Research Article

Physiological, Biochemical and Morphological Responses of Tomato Plants to lead Exposure

Mohammed I. Alkhalaf[1] Maryam[2] and Adel A. Rezk*[1]

[1]Agricultural Biotechnology Department, College of Agricultural and Food Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia; [2]Department of Environmental Sciences, The Government Sadiq College Women University Bahawalpur, Pakistan.

Abstract | lead (Pb) toxicity can cause damage to tomato seedlings growth and development which leads to serious threats to food safety and crop productivity on sustainable basis. Lead is considered most persistent, its continuous addition into existing agroecosystem is alarming for human health. Present experiment was aimed to assess the effects of lead on morphology, physiological and biochemical attributes of the tomato seedlings at various concentrations (0, 50, 100, 150, and 200 mg kg^{-1} soil) of lead (Pb) grown under controlled greenhouse conditions. From the results it was clearly observed that gradual reduction morphological parameters like plant height, stem diameter, leaf number, shoot biomass and root length was observed with the increase in lead (Pb) concentration, which indicates a dose dependent inhibition of vegetative growth. Physiological analyses revealed significant reductions in chlorophyll a, chlorophyll b, total chlorophyll, total soluble protein, relative water content, stomatal conductance, and photosynthetic rate at higher Pb concentrations, reflecting impaired photosynthetic capacity and water relations. Biochemical analysis depicted that elevated malondialdehyde (MDA) levels, indicating oxidative stress, along with increased proline accumulation and increase in antioxidant enzyme activities (superoxide dismutase, catalase, peroxidase), suggesting activation of defense mechanisms against lead (Pb) induced oxidative stress. Concludingly, these results indicate that lead (Pb) toxicity significantly compromises growth, photosynthetic activity and cellular membrane integrity in tomato plants, while inducing protective biochemical responses. The findings provide deep insight into lead (Pb) toxicity thresholds and the physiological and biochemical strategies adopted by tomato seedlings to mitigate heavy metal stress, with implications for sustainable cultivation in contaminated soils.

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***Correspondence** | Adel A. Rezk, Agricultural Biotechnology Department, College of Agricultural and Food Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia; Mohammed I. Alkhalaf, Agricultural Biotechnology Department, College of Agricultural and Food Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia; **Email:** arazk@kfu.edu.sa
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Introduction

Heavy metals contaminated soil causes serious issues to crop yield and food safety across the globe. Among the heavy metals lead (Pb) is considered as most persistent and non-essential element, which is continuously entering in agroecosystem through human activities like mining, industrial discharge and massive use of contaminated irrigation water or agrochemicals (Ramadan *et al.*, 2025). As plants uptake lead (Pb) through irrigated water and translocate it in plant system which not only disrupts plant physiology but also causes serious human health issues via the food chain (Zhou *et al*., 2024; Razak *et al*., 2024).

Tomato has key position among the vegetables as it is consumed as raw farm (salad) and also used as main ingredient in different cooked recipes. Tomato is being cultivated in diverse soil and climate across the globe, definitely some of these soils may be affected by heavy metal toxicity this make it necessary to study the effect of lead (Pb) on morphological, biochemical and physiological attributes of tomato seedlings (Razak *et al*., 2024). Recent study reveals that the lead (Pb) exposure to vegetables cause serious not only reduces total biomass and overall vegetative growth but also reduced chlorophyll content, and compromised water relations (Sharma *et al*., 2025). Similarly, in a recent experiment 'Pusa Ruby' cultivar of tomato was used to assess effect of increasing Pb concentrations results indicated that there is significant decrease in shoot and root lengths, fresh/dry weights and total chlorophyll content was recorded, indicating growth restriction and pigment degradation (Sharma *et al*., 2025).

Lead (Pb) toxicity not only creates disturbance in nutrient uptake as it interacts with cell membrane but also induces excessive generation of reactive oxygen species (ROS), which ultimately disturbs major metabolic pathways involved in biochemical activities (Zhou *et al*., 2024; Ramadan *et al*., 2025). Actually, lead (Pb) triggers activation of antioxidant enzymes (e.g., superoxide dismutase, catalase, peroxidase) and accumulation of osmolytes (e.g., proline) to mitigate oxidative damage (Zhou *et al*., 2024). However, when Pb concentrations exceed the threshold limit, which difficult situation for plant to detoxify, physiological and biochemical functions degrade, and morphological growth is significantly reduced.

