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Comparative Assessment of Lead and Copper Toxicity on Growth Dynamics of *Spinacia oleracea* L.

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ABSTRACT

Soil contamination with heavy metals is a serious environmental problem that negatively affects plant growth, crop productivity, and food safety. This study was conducted to evaluate the growth and stress responses of Spinacia oleracea L. under lead and copper stress conditions. Plants were treated with different concentrations of lead and copper salts at 0, 100, 200, and 300 ppm under controlled conditions. Several growth parameters were measured, including plant height, root and shoot length, number and size of leaves, fresh and dry biomass, and inflorescence development. The results showed that copper exhibited a concentration dependent effect on plant growth. At lower concentrations, copper slightly enhanced growth parameters, likely due to its essential role as a micronutrient involved in enzymatic activities and metabolic processes. However, higher concentrations led to growth inhibition. In contrast, lead exposure caused a significant and consistent reduction in all measured growth parameters, indicating strong phytotoxic effects. Lead toxicity likely interfered with physiological processes such as photosynthesis, nutrient uptake, and water balance, resulting in reduced biomass and overall plant vigor. Statistical analysis confirmed that these effects were significant compared to control treatments. The findings highlight that while copper may support plant growth at optimal levels, lead poses a severe risk even at relatively low concentrations. This study emphasizes the importance of monitoring and managing heavy metal contamination in agricultural soils. Future research should focus on understanding the physiological, biochemical, and molecular mechanisms underlying heavy metal stress tolerance and developing effective strategies to mitigate their adverse effects in crop plants.
Keywords: *Soil contamination, heavy metals, Spinacia oleracea, lead toxicity, copper stress, plant growth.*

Introduction

Environmental stress is a major constraint that limits plant growth and agricultural productivity worldwide. Stress is defined as a physiological condition caused by external or internal factors that disrupt normal cellular functions in plants (Hasan et al., 2020). Plant stress occurs when environmental or biological factors disturb physiological equilibrium, leading to significant changes in metabolic and biochemical processes such as photosynthesis, ion balance, and water uptake. These disruptions ultimately reduce plant growth and development (Kanwal et al., 2026).

Abiotic stress includes non-living environmental factors such as drought, temperature extremes, salinity, and pollutants. These stresses directly affect plant physiology and biochemistry, resulting in reduced productivity and yield losses (Raza et al., 2022; Jan et al., 2026). Among these stresses, heavy metal toxicity has emerged as a critical environmental issue due to its persistence and harmful effects on plant systems. Heavy metals alter key physiological and biochemical processes, including nitrogen and carbohydrate metabolism, photosynthesis, mineral nutrient assimilation, and plant water relations. Their accumulation disrupts cellular homeostasis, induces oxidative stress, and interferes with essential metabolic pathways (Hu et al., 2023; El-Sappah et al., 2024).

The increasing accumulation of toxic metals in soil is mainly driven by anthropogenic activities such as industrial discharge, mining operations, and the extensive use of agrochemicals. This contamination poses a serious risk to crop productivity, environmental sustainability, and food safety (Mohamed et al., 2025; Al-Obaidi et al., 2024). Lead is one of the most toxic heavy metals and has no known biological function in plants. Its accumulation in soil and water severely affects plant growth by disrupting physiological processes and reducing biomass production (Summaya et al., 2026).

In contrast, copper is an essential micronutrient required for normal plant growth and development. It plays a key role in enzymatic reactions and metabolic pathways. However, excess copper becomes toxic and can induce oxidative damage and metabolic imbalance. Plants have evolved complex mechanisms to regulate copper uptake, transport, and detoxification in order to maintain cellular homeostasis (Yruela, 2005).

Spinacia oleracea L. is an important leafy vegetable with high nutritional value and global consumption. However, its growth and productivity are highly sensitive to abiotic stresses such as salinity, drought, and temperature fluctuations (Morelock and Correll, 2018; Raphael et al., 2026). Salinity stress, in particular, disrupts ion balance and causes osmotic stress, leading to reduced plant vigor and yield (Amir et al., 2024). Despite extensive studies on abiotic stress, limited information is available on the comparative effects of lead and copper stress on the growth performance of spinach.

