Bangladesh J. Bot. **54**(3): 855-864, 2025 (September) Special DOI: https://doi.org/10.3329/bjb.v54i30.85163

EFFECTS OF DIFFERENT CONCENTRATIONS OF LEAD AND CADMIUM STRESS ON SPINACH GROWTH AND PHYSIOLOGY

NA WANG[1,2,3*], ZHE LIU[1,2,3], LU NAN[1,2,3] AND YUHU LUO[1,2,3]

Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co. Ltd., Shaanxi 710021, China

Keywords: Lead, Cadmium, Combined Stress, Spinach, Antioxidant enzyme activity, Chlorophyll degradation

Abstract

This study investigated the ecotoxicological effects of lead (Pb) and cadmium (Cd) stress, both individually and in combination, on spinach (*Spinacia oleracea*), by analyzying the growth characteristics, antioxidant responses, and photosynthetic damage mechanisms of spinach under controlled experimental conditions with different concentration gradients. Pb-Cd combined stress significantly inhibited spinach biomass, with a dose-dependent and synergistic enhancement, with the total dry weight decreasing by up to 62.8%. The root system was the primary target of stress, with dry weight loss (minimum 28.4% in control) being significantly higher than that of the aerial parts. The biomass differences between stems and leaves were closely associated with the differential antioxidant capacity of the organs. At low concentrations, the increase in leaf superoxide dismutase (SOD) activity (by 12.3-18.5%) partly alleviated oxidative damage, while a sharp increase in peroxidase (POD) activity in the roots (3.8-fold at Pb200 + Cd20) revealed a lignin-mediated cell wall reinforcement mechanism. The antioxidant system responded with a dynamic threshold effect: at low concentrations (Pb50/Cd5), SOD activity significantly increased (421.5 U/g FW), triggering primary defense, whereas at high concentrations (Pb200/Cd20), enzyme activity decreased to 382.4 U/g FW. Cd exerted a more pronounced destructive effect on chlorophyll a (decrease by 56.3%) compared to Pb (34.7%), with the mechanism involving chloroplast structural damage and Mg^{2+} competition inhibition. Combined stress, through a synergistic pathway (Cd inhibition of synthetase activity, Pb acceleration of pigment degradation), reduced chlorophyll a content to only 28.9%. This study quantifies the physiological tolerance thresholds of spinach to Pb-Cd combined stress and reveals a coupled mechanism of 'heavy metal interaction-antioxidant hierarchical response-photosynthetic damage synergy', providing a theoretical basis for ecological risk assessment of polluted farmland and safe spinach production.

Introduction

With the accelerating global industrialization and the development of intensive agriculture, soil heavy metal contamination has become a significant environmental issue threatening ecosystem health and food safety (Aparicio *et al.* 2022). Over the past few decades, global agricultural soil contamination by heavy metals has been expanding and intensifying due to mineral extraction, metal smelting, fossil fuel combustion, wastewater irrigation, and the long-term use of heavy metal-laden pesticides and fertilizers (Shen *et al.* 2017). According to the United Nations Environment Programme (UNEP), approximately 5 million polluted sites worldwide require remediation, with over 60% of these sites contaminated by heavy metals. The "National Soil Pollution Survey Bulletin" published in 2014 reported that the exceedance rate of soil pollution in agricultural land in China was as high as 19.4%, with exceedance rates for heavy metals Cd and Pb at 7.0 and 1.5%, respectively, making them the primary risk factors affecting

*Author for correspondence: <Na0113@126.com.>. [1]Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co. Ltd., Shaanxi 710021, China. [2]Shaanxi Agricultural Development Group Co., Ltd., Shaanxi 710075, China.[3]Key Laboratory of Degraded and Unused Land Consolidation Engineering, Ministry of Natural Resources.Shaanxi 710075, China

the quality and safety of agricultural products (Ministry of Environmental Protection 2014). Among various heavy metal pollutants, lead (Pb) and cadmium (Cd) have attracted considerable attention due to their unique physicochemical properties and biological toxicity (Li *et al.* 2024). These two heavy metals share common characteristics such as high toxicity, strong mobility, and bioaccumulation, yet their environmental behavior and toxicological mechanisms differ significantly. Pb primarily exists in soils in forms such as Pb^{2+}, $Pb(OH)^{+}$, and $PbCO_3$, with relatively low mobility, but it can be absorbed by plant roots and enter the food chain (Zong *et al.* 2024). In contrast, Cd predominantly exists as Cd^{2+} in soil environments, exhibiting higher solubility and mobility. Its absorption efficiency by plants is typically 10 to 100 times greater than that of Pb (Wang *et al.* 2023). From a toxicological perspective, Pb primarily damages the plant photosynthetic system and cell membrane structures, while Cd severely disrupts enzyme activity and mineral nutrient balance (Saqib *et al.* 2023). of particular concern is the potential for complex interactions when Pb and Cd coexist, which may either synergistically enhance toxicity or antagonistically mitigate damage. This combined pollution effect is far more complex and difficult to predict than single metal contamination.