In case of tomato lead is major threat for both crop productivity and human health, in current study a detailed assessment of how continuous increase in lead (Pb) levels influence its growth, physiological activities and biochemical defense mechanism is highly warranted. Therefore, the current experiment investigates how lead influences the tomato morphological, physiological and biochemical attributes. The aim is to define the dose-dependent impact of Pb stress on tomato, identify major response attributes and inform potential mitigation approaches.

Materials and Methods

Plant material and experimental design

Seeds of Tomato (*Solanum lycopersicum* L.) cultivar "Dominant F1" were purchased from local vegetable seed market, Bahawalpur followed by treating with 1 % sodium hypochlorite for 5 min, and washed with distilled water to remove surface dust particles. After washing seeds were sown in plastic trays containing autoclaved compost and pond soil (1:1 v/v) mixture. The plastic trays were kept in controlled conditions (22 ± 2 °C day/18 ± 2 °C night; 14 h photoperiod; ~60 % RH), after four weeks uniform seedlings were transplanted into 5 L pots filled with air-dried pond soil (sieved to 2 mm) physico-chemical properties of soil were assessed: pH 7.2, organic matter 1.8 %, electrical conductivity 0.24 dS m^{-1} in the laboratory department of horticultural sciences, the Islamia University of Bahawalpur, Pakistan. Lead nitrate $Pb(NO_3)_2$ solution was used at different levels to induce heavy metal stress to the seedlings. The following concentrations ware applied 0 (control), 50, 100, 150 and 200 mg Pb kg^{-1} soil. The experiment was conducted in Complete Randomized Design (CRD) with five replications. Irrigation and fertilizer application was provided as per recommended for tomato.

Morphological measurements

After six weeks of Pb exposure, plants were harvested at the vegetative stage. Plant height (cm) was measured from soil surface to shoot apex and the number of fully expanded leaves per plant was recorded. Stem diameter (mm) was measured at 2 cm above soil level using digital callipers. Shoots and roots were separated, lightly washed in deionised water, blotted dry, and fresh weights recorded. Root length (cm) was measured via a ruler from the root collar to the tip. Shoot biomass (g $plant^{-1}$) was determined after drying at 70 °C to constant weight.

Physiological analyses

Leaf samples (third fully expanded leaf from apex) were collected at mid-day, immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Chlorophyll a and chlorophyll b were extracted in 80% acetone and quantified spectrophotometrically according to Arnon's equation. Total chlorophyll (mg

g^{-1} fresh weight, FW) was calculated as the sum of both pigments. Total soluble protein was extracted in 50 mM phosphate buffer (pH 7.0) and determined by the Bradford method (Coomassie Reagent) with bovine serum albumin as standard. Relative water content (RWC,%) was calculated as: (FW – dry weight)/ (turgid weight – dry weight) ×100. Stomatal conductance (mmol m^{-2} s^{-1}) was recorded using a porometer on the abaxial surface of the same leaf, and photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) was measured between 10:00–12:00 h under ambient CO_2 using a portable photosynthesis system.

Biochemical assessments

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) via thiobarbituric acid reactive-substances (TBARS) assay (Heath and Packer, 1968). Proline accumulation was quantified using the acid-ninhydrin method and expressed as μmol g^{-1} FW. Antioxidant enzyme activities were measured in enzyme extracts prepared in 50 mM phosphate buffer (pH 7.8) containing 1 % polyvinylpyrrolidone and 0.1 mM EDTA (Bates *et al*., 1973). Superoxide dismutase (SOD) activity (U mg^{-1} protein) was assayed by inhibition of photochemical reduction of nitroblue tetrazolium (Giannopolitis and Ries, 1977), catalase (CAT) activity by H_2O_2 decomposition at 240 nm (Aebi, 1984), and peroxidase (POD) activity by guaiacol oxidation at 470 nm (Chance and Maehly, 1955).