Therefore, the present study was designed to evaluate the effects of lead and copper stress on key growth parameters of Spinacia oleracea under controlled conditions. This work aims to provide insight into the differential responses of spinach to essential and non-essential heavy metals and to contribute to strategies for managing heavy metal stress in crop production.

MATREIALS AND METHODS

Experimental design

The experiment was conducted at the Botanical Garden of Islamia College Peshawar. Pots were arranged in a randomized block design with three replicates. Each pot was filled with a mixture of clay, sand, and organic matter in a ratio of 2:2:1 (Jan *et al.*, 2026).

Seed preparation and sowing

Seeds of *Spinacia oleracea* were tested for viability by soaking in water for 2 to 3 hours. Healthy seeds were selected and sown in pots.

Metal treatments

Lead and copper solutions were prepared using lead nitrate $Pb(NO_3)_2$ and copper sulfate $CuSO_4$. Plants were treated with 0, 100, 200, and 300 ppm concentrations of each metal.

Growth measurements

Seed germination percentage was calculated using the formula (FGP = S/T x100). where S is the number of germinated seeds and T is the total number of seeds sown. Plant height, root length, and shoot length were measured using a meter ruler at harvest. Leaf number and size were recorded manually. Fresh weight was measured immediately after harvesting using a digital balance. Dry weight was measured after drying plant samples in an oven (Ologundudu *et al.*, 2014).

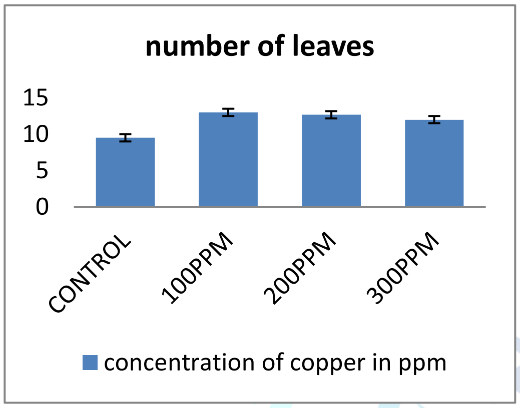
Statistical Analysis

Data were analyzed using Microsoft Excel and GraphPad Prism 9.

RESULTS AND DISCUSSION

Number of leaves

It is clear from the Fig. 1 that all the three concentrations of copper show positive effect on the length of leaves of spinach as compared to lead which has reduced the number of leaves. Lead (Pb) is a toxic metal that has negatively affected plant growth and development. It retarded many physiological processes e.g. cell division, photosynthesis process, and nutrient uptake, which ultimately leads to reduction in vegetative growth and results in a reduction in leaves number (Gupta *et al.*, 2024; Alkhatib *et al.*, 2019). At low concentrations, copper prop up the normal growth and help maintenance or increase in the number of leaves (Giannakoula *et al.*, 2021).



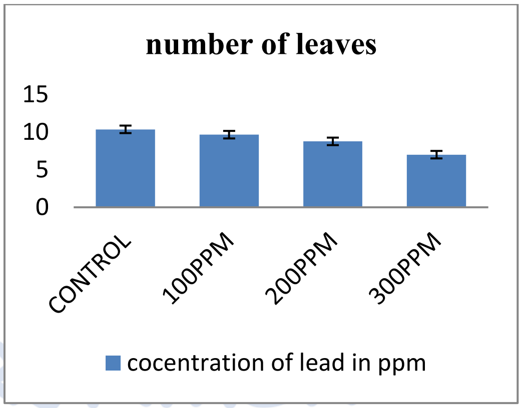
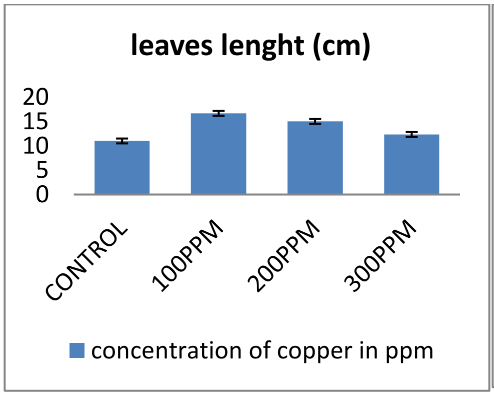


Fig 1. Effect of different concentrations of lead and copper on the number of leaves of *Spinacia oleracea*.

