots of CC. This is closely related to soil acidification caused by fertilization²⁸. Although current treatments can mitigate heavy metal accumulation, prolonged application may be required to achieve levels that ensure food safety and protect public health.

The BF and TF of Pb in vegetables under NF conditions are detailed in Table 4. After applying amendments, all TF values for Pb in vegetables decreased, except for CC. As illustrated in Fig. 5a, the application of amendments significantly reduced the Pb concentration in the roots of CC, which likely explains the observed results. The BF₁

Vegetables	Amendments	TF	BF ₁	BF ₂
WS	CK	0.67	0.05	0.075
	BC	0.59	0.04	0.069
	ZE	0.63	0.04	0.067
	CO	0.46	0.03	0.073
CC	CK	0.70	0.04	0.06
	BC	0.79	0.04	0.05
	ZE	1.03	0.05	0.05
	CO	0.82	0.04	0.05
LE	CK	0.65	0.07	0.10
	BC	0.46	0.04	0.08
	ZE	0.51	0.04	0.09
	CO	0.25	0.02	0.06
CH	CK	0.17	0.03	0.16
	BC	0.22	0.02	0.08
	ZE	0.21	0.02	0.11
	CO	0.15	0.01	0.08

Table 4. TF and BF of pb in vegetables eatable part under NF condition.

and BF₂ values for WS, LE, and CH also saw reductions compared to their respective controls. Numerous studies have verified that amendments can mitigate the risk of Pb transfer from soil to the edible parts of vegetables^{52,56}. The results indicate that BC and ZE reduce Cd and Pb accumulation in vegetables, with the greatest reduction observed in the CO treatment, thus enhancing food safety.

The effects on growth indices of vegetables

The growth indices of vegetables were showed in Table S6. Numerous studies affirm that soil amendments can positively impact vegetable yields by enhancing soil properties such as pH, CEC, SOM, and nutrient availability, as well as by reducing heavy metal migration rates^{47,57,58}. Our findings are consistent with these previous studies, as we observed that amendments such as CO and BC improved vegetable growth indices through alterations in soil characteristics. For example, under NF conditions, CO increased the dried weight of LE by 33.5%, showcasing its effectiveness in promoting growth by improving soil quality. However, BC, while beneficial for WS and CC, did not significantly enhance the growth of LE, and even inhibited certain indices such as plant height and fresh weight. This outcome suggests that the exclusive application of BC may have limitations for specific crops like LE, aligning with Steiner et al.'s²⁴ findings, which emphasized biochar's role as an amendment rather than a direct nutrient source due to its low inherent nutrient content. For WS and CC, BC treatment showed a significant positive effect on plant growth, which increased their dried weight by 52.7 and 29.7%, respectively. In addition, amendments reduced the dried weight of CH, and the use of them for planting CH need to be re-estimated for field production.

Zeolite, a natural mineral, is not enriched in N, P, and K. Despite this, it contributes to plant growth by mitigating toxic effects⁵⁹. In our study, the combined application of these amendments with fertilizers under TCF conditions improved plant growth indices more significantly than under NF conditions, suggesting a synergistic effect. For instance, compost increased the dried weights of WS, LE, and CH by 33.3%, 32.1%, and 14.5%, respectively, demonstrating its potential to promote healthier and higher-yielding crops. The above results indicate that the application of CO improved the growth index of vegetables, promoting healthier and higher-yielding crops.

Correlation between soil properties and heavy metal accumulation in vegetables

To explore the interactions between soil physicochemical properties and heavy metal accumulation in plants, we conducted a correlation analysis using SPSS software. The analysis results are presented in Table 5. The results indicate that the enrichment of Cd and Pb in vegetables shows a negative correlation with soil pH, CEC, and SOM.

The analysis indicates that the accumulation of Cd in WS shows a significant negative correlation with soil pH, CEC, and OM ($P < 0.05$), suggesting that an increase in these soil properties can reduce Cd accumulation in plants. Studies have shown that soil pH and organic matter are key factors in the migration and transformation of heavy metals such as cadmium and lead. Siddique et al.⁶⁰ found that higher pH levels promote the formation of insoluble metal complexes, thereby reducing cadmium bioavailability. Kwiatkowska-Malina et al.⁶¹ demonstrated that an increase in SOM content through biochar addition enhances complexation with Cd ions, reducing their mobility and subsequent accumulation in vegetables, which is consistent with the findings of this study. In CH, Cd accumulation shows a significant negative correlation with AK and AP ($P < 0.05$), indicating that higher levels of these nutrients decrease Cd bioavailability. This observation aligns with the findings of some scholars, who reported that available potassium and phosphorus can reduce Cd uptake by plants through competitive inhibition in the soil matrix^{62,63}.

		pH	CEC	SOM	AP	AK	Urease enzyme	Sucrase enzyme	Catalase enzyme
Cd	WS	-0.9704*	-0.987*	-0.9587*	-0.8366	-0.6088	-0.4911	0.6070	0.6663
	CC	-0.1295	-0.1683	-0.1025	0.0999	-0.1015	-0.1870	-0.3015	-0.1576
	LE	-0.9899*	-0.8372	-0.7549	-0.7950	-0.6380	-0.4545	-0.3804	0.2047
	CH	-0.7950	-0.6380	-0.7950	-0.9584*	-0.970*	-0.9149	0.0036	-0.6747
Pb	WS	-0.6323	-0.8740	-0.6300	-0.8366	-0.6088	-0.4911	0.6070	0.6663
	CC	-0.8956	-0.8410	-0.6826	-0.8783	-0.969*	-0.8457	0.8436	-0.9712*
	LE	-0.8362	-0.7277	-0.7020	-0.999**	-0.8651	-0.4783	-0.0381	-0.0267
	CH	-0.1398	-0.6729	-0.6962	-0.7461	-0.8328	-0.6562	0.1703	-0.4992

Table 5. Correlation between soil properties and heavy metal content.

In terms of Pb accumulation, Pb content in CC shows a significant negative correlation with catalase activity ($P < 0.05$), indicating that higher catalase activity in soil can reduce Pb mobility. Studies have shown that Pb adversely affects catalase activity, possibly due to Pb inhibiting the oxidation reactions facilitated by catalase in the soil⁶⁴. Additionally, the analysis indicates a significant negative correlation between Pb accumulation in lettuce and available phosphorus ($P < 0.01$). The addition of amendments increased available phosphorus in the soil, promoting the formation of lead-phosphate complexes, thereby reducing Pb bioavailability to plants⁶⁵.

Conclusion

This study confirms that the combined use of BC and ZE is an effective strategy for remediating Cd and Pb contaminated soils, particularly under fertilization conditions. The amendments enhanced soil pH, organic matter, cation exchange capacity, and nutrient availability, leading to reduced bioavailability of Cd and Pb. This stabilization effect was reflected in lower heavy metal accumulation in vegetable crops, demonstrating the amendments’ ability to mitigate metal uptake and promote safer agricultural production. The findings suggest that applying BC and ZE together can counteract soil acidification and heavy metal mobilization caused by fertilizers, making this combined amendment a promising approach for sustainable management of heavy metal-contaminated agricultural soils.

Data availability

All data generated or analyzed during this study are included in this published article.

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Author contributions

Z.B.C: Project administration, Writing – original draft, Writing – review & editing. L.Y.Q: Writing – review & editing, Methodology, Conceptualization. Z.X.J: Writing – review & editing. W.Z.L: Writing – review & editing. L.Q: Writing – review & editing. C.M: Funding acquisition, Supervision. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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