Spinach (*Spinacia oleracea* L.), an annual herbaceous plant in the Amaranthaceae family, is an important leafy vegetable crop widely cultivated around the world, with an annual production exceeding 26 million tons. From a scientific research perspective, spinach has unique advantages as a model plant for heavy metal pollution studies. It has a short growth cycle (approximately 40-60 days), high biomass, and simple cultivation management, making it ideal for controlled laboratory experiments. Additionally, spinach has large leaves with thin epidermis, making it an excellent material for studying foliar absorption and translocation of heavy metals. Studies have shown that under the same pollution conditions, the bioaccumulation coefficient for Cd in spinach can reach 0.03-0.38, much higher than that in cruciferous vegetables such as cabbage and rapeseed (Sheikh *et al.* 2025). This characteristic makes spinach a "sentinel" for linking soil pollution and food safety and an ideal material for studying plant-heavy metal interaction mechanisms. From a physiological and ecological perspective, Pb and Cd stress affect spinach growth, development, and metabolic processes through multiple pathways. In the photosynthetic system, heavy metals can damage the ultrastructure of chloroplasts, inhibit chlorophyll synthesis, and interfere with the electron transport chain. Research has shown that a 1000 μM Cd treatment can reduce chlorophyll a content in spinach by 53.49%, with a more than 40% decrease in photosynthetic rate (Zhang *et al.* 2015). In terms of antioxidant defense, reactive oxygen species (ROS) induced by heavy metals activate the activity of antioxidant enzymes such as SOD, POD, and CAT, triggering oxidative stress responses. Soil Cd stress significantly enhances the uptake of Mn, Zn, and Cu in spinach, while inhibiting Fe uptake (Aparicio *et al.* 2022). These physiological responses form a comprehensive defense network for spinach under heavy metal stress. However, the specific regulatory mechanisms, especially the response patterns under combined Pb-Cd stress, remain to be further elucidated.

Current research has primarily focused on the effects of single heavy metal stress, while the interaction mechanisms of Pb-Cd combined pollution are still poorly understood. Notably, Pb and Cd may exhibit entirely different interaction patterns depending on concentration combinations-at low concentrations, they may compete for absorption sites, leading to antagonism, while at high concentrations, they may synergistically damage membrane structures, enhancing toxicity. This concentration-dependent interaction pattern has yet to be fully elucidated. Furthermore, Bashir *et al.* (2024) demonstrated significant differences in the Cd accumulation in the aboveground parts of spinach across different genotypes (0.03-0.31 mg/kg), suggesting the presence of molecular mechanisms for active regulation of heavy metal uptake in plants. However, whether such mechanisms exist in spinach and how they function remain unclear. Based on the aforementioned scientific issues, this study aims to analyze the effects of single and combined Pb-Cd stress at different concentrations on the growth characteristics, antioxidant enzyme activity, and heavy metal accumulation patterns of spinach through a controlled experimental system. The study will focus on: (i) elucidating the dose-effect relationship of Pb-Cd combined stress, particularly the interaction types (synergistic/antagonistic) and their inflection points under different concentration combinations; (ii) revealing the hierarchical response mechanisms of the spinach antioxidant defense system, including SOD and POD; (iii) analyzing the migration and distribution patterns of heavy metals within the plant.

Materials and Methods

Spinach seeds were obtained from the Fengzhiyuan Seed Sales and Service Center, located in Fuping County, Shaanxi Province.

The experiment consisted of 10 treatments: (i) Control (CK, without the addition of any heavy metals); (ii) Pb at 50, 100, and 200 mg/kg; (iii) Cd at 5, 10, and 20 mg/kg; (iv) Combined Pb + Cd treatment at 50+5, 100+10, and 200+20 mg/kg. The experimental soil was fertilized with a base fertilizer following a N : P_2O_5 : K_2O ratio of 2 : 2 : 1 (with a nitrogen addition of 200 mg/kg), and mixed with the heavy metal solutions, then aged for 2 weeks.

Seeds were surface-sterilized, rinsed with distilled water, and germinated in plastic trays with nutrient solution. Two-leaf stage seedlings were transplanted into heavy metal-treated soil. After transplanting, soil moisture was monitored every 3 days, and water was added using the weighing method to maintain the soil water content at 60%. Thirty days after transplanting, relevant indices were measured.

