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EXOGENOUS GIBBERELLIC ACID (GA_3) ALLEVIATES LEAD-INDUCED PHYTOTOXICITY BY MODULATING GROWTH AND ANTIOXIDANT DEFENCE IN SPINACH (*SPINACIA OLERACEA* L.)

AYESHA MEHMOOD[1]*, ISHTIAQ AHMAD[2], MARYAM[3], ADEL A. REZK[4]*, HOSSAM S. EL-BELTAGI[4], BADER Y. ALSUBAIE[4], OTHMAN AL-DOSSARY[4], AHMED M. ISMAIL[5], KONSTANTIN KOROLEV[6] AND FARIDA SAFAROVA[7]

[1]Institute of Agro-Industry and Environment, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, 63100, Pakistan
[2]Department of Horticultural Sciences, The Islamia University of Bahawalpur, Pakistan
[3]Department of Environmental Sciences, The Govt. Sadiq College Women University, Bahawalpur 63100, Pakistan
[4]Agricultural Biotechnology Department, College of Agricultural and Food Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia
[5]Pests and Plant Diseases Unit, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia
[6]Department of Botany, Biotechnology and Landscape Architecture, School of Natural Sciences, Tyumen State University, Tyumen, 625003, Russia
[7]Department of Biology, Nakhchivan State University, Nakhchivan, Azerbaijan

*Corresponding author's email: ayeshamehmood957@gmail.com; arazk@kfu.edu.sa

Abstract

Lead (Pb) contamination is a serious threat to vegetable production as it adversely affects plant growth, disrupts physiological and oxidative balance. To assess the effects of Pb stress (20 mg L-1) and the mitigating role of gibberellic acid (GA3; 100 mg L-1) on Spinacia oleracea L., a pot experiment was conducted, laid out in a completely randomized design (CRD) with 5 replications. A significant reduction was observed in plant height, root length, fresh weight and dry weight by 34.6%, 20.7%, 25.3%, and 25.2%, respectively under Pb exposure, compared with the control ($p \leq 0.05$). Photosynthetic pigments also reduced with Pb stress, chlorophyll a (0.02 mg g^{-1} FW), chlorophyll b (0.28 mg g^{-1} FW), carotenoids (0.24 mg g^{-1} FW) and relative water content (56.2%), resulting in disruption of photosynthetic activity. Importantly, GA_3 application to plants grown under Pb stress significantly alleviated Pb-induced oxidative damage by restoring growth traits. Application of GA_3 significantly increased chlorophyll content, carotenoids and relative water content. Pb stress triggered the antioxidant enzymatic activities, including superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase, reflecting enhanced oxidative stress. GA_3 application under Pb stress further strengthened antioxidant defence, resulting in more efficient reactive oxygen species detoxification.

Key words: Heavy metal toxicity; Lead stress; Phytoremediation; Plant growth regulation; Leafy vegetable; Oxidative stress

Introduction

Spinach (*Spinacia oleracea* L.) is a popular leafy vegetable and contributes significantly to human nutrition (Iftikhar *et al*., 2023; Afzal *et al*., 2025). Vegetables are key for maintaining our health and preventing chronic diseases; they are sources of important vitamins and minerals, as well as many bioactive compounds (Sarma & Bhavya, 2024). Spinach has received special attention as an important dietary constituent with exceptional nutritional composition in a balanced diet (Naseem *et al*., 2023). It contains vitamin A, vitamin C, vitamin K and numerous B vitamins and essential minerals (those include iron, calcium and magnesium). These nutrients are involved in systems including immune response, bone health and antioxidant defence systems (Amjad *et al*., 2025). Protection against the oxidation is made by the phytochemicals like phenolics, carotenoids and organic acids (Nyonje *et al*., 2022). Spinach helps reduce inflammation and the risk of cardiovascular disease by boosting immune system, moreover, it is also a rich source of dietary fibre and food availability for low-income households (Basu *et al*., 2019; Arooj *et al*., 2025). It is not only beneficial for health but also has economic significance, as it is cultivated extensively across the globe due to its widespread use in salad and cooked recipes (Isaza *et al*., 2025; Roberts & Moreau, 2016).