Length and width of leaves

Fig. 2 and 3 revealed that when plant was treated with high concentrations of Pb, it cause the reduction in leaves length and width. While in case of copper it increases the length and width of leaves. Recent studies show that Pb toxicity disturbs the process of photosynthesis, cellular structure, and certain metabolic processes, eventually retarding leaf growth (Borkowska-Drela *et al.*, 2026). Copper (Cu) is an essential nutrient, but in excess it may be toxic. At optimal levels, Cu reinforce plant growth and normal leaf development (Zeb *et al.*, 2024).



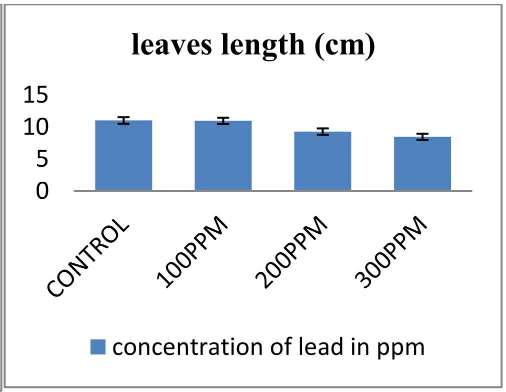


Fig 2 Effect of lead and copper treatments on leaf length of *Spinacia oleracea* under different metal concentrations.

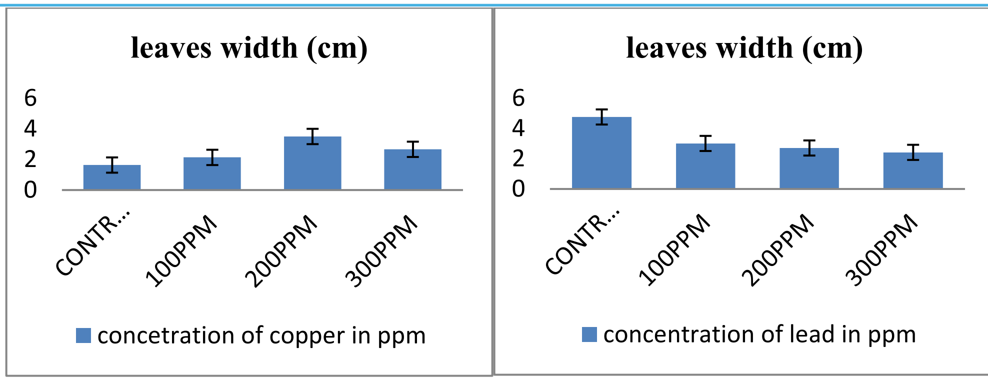


Fig 3. Effect of lead and copper treatments on leaf width of *Spinacia oleracea* at varying concentrations.

Plant height

Fig 4 demonstrate the effects of copper and lead salts in the overall height of plant species. It can be observed that copper promote the plant height while the lead salt has retarded the height of plant. Pb toxicity interrupts photosynthesis, water-solute balance, and uptake of nutrients, resulting in stunted growth of plants (Hu *et al.*, 2023). Copper (Cu) is fundamental requirement for various plant metabolic processes. Cu supports enzyme activity and normal growth, helping maintain plant height when it was applied at normal level (Zeb *et al.*, 2024).

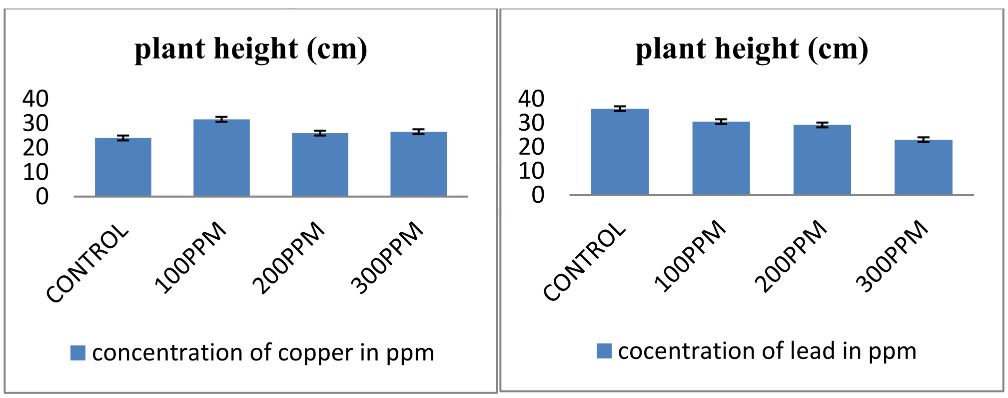


Fig 4. Effect of lead and copper stress on plant height of *Spinacia oleracea* grown under controlled conditions.