The plants were thoroughly washed with deionized water, and surface moisture was removed using filter paper. The roots, stems, and leaves of the plants were separated and cut using scissors. The samples were oven dried at 105°C for 20 min, followed by drying at 80°C to constant weight. The biomass of the roots, stems, and leaves was measured using an electronic balance.

The concentrations of Cd and Pb in the plant tissues were determined by graphite furnace atomic absorption spectrometry (GFAAS), after nitric acid and perchloric acid digestion (0.3000 g of dried sample with 10 ml nitric acid and 5 ml perchloric acid). The detection limits for Cd and Pb were 0.01 and 0.2 µg/L, respectively, with recoveries of 94.7 and 96.2%.

Superoxide dismutase (SOD), peroxidase (POD) and chlorophyll content were assayed by the nitroblue tetrazolium (NBT) photochemical reduction, the guaiacol and an ethanol extraction methods, respectively.

Data were analyzed with SPSS 20.0. The normality and variance homogeneity were verified using the Shapiro-Wilk and Levene test, respectively. One-way ANOVA followed by Duncan's test ($\alpha = 0.05$) was used. Experimental results were presented as mean ± SD, with different letters indicating significant differences ($P < 0.05$). Graphs were prepared with Origin 2021.

Results and Discussion

Both single and combined Pb and Cd stress significantly inhibited dry matter accumulation in all plant organs ($P < 0.05$) (Table 1). Compared to the control (CK), with increasing concentrations of heavy metals, the dry mass of the roots, stems, leaves, and total biomass of spinach decreased, with the combined treatments showing significantly stronger inhibitory effects than the single treatments. In the single Pb treatment, the Pb50 treatment had no significant effect on spinach growth ($P > 0.05$); however, when the Pb concentration was increased to 200 mg/kg, the total dry biomass of spinach decreased significantly by 31.2% compared to CK. The Cd treatment exhibited stronger toxic effects, with the lowest concentration of Cd5 resulting in an 18.7% reduction in total dry biomass, and the Cd20 treatment causing a 46.3% decrease. The combined treatment showed a clear synergistic inhibitory effect. The Pb50 + Cd5 treatment reduced the total dry biomass of spinach by 27.5% compared to CK, while the Pb200 + Cd20 treatment caused a 62.8% reduction, which was significantly greater than the reductions observed in the single Pb200 (31.2%) or Cd20 (46.3%) treatments ($P < 0.05$).

The sensitivity of different plant organs to heavy metal stress varied. The roots were the most sensitive to heavy metal stress, with the root dry biomass in the Pb200 + Cd20 treatment being only 28.4% of that in CK. The stems were less sensitive, with the stem dry biomass under the same treatment being 35.7% of that in CK. The leaves exhibited relatively higher tolerance to stress, with a dry biomass retention rate of 42.1%. These differences may be related to the varying levels of heavy metal accumulation and antioxidant capacity in each organ.

Table 1. Effects of different Cd and Pb treatment on dry mass of spinach organs.

Treatment	Root mass/g	Stem quality/g	Leaf stem quality/g	Total dry weight/g
CK	0.48 ± 0.03a	0.62 ± 0.04a	1.25 ± 0.08a	2.35 ± 0.15a
Pb50	0.45 ± 0.03ab	0.59 ± 0.04ab	1.18 ± 0.08ab	2.22 ± 0.14ab
Pb100	0.39 ± 0.03bc	0.51 ± 0.03bc	1.02 ± 0.07bc	1.92 ± 0.13bc
Pb200	0.33 ± 0.02cd	0.43 ± 0.03cd	0.86 ± 0.06cd	1.62 ± 0.11cd
Cd5	0.38 ± 0.03bc	0.50 ± 0.03bc	1.01 ± 0.07bc	1.89 ± 0.13bc
Cd10	0.31 ± 0.02de	0.41 ± 0.03de	0.82 ± 0.06de	1.54 ± 0.10de
Cd20	0.26 ± 0.02ef	0.34 ± 0.02ef	0.68 ± 0.05ef	1.28 ± 0.09ef
Pb50 + Cd5	0.35 ± 0.02cd	0.45 ± 0.03cd	0.90 ± 0.06cd	1.70 ± 0.11cd
Pb100 + Cd10	0.27 ± 0.02ef	0.36 ± 0.02ef	0.72 ± 0.05ef	1.35 ± 0.09ef
Pb200 + Cd20	0.14 ± 0.01g	0.22 ± 0.01g	0.53 ± 0.04g	0.89 ± 0.06g

Different lowercase letters following the data in each column indicate significant differences between treatments ($P < 0.05$, Duncan's test) (same for the following sections).