However, serious problems have been observed in spinach growth due to lead (Pb) toxicity which is a heavy metal (HM) and non-essential for plant growth but causes serious human health issues. Basically, the primary mechanism of lead uptake occurs through the roots from the soil; after entering the plant system, it disturbs various physiological processes (Zhao *et al*., 2025; Maryam *et al*., 2025; Teoh *et al*., 2024). Lead-contaminated soil showed serious issues to food safety and crop yield. According to international standards, permissible limits for lead contamination ranges from 140 to 400 mg/kg, moreover it depends on local standards for soil physical properties and permissible limits (Ahmad *et al*., 2020; Yi *et al*., 2022). Maximum limit of lead contamination ranges up to 0.3 mg/kg fresh weight that is acceptable for leafy vegetables including spinach, as set by Codex Alimentarius Commission in their session 2024 (Anon., 2024). Generally,

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lead a toxic heavy metal, causes severe yield losses through affecting photosynthetic activity and exerting oxidative stress (Ashar *et al*., 2024; Rahmdel *et al*., 2018). Studies have demonstrated that Pb contamination may have detrimental effects on plant morphology and chlorophyll contents, as controlled by oxidative damage reactions, such as increased production of lipid peroxides and reactive oxygen species (ROS) (Emanuil *et al*., 2020; Manzoor *et al*., 2025; Tanhan *et al*., 2025).

Heavy metals disrupt essential physiological processes, which decreases the plant germination and growth (Muhammad *et al*., 2024). Heavy metals in soil (such as lead) are transported quickly through the various plant tissues of spinach, where the maximum concentration has been observed in the roots in most instances due to their uptake tendency (Ahmad *et al*., 2023; Wang *et al*., 2023). Physiological stress induced by Pb exposure is lethal for spinach production, which signifies the need to develop strategies that could improve its tolerance against Pb toxicity (Bibi *et al*., 2024). Plants absorb Pb mainly through the roots from contaminated soils or water, which leads to the disturbance of ionic homeostasis and nutrient uptake. This blockage results in decreased chlorophyll content, stunted growth and production of lipid peroxidation, damaging the cell membranes and integrity of cells. Especially, Pb mimics essential nutrients and disrupts functions that depends on metal ions homeostasis in the plant body (Gupta *et al*., 2024). Pb imposes oxidative stress through the generation of ROS which causes cell disruption in plants in a chain reaction leading to lipid degradation and protein denaturation that finally ending with necrosis in plant tissues (Ilyas *et al*., 2024; Wang *et al*., 2024).

Several studies explained how lead toxicity, how gibberellic acid (GA_3, CAS no. 77-06-5) manage antioxidant enzyme activity and increase oxidative damage resistance caused by lead ions (Ghani *et al*., 2021). Physiologically, GA_3 enhances chlorophyll formation, which aids in photosynthesis, as well as controls numerous stress response genes (Vasyuk, 2025; Abubakar *et al*., 2023). Beyond, reducing in oxidative stress GA_3 is helpful to improve the crop quality and yield, it is also considered as a promising tool in precision agriculture on sustainable basis. Mitigation of heavy metal toxicity can be addressed through judicious use of GA_3, through improving nutrient uptake on sustainable basis while minimizing the carcinogenic effects of soil pollutants (Ahmad *et al*., 2025).

Gibberellins (GAs) are a family of growth-promoting plant hormones, but the key biochemical and molecular signalling regulatory mechanisms controlling plant growth, developed from changes in hormone metabolism, remain largely unknown (Saqib *et al*., 2025). It is believed that these hormones suppress the harmful effects of ROS formation resulting from exposure to heavy metals (Arru *et al*., 2021). This is attributed to the activation of the synthesis of phytohormones and secondary metabolites by GAs, which stimulates stress tolerance pathways, including detoxification pathways for heavy metals chelation (Ghani *et al*., 2025). The significant improvement in chlorophyll content combined with increased biomass accumulation demonstrates the efficiency of GA_3 treatment to increase spinach productivity even in contaminated natural soil (Ismail *et al*., 2023).