Statistical analysis

All data were expressed as mean ± standard deviation (n = 5). Statistical differences among treatments were evaluated using one-way ANOVA followed by Tukey's HSD test at $p < 0.05$. Correlations between lead concentration and measured parameters were determined using Pearson's correlation coefficient. Statistical analyses were performed in SPSS v26.0.

Results

The results presented in Table 1 are summarized morphological behavior of tomato seedlings in response of lead (Pb) induced stress. Progressive decline in seedlings height was measured from 45.2 ± 1.5 to 23.4 ± 1.0 cm as compared to highest level of 200 mg/kg Pb, depicting that Pb stress notably restricts seedlings height. Furthermore, lead stress also influences number of leaves per seedling, as significant decrease in number of leaves from 12 ± 1 to 6 ± 1 was observed, while reduction in stem thickness was recorded from 5.2 ± 0.3 mm to 3.1 ± 0.2 mm as compared to control, showcasing prohibited leave emergence and stem diameter under higher Pb levels. Reduction in total shoot biomass was recorded from 18.5 ± 0.8 g/plant to 7.1 ± 0.3 g/plant as compared to control with highest concentration of lead, moreover, severe decline in root length was also observed from 20.4 ± 1.2 cm to 9.4 ± 0.7 cm, signifying the inhibitory effects of lead on both areal and underground parts of tomato seedlings. These findings provide a conclusion that lead (Pb) stress not only impairs cell division and elongation but also reduces nutrient and water uptake, and concludingly minimizes overall vegetative growth. The continuous reduction in all morphological attributes indicates a dose-dependent response to soil Pb stress.

The results displayed in Table 2 clearly depicting physiological responses of tomato seedlings to increasing lead (Pb) concentrations. Chlorophyll a content decreased from 2.45 ± 0.05 mg/g FW in the control to 1.05 ± 0.02 mg/g FW at 200 mg/kg Pb, chlorophyll b from 1.12 ± 0.03 mg/g FW to 0.55 ± 0.01 mg/g FW, and total chlorophyll from 3.57 ± 0.06 mg/g FW to 1.60 ± 0.03 mg/g FW, indicating a strong inhibition of chlorophyll synthesis under Pb stress.

Table 1: *Effect of lead stress on morphological attributes of tomato seedling*

Lead concentration (mg/kg soil)	Plant height (cm)	Leaf number	Stem diameter (mm)	Shoot biomass (g/plant)	Root Length (cm)
0 (Control)	45.2 ± 1.5	12 ± 1	5.2 ± 0.3	18.5 ± 0.8	20.4 ± 1.2
50	39.1 ± 1.3	10 ± 1	4.7 ± 0.2	15.2 ± 0.6	17.8 ± 1.0
100	33.5 ± 1.2	8 ± 1	4.1 ± 0.3	12.4 ± 0.5	14.6 ± 0.9
150	28.7 ± 1.1	7 ± 1	3.6 ± 0.2	9.8 ± 0.4	12.1 ± 0.8
200	23.4 ± 1.0	6 ± 1	3.1 ± 0.2	7.1 ± 0.3	9.4 ± 0.7

Table 2: *Effect of lead stress on physiological attributes of tomato seedling*

Lead Concentration (mg/kg soil)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Total soluble Protein (mg/g FW)	Relative water Content (%)	Stomatal conductance (mmol/m²/s)	Photosynthetic Rate (µmol CO_2/m²/s)
0 (Control)	2.45 ± 0.05	1.12 ± 0.03	3.57 ± 0.06	25.3 ± 0.9	88.4 ± 2.1	0.31 ± 0.02	12.5 ± 0.6
50	2.10 ± 0.04	0.95 ± 0.02	3.05 ± 0.05	21.7 ± 0.7	81.2 ± 1.8	0.27 ± 0.02	10.2 ± 0.5
100	1.78 ± 0.03	0.82 ± 0.03	2.60 ± 0.06	18.5 ± 0.6	75.5 ± 1.5	0.23 ± 0.01	8.7 ± 0.4
150	1.42 ± 0.02	0.69 ± 0.02	2.11 ± 0.04	15.2 ± 0.5	69.8 ± 1.3	0.18 ± 0.01	6.9 ± 0.3
200	1.05 ± 0.02	0.55 ± 0.01	1.60 ± 0.03	12.0 ± 0.4	63.7 ± 1.2	0.13 ± 0.01	5.2 ± 0.3