Both single and combined Pb and Cd stress significantly affected the SOD activity in spinach leaves ($P < 0.05$) (Table 2 and Fig. 1). Compared to the control (CK), low concentrations of heavy metals (Pb50, Cd5) induced a 12.3 and 18.5% increase in SOD activity, respectively, demonstrating a clear stress response. As the stress concentration increased, SOD activity initially increased and then decreased, reaching a peak at Pb100 and Cd10 treatments (436.7 and 458.3 $U \cdot g^{-1}$ FW, respectively), which were significantly higher by 24.6 and 30.8% compared to CK. Higher concentrations of stress (Pb200, Cd20) led to a decline in SOD activity, although it remained significantly higher than CK levels ($P < 0.05$). Notably, in the combined stress treatments, the SOD activity of Pb50 + Cd5 (421.5 $U \cdot g^{-1}$ FW) was significantly higher than that of the single treatments ($P < 0.05$), while the SOD activity of the highest concentration combined treatment (Pb200 + Cd20, 382.4 $U \cdot g^{-1}$ FW) was significantly lower than that of the corresponding single treatments.

Both single and combined Pb and Cd stress significantly affected the POD activity in various spinach organs ($P < 0.05$) (Table 3 and Fig. 2). In terms of root response, single Pb stress showed a clear dose-dependent effect, with POD activity increasing 2.1-fold compared to the control (CK) when the treatment concentration reached 200 mg/kg. Cd stress exhibited a biphasic response, with the activity reaching its peak at 10 mg/kg (156.3% higher than CK), but decreasing at the high concentration of 20 mg/kg. Notably, the combined stress treatment at the highest concentration combination (Pb200 + Cd20) produced a significant synergistic effect. The POD activity in the roots was not only 3.8 times higher than that of CK but also significantly exceeded the sum of the corresponding single treatments ($P < 0.05$).

Table 2. SOD activity in different organs of spinach under Pb and Cd stress (U/g FW).

Treatment	Root	Stem	Leaf
CK	85.3 ± 3.2a	62.1 ± 2.8a	73.6 ± 4.1a
Pb50	102.4 ± 4.1b	71.5 ± 3.6b	84.2 ± 3.9b
Pb100	121.5 ± 5.3c	68.7 ± 2.9b	89.7 ± 4.7c
Pb200	78.9 ± 3.6d	63.8 ± 3.1a	91.2 ± 5.1c
Cd5	110.2 ± 4.7b	70.3 ± 3.4b	105.4 ± 6.2d
Cd10	117.4 ± 5.1c	73.8 ± 3.8b	118.6 ± 7.3e
Cd20	96.8 ± 4.3b	65.2 ± 2.7a	122.9 ± 6.9e
Pb50 + Cd5	95.6 ± 4.0b	60.4 ± 2.5a	98.7 ± 5.4d
Pb100 + Cd10	80.2 ± 3.4d	54.3 ± 2.3c	85.1 ± 4.2b
Pb200 + Cd20	61.3 ± 2.8e	48.9 ± 2.1d	69.5 ± 3.7a

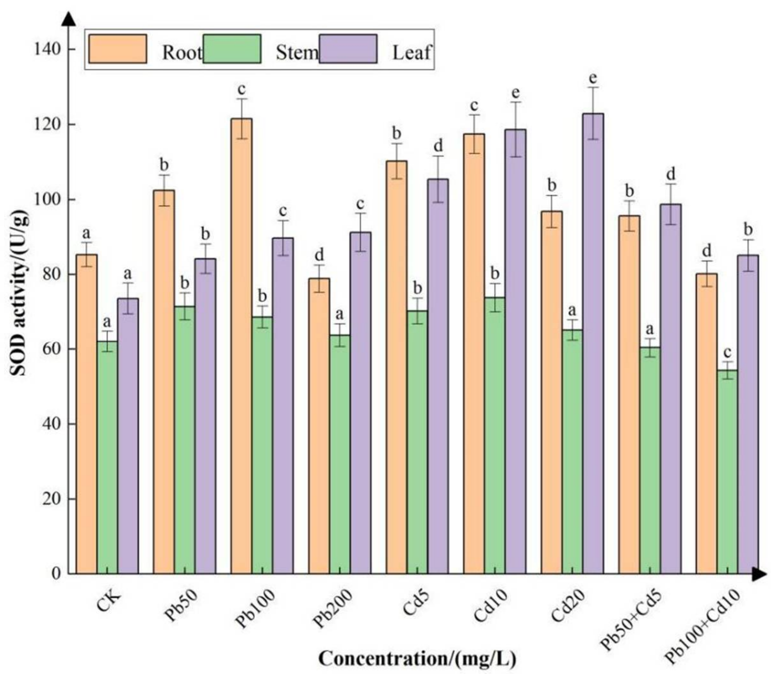

Fig. 1. The superoxide dismutase (SOD) activity in spinach under cadmium (Cd) and lead (Pb) stress.