Studies have also demonstrated that GAs is involved in cellular mechanisms that alleviate the harmful impacts of HMs through the induction of adaptive growth strategies (Badar *et al*., 2022). Hydroxyproline (Hyp) is one of the vital amino acids required for the stress tolerance capacity of plants. Therefore, hydroxyproline is frequently involved in improving plant defense mechanism against stress (Hammoud *et al*., 2025). However, the use of GAs has been found to sustain antioxidant enzyme activity, increase production of kairomones and alleviate oxidative stress. This antioxidant defence pathway is crucial in upholding plant structural integrity when confronted with Pb and is activated by gibberellin signalling to encourage the expression of genes (Liu *et al*., 2019; Shakeel *et al*., 2025). Also, during the vegetative stage, GA_3 signalling has been linked to stress mitigation strategies, which also involve other phytohormones like abscisic acid. These elevated concentrations of abscisic acid (ABA), as customary, are a physiological response to abiotic cues that are capable of amplifying the positive role of GAs by inducing the expression of GA_3 biosynthesis genes or potentially in a synergistic fashion through the activation of growth-associated pathways (Saleem *et al*., 2025).

The hypothesis was that GA_3 application could alleviate lead toxicity in spinach by enhancing the plant stress tolerance mechanism. Thus, the present study evaluated the effectiveness of GA_3 in enhancing growth and biomass accumulation of spinach under Pb stress.

Materials and Methods

A pot experiment was executed on *S. oleracea* L. to investigate its response to Pb stress and the role of GA_3 in growth regulation. Twenty plastic pots (59 cm in diameter, 38 cm in height) were filled with 20 kg of loamy soil. The soil samples were also analysed, and the results are shown in Table 1.

Table 1. Physico-chemical properties of soil after the experiment.

Soil texture	Silt loam
Saturation (%)	27
pH	8.0
Electrical conductivity ($mScm^{-1}$)	1.79
Organic matter (%)	0.37
P (mg/ kg)	3.47
K (mg/ kg)	114
Na (mg/ kg)	188
Cl (mg/ kg)	137

Experimental layout: A completely randomized design (CRD) was conducted with one GA_3 concentration (100 ppm) and one Pb concentration (20 ppm) via foliar sprays, with deionized water spray as control, with 5 replications. Every single pot had 1 plant. $Pb(NO_3)_2$ was used as the Pb source, and GA_3 was dissolved in water.

Preparation of Pb solution: Pb levels were prepared in deionized water by dissolving the required amounts of Pb. 20 mg of Pb as nitrate salt (32 mg) was diluted to 1000mL of distilled water for the preparation of a 20 ppm Pb^{2+} solution.

Preparation of GA_3: GA_3 levels were prepared in deionized water by dissolving the required amount of GA_3. 100 mg of GA_3 powder in 1000 mL of distilled water was used for the preparation of a 100-ppm solution.

Treatment application: After 15 days of germination, Pb stress was applied to the soil by adding a freshly prepared aqueous solution of lead salt at the required concentration. Thirty days after germination, the GA_3 treatment was performed using a solution of 100 ppm (100 mg GA_3 dissolved in 1000 mL deionized water as described above). After 60 days, the spinach leaves were collected, and morphological, physiological, and biochemical parameters were measured.

Morphological parameters: Morphological attributes were assessed by harvesting plant samples 60 days after transplanting in the field, followed by gentle rinsing with deionized water for removal of surface particulate matter. After harvesting plant samples were brought to the laboratory and roots and shoots were separated for further analysis. Digital balance (Ohaus PR64/E) was used to measure the fresh weight of plant samples. Finally, to remove dust particles distilled water was used for rinsing of plant samples followed by were oven-dried at 70°C for three days (Haider *et al*., 2022).

Plant height (cm): The plant height was measured using measuring scale, plants were selected randomly from each treatment and measurements were taken from the base of the plant at soil level up to tallest leaf of the plant.

Root length (cm): Using a scale, the length of the roots of randomly chosen plants was measured from the tip of the longest root to the point where the root and shoot join.

Fresh weight (g): Fresh weight was determined by carefully uprooting the plant, followed by washing to remove surface soil, blotting excess moisture with tissue paper, and weighing the plant samples using a digital electronic balance.

Dry weight (g): Dry weight of harvested plant sample was done by placing the plant samples in paper bags followed by placing in oven while maintaining the temperature at 70°C for 3 days till constant weight was attained and then weighed using a digital balance in g.

Physiological Parameters

Chlorophyll determination: Chlorophyll contents were determined by harvesting of fresh plant leaves (0.5 g) randomly from each treatment followed by washing with distilled water to remove dust particles from the sample. After washing the known weight of plant sample was ground by adding 80% acetone with a pestle and mortar. The ground material was centrifuged at 10000 rpm for 10 minutes followed by separating the supernatant for further analysis. Residue of acetone was removed by washing three times with distilled water, and the extract was subjected to spectrophotometer absorbance at 480, 645, and 663 nm (Guamán-Rivera *et al*., 2025). The following formulae were used (OD = optical density, subscript = wavelength).