Table 3: *Effect of lead stress on biochemical attributes of tomato seedling*

Lead concentration (mg/kg soil)	Malondialdehyde (MDA, µmol/g FW)	Proline (µmol/g FW)	Superoxide dismutase (SOD, U/mg protein)	Catalase (CAT, U/mg protein)	Peroxidase (POD, U/mg protein)
0 (Control)	1.85 ± 0.08	4.2 ± 0.3	35.2 ± 1.5	22.5 ± 1.2	18.3 ± 0.9
50	2.34 ± 0.10	6.5 ± 0.4	42.1 ± 1.7	27.4 ± 1.3	22.8 ± 1.1
100	2.89 ± 0.12	8.9 ± 0.5	48.6 ± 1.9	32.7 ± 1.4	27.6 ± 1.2
150	3.41 ± 0.14	11.2 ± 0.6	53.9 ± 2.0	38.1 ± 1.5	32.5 ± 1.3
200	4.05 ± 0.16	14.0 ± 0.7	59.5 ± 2.2	43.7 ± 1.6	38.2 ± 1.4

There is continuous decline was observed in total soluble protein content from 25.3 ± 0.9 to 12.0 ± 0.4 mg/g FW as compared to control as least concentration was recorded when lead was applied 200 mg/kg soil. Similarly, Relative water content was reduced from 88.4 ± 2.1% to 63.7 ± 1.2%, indicating decreased water retention. Stomatal conductance dropped from 0.31 ± 0.02 mmol/m²/s to 0.13 ± 0.01 mmol/m²/s, and photosynthetic rate fell from 12.5 ± 0.6 µmol CO_2/m²/s to 5.2 ± 0.3 µmol CO_2/m²/s, indicating reduced gas exchange and photosynthetic efficiency under elevated Pb concentrations. Current findings concluded that continuous increase in lead concentration in the soil damages the physiological activities of tomato seedlings, as it tremendously affects the protein levels, pigment content, water status and photosynthetic activity as shown in (Table 2).

Summary of biochemical responses of tomato seedlings (*Solanum lycopersicum*) in response of lead (Pb) induced stress is presented in Table 3. Results clearly indicated that progressively increase in Malondialdehyde (MDA) with the increase in lead (Pb) concentration was recorded from 1.85 ± 0.08 µmol/g FW to 4.05 ± 0.16 µmol/g FW as compared to control which indicates enhanced oxidative stress and membrane damage under higher Pb levels.

Similarly, significant increase in proline content was also recorded from 4.2 ± 0.3 µmol/g FW to 14.0 ± 0.7 µmol/g FW as compared to highest Pb concentration, reflecting the plant's adaptive response to mitigate osmotic and oxidative stress. Antioxidant enzyme activities showed a similar trend: superoxide dismutase (SOD) increased from 35.2 ± 1.5 to 59.5 ± 2.2 U/mg protein, catalase (CAT) from 22.5 ± 1.2 to 43.7 ± 1.6 U/mg protein, and peroxidase (POD) from 18.3 ± 0.9 to 38.2 ± 1.4 U/mg protein. Significant increase in enzymatic activity indicating that activation of the plant's defense mechanism to scavenge reactive oxygen species generated by Pb induced stress in tomato seedling. Concludingly, these findings presented in Table 3 indicate that lead (Pb) induced stress causes oxidative damage in tomato seedlings and also activating biochemical defense mechanisms of plants, like proline accumulation and upregulation of antioxidant enzymatic activity. Apart from this protective behavior of tomato seedlings, the increase in levels of MDA signify that oxidative stress outweighs defense capacity at higher Pb concentrations, contributing to reduced plant growth and physiological performance.