The response pattern in the stem tissue differed from that of the roots. At low concentrations, Pb50 (50 mg/kg) and Cd5 (5 mg/kg) induced increases in POD activity of 32.7 and 28.4%, respectively. However, no significant differences were observed at higher concentrations (Pb200 and Cd20). The combined treatment exhibited a dose-dependent synergistic enhancement effect, particularly with the Pb100+Cd10 combination, which resulted in a POD activity 118.5% of the average value of the single treatments, showing a clear interactive promotion. Leaf tissue, being the main photosynthetic organ, was more sensitive to Cd stress. Cd20 treatment caused a dramatic increase in POD activity, reaching 3.2 times the CK level, significantly higher than the 1.9-fold increase observed with Pb200 treatment. Under combined stress (Pb200 + Cd20), POD activity was 1.3 times greater than the single treatments, indicating activation of antioxidant defenses through multiple pathways.

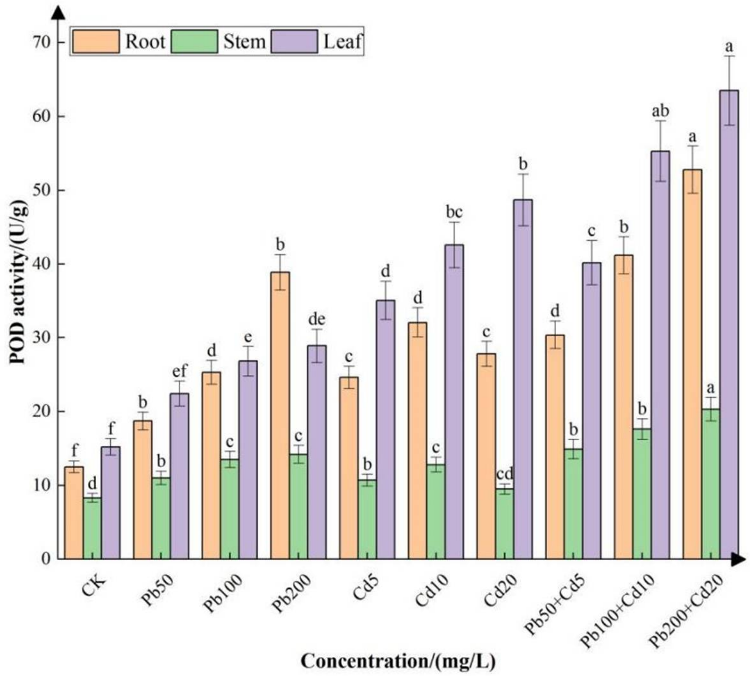

Fig. 2. The peroxidase (POD) activity of spinach under Cd and Pb stress.

Table 3. POD activity in different organs of spinach under Pb and Cd stress (U/g FW).

Treatment	Root	Stem	Leaf
CK	12.5 ± 0.8f	8.3 ± 0.6d	15.2 ± 1.1f
Pb50	18.7 ± 1.2b	11.0 ± 0.9b	22.4 ± 1.7ef
Pb100	25.3 ± 1.6d	13.5 ± 1.1c	26.8 ± 2.0e
Pb200	38.9 ± 2.4b	14.2 ± 1.2c	28.9 ± 2.3de
Cd5	24.6 ± 1.5c	10.7 ± 0.8b	35.1 ± 2.6d
Cd10	32.1 ± 2.0d	12.8 ± 1.0c	42.6 ± 3.1bc
Cd20	27.8 ± 1.7c	9.5 ± 0.7cd	48.7 ± 3.5b
Pb50 + Cd5	30.4 ± 1.9d	14.9 ± 1.3b	40.2 ± 3.0c
Pb100 + Cd10	41.2 ± 2.5b	17.6 ± 1.4b	55.3 ± 4.1ab
Pb200 + Cd20	52.8 ± 3.2a	20.3 ± 1.6a	63.5 ± 4.7a

The impact of heavy metal stress on chlorophyll synthesis in spinach exhibited significant and regular patterns of change ($P < 0.05$) (Table 4 and Fig. 3). Cd20 reduced chlorophyll a by 56.3%, more than Pb200 (34.7%). Under combined stress (Pb200 + Cd20), chlorophyll a dropped to 28.9% of the control level, showing a strong synergistic inhibition.