Chlorophyll (a) = $[(12.7 \times O.D_{663}) - (2.69 \times OD_{645})] \times V/1000 \times W$ (1)

Chlorophyll (b) = $[(22.9 \times OD_{645}) - (4.68 \times OD_{663})] \times V/1000 \times W$ (2)

Total chlorophyll = $[(20.2 \times OD_{645} + (8.02 \times OD_{663})] \times V/1000 \text{ x } W$ (3)

Carotenoids (Car) = $[OD_{480} + (0.114 \times OD_{663})] - (0.638 \times OD_{645})$ (4)

Where:

- OD663, OD645 = Optical density at 663 nm and 645 nm respectively
- 12.7 and 2.69 = coefficients of specific absorption
- V = Volume of extract (mL)
- W = Weight of fresh leaf sample (g)
- 1000 = converts µg to mg if needed, depending on units used.

Relative water content: Fully matured leaves from each treatment were gently separated from the lamina base followed by sealing in plastic labelled bags for analysis. After harvesting leaves were soaked in deionized water for 16 to 18 hours to obtain turgid weight (TW). Measurement of dry weight (DW) was done after 3 days oven dry at 70°C. RWC was calculated using the mathematical formula created by (Arnon, 1949).

Biochemical parameters: A homogenized mixture of 1gm plant sample was made by grinding in 5 mL of chilled extraction buffer (K_2HPO_3, pH 7.0) composed of 1 mM EDTA and 1% PVP (polyvinylpyrrolidone), followed by centrifugation at 15,000 rpm for 15 minutes maintaining the temperature at 4°C. The supernatant was separated into layers for further biochemical analysis (Lazcano-Ferrat and Lovatt, 1990). Superoxide dismutase (SOD) activity was assessed as described by Zhang *et al*., (2017). Catalase (CAT) activity was evaluated by monitoring the degradation of H_2O_2 at 240 nm, using an extinction coefficient (ε) of 39.4 $mM^{-1} cm^{-1}$. Peroxidase (POD) activity was assessed through guaiacol oxidation at 470 nm, and ascorbate peroxidase (APX) activity was measured by the decline in ascorbate absorbance at 290 nm ($\varepsilon = 2.8 \, mM^{-1} cm^{-1}$).

Statistical analysis

Data regarding various morphological and biochemical attributes was recorded and arranged for further statistical analysis. For the assessment of significance among the treatments analyses were performed in Statistix 8.1. Significance among the treatment means was compared by using the Least Significant Difference (LSD) test at the 5% significance level ($p \leq 0.05$).

Results

Effects of Pb stress and GA_3 on morphological traits of *S. oleracea*: Morphological performance of *S. oleracea* was severely affected due to Pb exposure as presented in Table 2, however application of GA_3 significantly improved growth and partially alleviated Pb-induced oxidative damage ($p<0.05$). It was noticed that under control conditions, plants gained a height of 15.6 ± 0.92 cm, root length of 8.10 ± 0.94 cm, fresh weight of 40.14 ± 6.64 g and dry weight of 7.22 ± 0.25 g. Exogenous application of GA_3 significantly improved all growth traits, as maximum plant height was attained (21.0 ± 1.0 cm), longest roots (10.0 ± 0.15 cm) and the greatest biomass accumulation (50.20 ± 2.55 g fresh weight and 9.10 ± 0.16 g dry weight) as shown in Table 2. Compared with the control, GA_3 increased plant height by approximately 34.6%, root length by 23.5%, fresh weight by 25.1%, and dry weight by 26.0%.

In contrast, Pb stress severely reduced growth. Plant height declined to 10.2 ± 1.46 cm, root length to 6.42 ± 0.44 cm, fresh weight to 30.00 ± 1.52 g, and dry weight to 5.40 ± 0.33 g, all significantly lower than the control ($p<0.05$). Those plants did not receive GA_3, Pb exposure caused reductions of 34.6% in plant height, 20.7% in root length, 25.3% in fresh weight and 25.2% in dry weight, confirming the strong inhibitory effect of Pb on vegetative growth and biomass production.