Number of inflorescences

When the concentration of copper was increased it causes a rise in number of inflorescence while higher lead concentration negatively affected the number of inflorescences in average flowers as shown in the Fig. 5 Studies show that heavy metal stress delays flowering and suppresses reproductive development, thereby decreasing inflorescence formation (Hu *et al.*, 2023). Copper (Cu) is involved in reproductive development in plant body, but in excess it becomes toxic. At low concentrations, Cu supports many activities of enzymes and normal flowering (Hussain *et al.*, 2024).

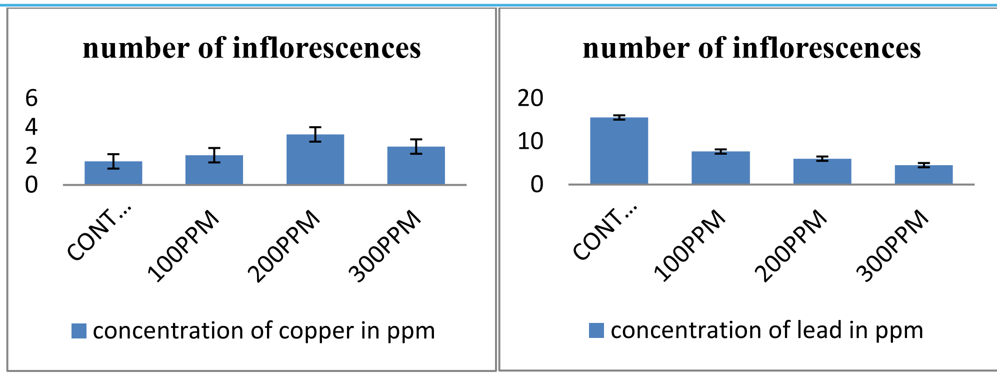


Fig 5. Effect of increasing concentrations of lead and copper on the number of inflorescences of *Spinacia oleracea*.

Fresh weight

Increase in concentration of copper cause the rise the fresh weight of the plant when weighted with digital balance while when concentration of Pb was increased it cause the reduction in fresh weight of plant (Fig. 6). Exposure to Pb induce stress reduces water uptake, disturb nutrient balance, and inhibits photosynthesis, which result in decreases fresh weight of plants (Gupta *et al.*, 2024). Low concentration of cupper contamination promotes enzymatic functions and may maintain or slightly increase fresh weight of plant (El-Sappah *et al.*, 2024).

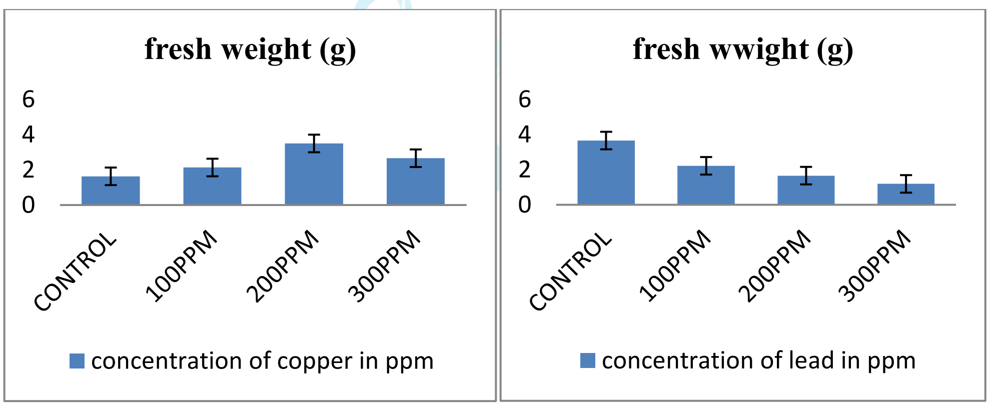


Fig 6. Effect of lead and copper treatments on fresh weight of *Spinacia oleracea* plants at different concentrations.