For chlorophyll b, low concentration treatments (Pb50 and Cd5) did not result in significant changes ($P > 0.05$), but high concentrations of Pb200 and Cd20 led to reductions of 41.2 and 62.8%, respectively. The Pb100 + Cd10 combination caused an additional 21.5% decline compared with single treatments, confirming a synergistic inhibition. Total chlorophyll followed a similar trend, with Pb200 + Cd20 causing a drastic 72.1% decrease, highlighting strong synergistic pigment degradation.

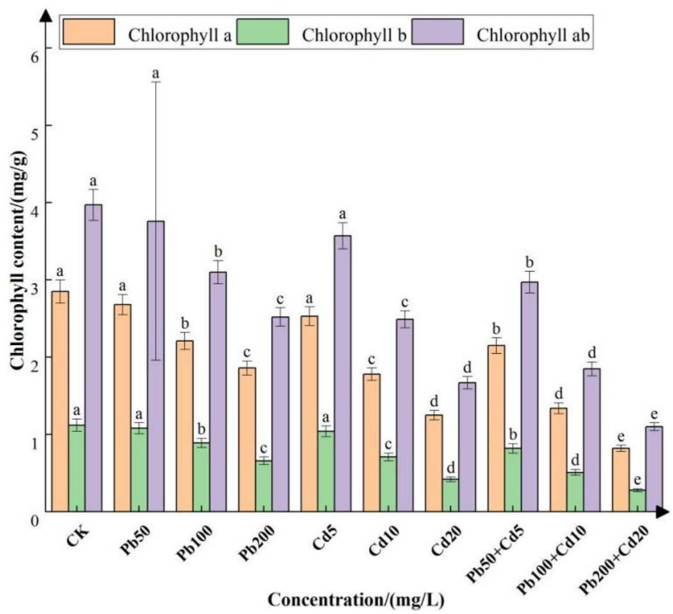

Fig. 3. Effects of Pb and Cd Stress on Chlorophyll Content in Spinach.

Table 4. Chlorophyll content of spinach leaves under Pb and Cd stress (mg/g).

Treatment	Chlorophyll a	Chlorophyll b	Chlorophyll ab
CK	2.85 ± 0.15a	1.12 ± 0.08a	3.97 ± 0.20a
Pb50	2.68 ± 0.13a	1.08 ± 0.07a	3.76 ± 0.18a
Pb100	2.21 ± 0.11b	0.89 ± 0.06b	3.10 ± 0.15b
Pb200	1.86 ± 0.09c	0.66 ± 0.05c	2.52 ± 0.12c
Cd5	2.53 ± 0.12a	1.04 ± 0.07a	3.57 ± 0.17a
Cd10	1.78 ± 0.08c	0.71 ± 0.05c	2.49 ± 0.11c
Cd20	1.25 ± 0.06d	0.42 ± 0.03d	1.67 ± 0.08d
Pb50 + Cd5	2.15 ± 0.10b	0.82 ± 0.06b	2.97 ± 0.14b
Pb100 + Cd10	1.34 ± 0.07d	0.51 ± 0.04d	1.85 ± 0.09d
Pb200 + Cd20	0.82 ± 0.04e	0.28 ± 0.02e	1.10 ± 0.05e

The inhibitory effects of heavy metals Pb and Cd, both individually and in combination, on spinach growth exhibited significant dose-dependent and synergistic characteristics. With increasing stress concentration, the dry matter accumulation in the root, stem, and leaf of spinach significantly decreased, with the combined treatments showing stronger inhibition than the single treatments (e.g., the total dry weight under the Pb200 + Cd20 treatment decreased by 62.8%). These findings are consistent with biomass responses of crops such as maize and wheat under heavy metal stress (Wan *et al.* 2022). The root, being the primary organ for heavy metal absorption, exhibited the largest reduction in dry weight (Pb200 + Cd20 treatment was 28.4% of the control), which may be related to its direct soil contact with the higher accumulation of heavy metals (Khanthom *et al.* 2022). The differential sensitivity of the stem and leaf could be attributed to spatial variations in antioxidant capacity between organs, for example, the higher SOD activity in the leaf may alleviate some oxidative damage by scavenging reactive oxygen species (ROS) (Hang *et al.* 2025).