Surprisingly, it was observed that combined application (Pb + GA_3) significantly mitigated Pb-induced growth suppression. Plants under Pb + GA_3 reached a height of 15.2 ± 1.07 cm and root length of 7.20 ± 0.28 cm, which were statistically comparable to the control for plant height (same letter "b") but remained lower than GA_3 alone. Similarly, fresh weight (39.76 ± 2.93 g) and dry weight (7.85 ± 0.32 g) were significantly higher than Pb treatment alone and approached control values. Compared with Pb-stressed plants, GA_3 supplementation under Pb improved plant height by 49.0%, root length by 12.1%, fresh weight by 32.5%, and dry weight by 45.4%. Conclusively, Pb stress significantly suppressed morphological traits in *S. oleracea*, however, application of GA_3 not only stimulated growth but also substantially alleviated Pb-induced growth inhibition.

Effect of Pb stress and GA_3 on photosynthetic pigments (mg g^{-1} FW) and relative water content (%) of *S. oleracea*: Lead (Pb) stress markedly impaired the physiological performance of *Spinacia oleracea* (Table 3). Plants exposed to Pb showed a significant reduction in chlorophyll a (0.02 mg g^{-1} FW), chlorophyll b (0.28 mg g^{-1} FW), and carotenoids (0.24 mg g^{-1} FW) compared with the control. Relative water content (RWC) was also declined sharply to 56.20%, indicating severe disturbance of plant water status under Pb toxicity.

Application of GA_3 significantly improved the accumulation of photosynthetic pigments and water relations, with maximum values recorded for chlorophyll a (0.08 mg g^{-1} FW), chlorophyll b (0.43 mg g^{-1} FW), carotenoids (0.52 mg g^{-1} FW), and RWC (97.40%). Surprisingly, GA_3 application on plants receiving Pb stress restored these traits. Chlorophyll a, chlorophyll b and carotenoid contents were increased to 0.04, 0.38, and 0.38 mg g^{-1} FW, respectively, while RWC was improved to 81.80%, compared with the Pb treatment alone, where no GA_3 was applied.

Effects of Pb stress and GA_3 on enzymatic activity of *S. oleracea*: A significant activation of the antioxidant defense system of *Spinacia oleracea* was observed due to Lead (Pb) stress (Fig. 1a–d). Superoxide dismutase (SOD) activity (µg mg^{-1} protein) was increased from approximately 75 in control plants to 88 under Pb stress, indicating enhanced production of superoxide radicals. Exogenous application of GA_3 under Pb stress further increased SOD activity to about 95 µg mg^{-1} protein, suggesting improved dismutation of superoxide ions into hydrogen peroxide.

Peroxidase (POD) activity (U mg^{-1} protein min^{-1}) also showed a significant increase, rising from 80 in control plants to 95 under Pb stress. The highest POD activity (approximately 105 U mg^{-1} protein min^{-1}) was recorded in Pb + GA_3–treated plants, reflecting enhanced hydrogen peroxide detoxification.

Catalase (CAT) activity (mmol H_2O_2 mg^{-1} protein) was increased markedly under Pb stress, reaching 11 mmol mg^{-1} protein compared with 6 mmol mg^{-1} protein in the control. GA_3 application further elevated CAT activity to 14 mmol mg^{-1} protein under Pb stress, indicating a stronger capacity for hydrogen peroxide scavenging.

Similarly, ascorbate peroxidase (APX) activity (mmol mg^{-1} protein) increased from 40 in control plants to 50 under Pb stress and reached a maximum value of 55 mmol mg^{-1} protein in Pb + GA_3–treated plants. This enhancement reflects a more efficient ascorbate–glutathione cycle under GA_3 supplementation.

Table 2. Effects of Pb stress and GA_3 on morphological parameters of *S. oleracea*.

Treatment	Plant height (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Control	15.6 ± 0.92b	8.10 ± 0.94b	40.14 ± 6.64 b	7.22 ± 0.25b
GA_3	21.0 ± 1.0a	10.0 ± 0.15a	50.20 ± 2.55a	9.10 ± 0.16a
Pb	10.2 ± 1.46c	6.42 ± 0.44c	30.00 ± 1.52c	5.40 ± 0.33c
Pb + GA_3	15.2 ± 1.07b	7.20 ± 0.28b	39.76 ± 2.93b	7.85 ± 0.32 b

Mean values sharing different letters are statistically significant at $p<0.05$ probability

Table 3. Effects of Pb stress and GA_3 on physiological parameters of *S. oleracea*.