Discussion

The present study demonstrates that lead (Pb) stress exerts a strong inhibitory effect on tomato (*Solanum lycopersicum* L.) growth, physiological performance, and biochemical responses, consistent with previous findings in vegetable crops exposed to heavy metals (Zhou *et al*., 2024; Razak *et al*., 2024). Morphological parameters, including plant height, leaf number, stem diameter, shoot biomass, and root length, declined progressively with increasing Pb concentration (Table 1). The reduction in plant height and biomass may be attributed to the disruption of cell division and elongation processes by Pb, as heavy metals interfere with nutrient uptake and hormonal balance, limiting meristematic activity (Ramadan *et al*., 2025). Root growth was particularly affected, which is significant because roots are the primary site of Pb uptake and accumulation, leading to impaired water and nutrient transport to areal parts.

It was observed that physiological characters also interrupted in tomato seedlings grown under Pb stress (Table 2). Significant decrease was observed in chlorophyll a, chlorophyll b, and total chlorophyll content with increasing in lead (Pb) concentration, which is indication of destruction in chloroplast structure and indirectly inhibition of pigment biosynthesis. The destruction in chloroplast resulted in decline of photosynthetic activity, stomatal conductance and water relations. Reduced total soluble protein under Pb stress indicates a decline in metabolic processes and may result from lead (Pb) induced denaturation of proteins or restriction of protein synthesis process (Ahmad *et al*., 2025). The overall decrease in photosynthetic activity supports the hypothesis that lead (Pb) not only affects pigment content but also inhibits enzymatic activity involved in carbon assimilation which results in yield losses (Shoukat *et al*., 2025).

Lead (Pb) induced stress also significantly interrupts the biochemical responses through oxidative damage, as evidenced by a notable increase in malondialdehyde (MDA) content (Table 3). Increased MDA concentration depicts lipid peroxidation and damage at membrane level, a major effect of heavy metal-induced oxidative stress (El-Sappah *et al*., 2024). Accumulation of proline in tomato plants, which act as osmo-protectants and reactive oxygen species (ROS) scavengers, helping to maintain cellular homeostasis is result of lead oxidative stress. Activities of antioxidant enzymessuperoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)increased significantly under Pb exposure, reflecting the activation of defense mechanisms to mitigate ROS-mediated damage. However, despite these protective responses, the progressive increase in MDA suggests that the defense capacity was insufficient at higher Pb concentrations. Overall, the results indicate that Pb stress induces a dose-dependent negative effect on tomato morphology, physiology, and biochemistry. Morphological reductions compromise growth and biomass production, physiological impairments reduce photosynthetic efficiency and water status, and biochemical changes represent a partial protective response against oxidative damage. These findings highlight the vulnerability of tomato plants to Pb contamination and underscore the need for strategies to mitigate Pb accumulation in soils to ensure sustainable production and food safety.

Conclusions and Recommendations

Lead (Pb) induced stress notably reduces tomato (*Solanum lycopersicum* L.) growth, photosynthetic activity and water relations, while inducing oxidative stress as demonstrated by elevated enzymatic activities like (MDA, SOD, CAT, POD) levels. The increase in proline content depicts the activation of defense mechanisms of tomato plant; however, activation of this mechanism cannot mitigate the damaging effect of oxidative stress caused by higher Pb concentrations. Overall, the study proves that continuous increase in lead (Pb) concentration damages the crop productivity and physiological processes, there is need to adopt such strategies to mitigate the oxidative stress caused by lead (Pb) for food safety and crop sustainability.

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Novelty Statement

Present investigation depicts that how lead (Pb) is creating disruption in morphological, physiological, and biochemical attributes of tomato seedlings while continuous entering in agroecosystem due human activities. Furthermore, providing new insights into Pb toxicity thresholds and adaptive responses for sustainable crop productivity.

Author's Contribution

Mohammed I. Alkhalaf: Presented the idea of the research.
Maryam: Wrote the draft of manuscript
Adel A. Rezk: Statistical analysis

Generative AI or AI assisted technology statement
The authors declare that they have not used generative AI or AI-assisted technologies in this manuscript.

Conflict of interest
There is no conflict of interest regarding current study.

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