At low concentrations of Cd and Pb (e.g., Cd5, Pb50) significantly induced an increase in SOD activity (increased by 18.5 and 12.3%, respectively), indicating that plants activate their antioxidant systems to cope with the initial stress. However, high concentrations of stress (e.g., Cd20, Pb200) led to a decline in SOD activity, possibly due to excessive ROS causing structural damage to enzyme proteins or metabolic exhaustion (Ding *et al.* 2025). Combined lower concentrations (Pb50 + Cd5) enhanced SOD activity (421.5 U/g FW) synergistically, while the inhibitory effect in the high concentration combination (Pb200 + Cd20) (382.4 U/g FW) may reflect oxidative damage exceeding the defense threshold, which is similar to the dynamic changes in enzyme activity observed in *Ageratina adenophora* under Cd-Pb combined stress (Li *et al.* 2023). The response pattern of POD activity further revealed organ specificity, as the root showed a 3.8-fold increase in POD activity under the Pb200 + Cd20 treatment, significantly higher than the sum of the single treatments, possibly through lignin synthesis to strengthen the cell wall and compartmentalize heavy metals (Soto-Ramírez *et al.* 2024). In contrast, the leaf exhibited a high sensitivity to Cd, with POD activity under Cd20 treatment being 3.2 times that of the control, which may be related to the special response of chloroplasts to Cd toxicity (Singh *et al.* 2022).

Chlorophyll synthesis inhibition is a significant marker of heavy metal toxicity. In this study, the Cd20 treatment caused a 56.3% decrease in chlorophyll a content, which was significantly higher than the 34.7% reduction observed under Pb200 treatment, which may be due to the direct disruption of chloroplast structure by Cd and its competitive inhibition of Mg^{2+} (Zhang *et al.* 2020). The synergistic effect of combined stress (Pb200 + Cd20), where chlorophyll a content was only 28.9% of the control, may result from a dual mechanism. On one hand, Cd interferes with the activity of chlorophyll synthase, and on the other, Pb induces ROS bursts that exacerbate pigment degradation (Hachani *et al.* 2020). The tolerance of chlorophyll b to low concentration stress (Pb50, Cd5) ($P > 0.05$) suggests that its biosynthetic pathway is less sensitive to heavy metals than chlorophyll a, but the synergistic inhibition under high concentration combined treatments (Pb100 + Cd10), with a reduction of 21.5%, indicates that the two heavy metals may interfere with different sites in the chlorophyll metabolic network (Souahi *et al* .2021).

This study systematically revealed the dose-effect relationship and synergistic mechanisms of lead (Pb) and cadmium (Cd) single and combined stress on the growth and physiology of spinach. The main conclusions are as follows: (i) Pb and Cd combined stress caused concentration-dependent and synergistic biomass inhibition, with total dry weight reduced by up to 62.8%. The root, as the primary target of heavy metal stress, experienced a significant loss in dry weight (with a minimum of 28.4% of the control), which was much higher than that in the aerial parts, indicating the dual effects of direct soil exposure and and limited compartmentalization. The differences in biomass in the stem and leaf were closely related to the variation in antioxidant capacity among organs, with the leaf repairing some damage through an increase in SOD activity (12.3-18.5% at low concentrations), while the root exhibited a 3.8-fold increase in POD activity under combined stress, revealing the lignin-mediated cell wall strengthening. (ii) Low concentration stress (Pb50/Cd5) induced a primary antioxidant response by enhancing SOD activity (421.5 U/g FW), while high concentrations (Pb200/Cd20) led to a decline in enzyme activity to 382.4 U/g FW, confirming that oxidative damage exceeded the plant's defense threshold. (iii) Cd had a more significant destructive effect on chlorophyll a (a decrease of 56.3%) compared to Pb (34.7%), which can be attributed to its direct damage to the chloroplast structure and competitive inhibition of Mg^{2+}. Combined stress aggravated chlorophyll degradation through dual pathways: Cd inhibited chlorophyll synthase activity, while Pb induced accelerated pigment breakdown, resulting in chlorophyll a content of only 28.9% in the combined treatment group. Chlorophyll was less sensitive at lower stress but showed 21.5% reduction under higher combined stress to heavy metals.

Acknowledgments

This study was supported by the Young Talent Fund of Association for Science and Technology in Shaanxi, China (20240517).

References

Aparicio JD, Raimondo EE, Saez JM, Costa-Gutierrez SB, Álvarez A, Benimeli CS and Polti MA 2022. The current approach to soil remediation: A review of physicochemical and biological technologies, and the potential of their strategic combination. J. Environ. Chem. Eng. **10**: 107141.

Bashir MH, Farhan M, Samreen T and Shehzad MT 2024. Effectiveness of constructed wetland technology-treated industrial wastewater on the spinach (*Spinacia oleracea*) health risks and biochar efficiency. Environ. Geochem. Health **46**: 469.

Ding L, Gao J, Meng D, Zeng J, Yuan M, Yang J, Lyu G and Hu Q 2025. Assessment of ecological and human health risks of heavy metals in soils and *Polygonatum sibiricum* plants from various cultivation areas in China. Environ. Geochem. Health **47**: 163.