Treatment	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Relative water content (%)
Control	0.04 ± 0.04b	0.36 ± 0.01b	0.41± 0.01b	86.00 ± 2.77 c
GA_3	0.08 ± 0.03a	0.43± 0.06a	0.52 ± 0.06a	97.40 ± 1.69a
Pb	0.02 ± 0.06c	0.28 ± 0.03c	0.24 ± 0.03d	56.20 ± 2.83d
Pb + GA_3	0.04 ± 0.12b	0.38 ± 0.02b	0.38 ± 0.02c	81.80 ± 2.97b

Mean values sharing different letters are statistically significant at $P < 0.05$ probability

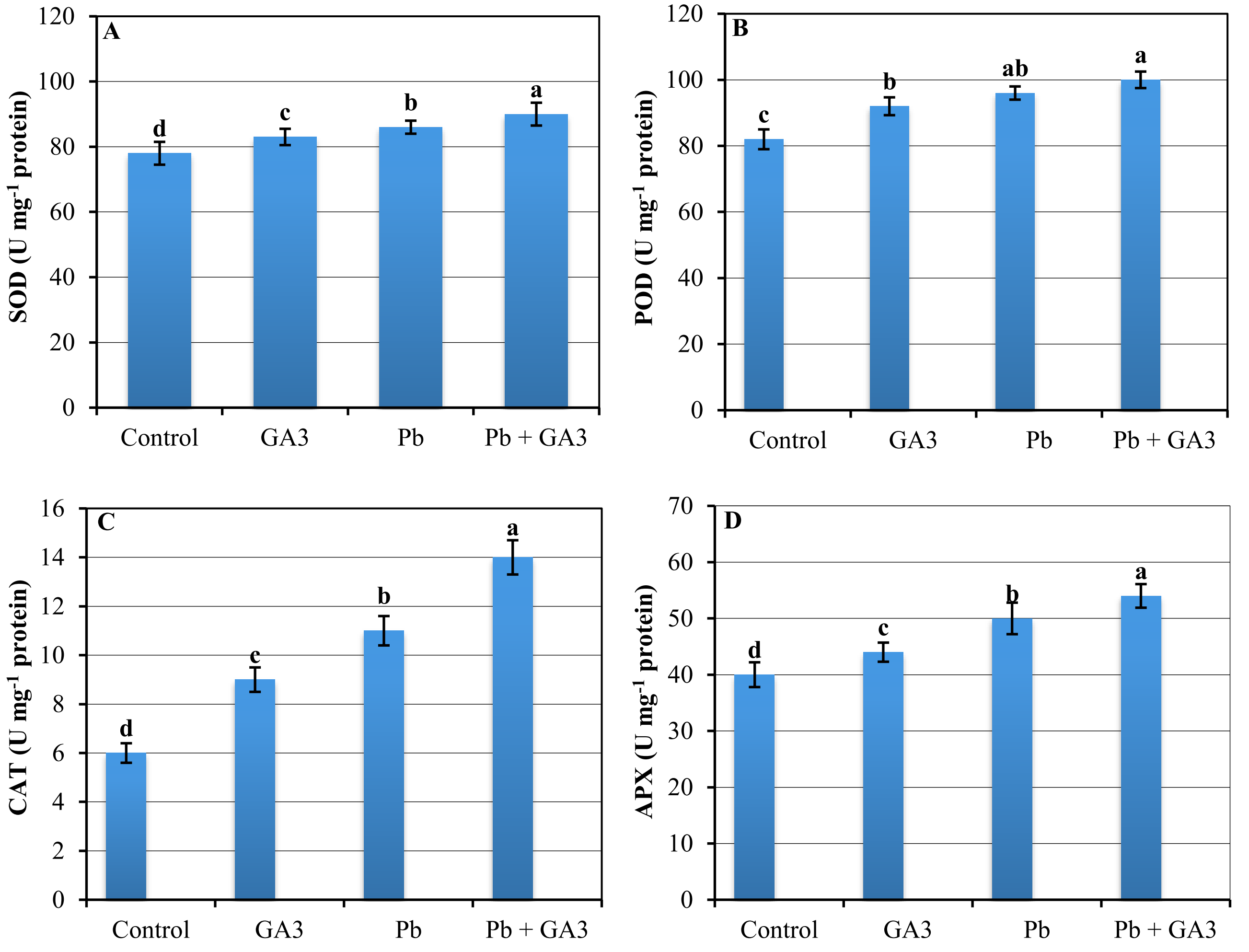

Fig. 1. **(a)** Impact of gibberellic acid (GA_3) on SOD (ug g^{-1} Fw^{-1}) of *S. oleracea* under lead stress **(b)** Impact of gibberellic acid (GA_3) of POD (min^{-1} g^{-1} FW) *S. oleracea* under lead stress **(c)** Impact of gibberellic acid (GA_3) of CAT (mmol g^{-1} Fw) *S. oleracea* under lead stress **(d)**. Impact of gibberellic acid (GA_3) of APX (mmol g^{-1} Fw) *S. oleracea* under lead stress

Discussion

Among the heavy metals lead (Pb) is recognized as a vital abiotic stressor that interferes with plant growth and development by disrupting nutrient uptake, damaging cellular structures and inducing oxidative stress (Gupta *et al*., 2024; Qiu *et al*., 2024), leading to limited vegetative growth, reduced biomass and impaired physiological processes (Meena *et al*., 2019). Results of current study revealed that Pb stress significantly reduced morphological traits including plant height, root length, fresh weight and dry weight of *Spinacia oleracea*, authenticating that Pb hampers cell division which indirectly restricts growth and development. These results are consistent with recent findings in other plant systems (Wang *et al*., 2023; Zhao *et al*., 2026), where Pb exposure led to deterioration in morphological and biomass traits due to inhibition of photosynthetic and metabolic activities (Haider *et al*., 2022) as Pb exposure interrupts the membrane integrity at cellular level indirectly disrupts the nutrient uptake results compromised chloroplast development.

Gibberellic acid (GA_3) has been extensively documented as a key phytohormone that promotes cell elongation, division, and overall plant growth (Fisher *et al*., 2023). In the current work, GA_3 application significantly enhanced all morphological traits under both control and Pb stress conditions (She *et al*., 2025). GA_3–treated plants attained greater heights, longer roots, and higher fresh and dry weights compared with controls, reflecting stimulation of growth processes. Similar promotive