Dry weight

Same like incase of fresh weight, high concentration of copper cause increase in dry weight of the plant. On the other hand, when lead concentration was increased it causes a significant reduction in plant dry weight (Fig. 7). Lead (Pb) is a toxic heavy metal that significantly reduces plant biomass. It disrupt many key physiological processes such as photosynthesis and nutrient uptake, which leads to a decrease in dry weight (Jan *et al.*, 2026). Cu enhances several physiological and biochemical processes, leading to an increase in dry weight. It acts as a cofactor for many enzymes that are involved in photosynthesis, respiration, and carbohydrate metabolism, which promotes biomass accumulation (Yruela, 2009).

Recent advances in sustainable agriculture highlight the growing integration of nanotechnology, microbial systems, and eco-friendly approaches to improve plant performance under stress conditions. Nanocomposite based fertilizers have shown strong potential to enhance nutrient use efficiency and crop productivity while reducing environmental impact (Ullah *et al.*, 2025). In parallel, plant growth promoting bacteria are gaining attention for their role in biodegradation processes and soil health improvement, which directly supports sustainable crop production systems (Maqbool *et al*., 2025). The application of green nanotechnology, particularly metal based nanoparticles and their composites, offers promising strategies for improving plant resilience and protection against biotic and abiotic stresses (Khan *et al*., 2025; Khan *et al*., 2026). In addition, soil microbial interactions play a key role in suppressing phytopathogens and maintaining ecosystem stability, which is critical for sustainable vegetable production (Khan *et al*., 2026). Certain microbial genera such as *Paenibacillus* have demonstrated multifunctional roles in heavy metal detoxification, pesticide degradation, and stress mitigation, making them valuable tools for future agricultural systems (Khan *et al*., 2025). Endophytic bacteria also contribute significantly to plant growth promotion and stress tolerance through various biochemical and physiological mechanisms (Iqbal *et al*., 2026). Moreover, recent studies on phytoremediation and nanoparticle synthesis highlight the potential of combining biological and nanotechnological approaches for environmental cleanup and crop improvement (Ullah et al., 2026). These emerging strategies indicate that integrated approaches involving nanomaterials, beneficial microbes, and plant systems will play a critical role in developing resilient and sustainable agricultural practices in the future.

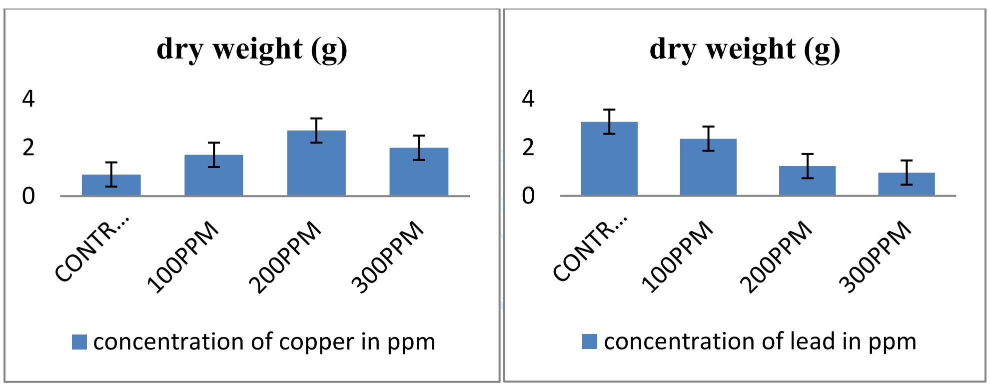


Fig 7. Effect of lead and copper stress on dry weight of *Spinacia oleracea* under varying treatment levels.

CONCLUSION

Heavy metal pollution is a major global issue Heavy metal stress significantly affects the growth of *Spinacia oleracea*. Lead showed strong toxicity and reduced all growth parameters. Copper showed a concentration dependent response, with slight growth improvement at lower levels and toxicity at higher levels. These results highlight the importance of controlling heavy metal contamination in agricultural soils. Future research should focus on physiological and molecular mechanisms of stress tolerance and develop strategies to reduce heavy metal toxicity in crop production.

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