.Hachani C, Lamhamedi MS, Zine El Abidine A, Abassi M, Khasa DP and Béjaoui Z 2021. Water relations, gas exchange, chlorophyll fluorescence and electrolyte leakage of ectomycorrhizal *Pinus halepensis* seedlings in response to multi-heavy metal stresses (Pb, Zn, Cd). Microorganisms **10**: 57.

Hang S, Wu C, Peng G, Li F, Ge F, Gan J, Li L and Li J 2025. Rhizosphere endophytes as allies in plant defense against heavy metals and organic pollutants in soil: Advances and applications. Appl. Soil Ecol. **211**: 106119.

Khanthom S, Stewart TN and Prapagdee B 2021. Potential of a rhizobacterium on removal of heavy metals from aqueous solution and promoting plant root elongation under heavy metal toxic conditions. Environ. Technol. Inno. **22**:101419.

Li S, Wen Y, Wang Y, Liu M, Su L, Peng Z, Zhou Z and Zhou N 2024. Novel α-amino acid-like structure decorated biochar for heavy metal remediation in acid soil. J. Hazard. Mater. **462**: 132740.

Li Y, Shi X, Tan W, Ling Q, Pei F, Luo S, Qin P, Yuan H, Huang L and Yu F 2023. Metagenomics combined with metabolomics reveals the effect of *Enterobacter* sp. inoculation on the rhizosphere microenvironment of *Bidens pilosa* L. in heavy metal contaminated soil. J. Hazard. Mater. **458**: 132033.

Ministry of Environmental Protection 2014. Bulletin on the National Soil Pollution Status Survey [EB/OL].

Saqib M, Shahzad U, Zulfiqar F, Tiwari RK, Lal MK, Naz S, Jahan MS, Awan ZA, El-Sheikh MA and Altaf MA 2023. Exogenous melatonin alleviates cadmium-induced inhibition of growth and photosynthesis through upregulating antioxidant defense system in strawberry. South Afr. J. Bot. **157**: 10-18.

Shen F, Liao R, Ali A, Mahar A, Guo D, Li R, Sun x, Awasthi MK, Wang Q and Zhang Z 2017. Spatial distribution and risk assessment of heavy metals in soil near a Pb/Zn smelter in Feng County, China. Ecotox. Environ. Safe. **139**: 254-262.

Sheikh L, Naz N, Oranab S, Younis U, Alarfaj AA, Alharbi SA and Ansari MJ 2025. Minimization of cadmium toxicity and improvement in growth and biochemical attributes of spinach by using acidified biochar. Sci. Rep. **15**: 5880.

Singh H, Kumar D and Soni V 2022. Performance of chlorophyll a fluorescence parameters in *Lemna minor* under heavy metal stress induced by various concentration of copper. Sci. Rep. **12**: 10620.

Soto-Ramírez R, Vlatten N, Ruz F, Tavernini L and Lobos MG 2024. Engineering the cell wall reactive groups of plant growth promoting *Rhizobacteria* by culture strategy for heavy metal removal. J. Biotechnol. **394**: 125-134.

Souahi H 2021. Impact of lead on the amount of chlorophyll and carotenoids in the leaves of *Triticum durum* and *T. aestivum, Hordeum vulgare* and *Avena sativa*. Biosyst. Diversity **29**: 207-210.

Wan F, Jiang N, Yu L, Zang K, Liu S, He W, Hu Z, Fan H, Li H, Wang H and Pan H 2022. Heavy metal ecological-health risk assessment under wheat-maize rotation system in a high geological background area in eastern China. Sci. Rep. **12**: 17912.

Wang CC, Zhang QC, Yan CA, Tang GY, Zhang MY, Ma LQ, Gu RH and Xiang P 2023. Heavy metal (loid) s in agriculture soils, rice, and wheat across China: Status assessment and spatiotemporal analysis. Sci. Total Environ. **882**: 163361.

Zhang H, Xu Z, Guo K, Huo Y, He G, Sun H, Guan Y, Xu N, Yang WS and un G 2020. Toxic effects of heavy metal Cd and Zn on chlorophyll, carotenoid metabolism and photosynthetic function in tobacco leaves revealed by physiological and proteomics analysis. Ecotox. Environ. Safe. **202**: 110856.

Zhang X 2015. Study on Cadmium Accumulation and Physiological Effects in Spinach [D]. Shandong Normal University, Jinan, Shandong Province, China. DOI:10.7666/d.D206651. (In Chinese)

Zong X, Liu Y, Lin X, He D, Dong Z, Guo T, Li J, Li H and Wang F 2024. Foliar spraying of lanthanum activates endocytosis in lettuce (*Lactuca sativa* L.) root cells, increasing Cd and Pb accumulation and their bioaccessibility. Sci. Total Environ. **908**: 168374.

(Manuscript received on 26 May 2025; revised on 11 October 2025)