effects of GA_3 on plant growth under heavy metal stress have been reported in other studies; for example, combinations of GA_3 with rhizobacterial inoculation improved growth and chlorophyll content in Pb-stressed *Solanum melongena* (Pant *et al*., 2020), and GA_3 alleviated Cu stress in spinach by enhancing antioxidant responses and biomass (Mitra *et al*., 2019). The growth promoting ability of GA_3 is biologically associated with cell division and elongation, in addition to this GA_3 activate relevant genes responsible for loosening and expansion the cell wall.

Importantly, GA_3 significantly ameliorated Pb-induced growth suppression in *S. oleracea*. Morphological performance in Pb + GA_3–treated plants was significantly higher Compared to plants with Pb only and, for plant height, statistically comparable to control levels (Srivastava & Srivastava, 2023). This indicates that GA_3 not only stimulates growth but also confers a protective effect under Pb toxicity, likely through enhanced hormonal balance, improved nutrient acquisition, and modulation of stress responses (Shahzad *et al*., 2024). Enhanced antioxidant enzyme activities and improved physiological parameters observed in this study further support the notion that GA_3 aids in mitigating Pb stress by reducing oxidative damage and preserving cellular integrity, thereby maintaining growth dynamics even under adverse conditions.

Conclusions

This study shows that lead (Pb) stress markedly restricts growth and disrupts physiological and biochemical processes in *Spinacia oleracea*, as evidenced by reduced biomass, lower photosynthetic pigment levels, poor water status, and enhanced oxidative stress. Exogenous application of gibberellic acid (GA_3) not only promoted plant growth under normal conditions but also effectively alleviated Pb-induced damage by improving morphological traits, maintaining chlorophyll content and relative water content, and strengthening the antioxidant defense system. The coordinated enhancement of growth and redox balance under GA_3 treatment suggests that this phytohormone plays an important protective role against Pb phytotoxicity. Overall, GA_3 emerges as a promising and practical approach to improve spinach tolerance to Pb stress, although further studies under field conditions and assessments of Pb accumulation in edible tissues are needed before broader agricultural application.

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