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Lead induced changes in biomimetic and physiological attributes of Soybean (*Glycine max*)

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Abstract

Glycine max is a plant that is widely used in traditional medicine. The aim of this study was to assess the growth, physiological responses, and bioaccumulation and translocation of lead (Pb) in different tissues of soybean under different doses of Pb. In this study, plants were irrigated with Pb contaminated water, and different treatments of contaminated water were prepared by adding 20, 40, 60, 80, 100 mg Pb L^{-1} along with distilled water as a control. The plants were subjected to treatment in three phases (i) 65 days after sowing, (ii) 72 days after sown, and (iii) 79 days after sowing. The pots were placed in randomized complete design (CRD), and each treatment was replicated three times. Results show that biomasses of shoot, root, and pods were significantly ($p < 0.05$) reduced by 63.88 to 78.98% in comparison to control treatment. Likewise, 100 mg Pb L^{-1} treatments resulted in a decrease in the number of pods, number of seeds, and 100 seed weight by 46-67 to 66.70% in comparison to control treatment. In this study, Pb treatments significantly reduced different gaseous traits of soybean i.e., net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (Gs) thereby increased CO_2 concentration in the plant tissues (Ci). Higher dose of Pb (e.g. 100 mg Pb L^{-1}) reduced the value of A by 58.55% as compared to the control treatment. Similarly, 100 mg Pb L^{-1} treatment decreased chlorophyll score by 65.13% over that of the control treatment. Furthermore, we observed higher bioaccumulation factors (BAF) and reduced translocation factors (TF) under higher doses of Pb. Moreover, Pb stress negatively correlated (r ranged from -0.95 to -0.99) with A, E, Gs and chlorophyll scores. It is concluded that Glycine *max* L. Merrill had a low tolerance against Pb toxicity. Moreover, Pb toxicity due to accumulation seeds could pose a health risk.

Keywords: Bioaccumulation, Chlorophyll score, *Glycine max,* Stomatal conductance, Translocation factor

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Introduction

Agricultural lands are vulnerable to environmental pollution, particularly heavy metals pollution. Heavy metal (HM) pollution in agricultural land is a major problem for developing countries, which affects food security and human health (Anuoluwa et al., 2025). Pb is one of the most hazardous metals on the earth which affects human health in several ways (Jomova et al., 2025) as Pb in body may cause neurological, cardiovascular and vascular disorders in humans (Wang et al., 2020). On the other hand, Pb toxicity influences soil fertility as well as morphological, physiological, and biochemical traits of plants (Chen et al., 2022; Wei et al., 2025). It disrupts cell growth, photosynthesis, photosynthetic pigments, gas exchange parameters, mineral uptake and enzymatic functions (Chen et al., 2022; Wei et al., 2025). It also causes oxidative damage which results into disturbances in plant growth, metabolism and membrane permeability (Pirzadah et al., 2020; Amari et al., 2017; Sofy et al., 2020). In Pakistan, chemical fertilizers, insecticides, wastewater, and vehicles are the primary contributors of Pb to the environment (Rehman et al. 2017; Ahmad et al., 2020).

The permissible limit of Pb in the soil is 85 mg kg^{-1} with a corresponding limit of 2 mg kg^{-1} in the plant (WHO, 1996). In the previous studies, the agricultural lands irrigated with wastewater were found to accumulate greater concentrations of Pb in food crops which exceeded the permissible limit set by the WHO/FAO (Akhtar et al., 2022; Atta et al., 2023). Furthermore, an exceeded level of Pb was recorded in vegetables grown along the roadsides which in the food chain is a high-risk cancer factor (Mabood et al., 2022). Soybean (*Glycine max*), belonging to the family *Leguminosae*, is a rich source of protein (Alleza et al., 2025). On a global scale, *Glycine max* stands as the 9th most vital food crop (Raza et al., 2021). In recent decades, there has been a marked increase in the cultivation of *Glycine max* (Pan et al., 2019) in the world. Globally, the US is the primary producer of soybeans with 45% of the production in the world, followed by

Brazil and China with 20% and 12% of production worldwide (Cheng & Rosentrater, 2017). However, it is a nonconventional crop in Pakistan that was introduced in 1970 but did not gain popularity as compared to other countries (Ali et al., 2013; Asad et al., 2020).

Abiotic stresses such as high temperature, drought, salinity, cold and heavy metal toxicity can severely limit the growth, development, and overall productivity of agricultural crops by disrupting physiological and biochemical processes (Zaman & Qureshi, 2018; Shah et al., 2019; Iqbal & Qureshi, 2021; Noroz et al., 2021). These stresses often impair photosynthesis, nutrient uptake, and cellular integrity, ultimately reducing crop yield and quality (Zia et al., 2023; Omokhafe et al., 2024; Batool et al., 2025; Abdullah et al., 2025). Soybean, like many major crops, is particularly sensitive to these stressors, which can significantly impact its growth, seed quality, and overall productivity (Bilal et al., 2018). Although several studies have evaluated the effect of Pb on the growth and productivity of soybeans (Fatoba et al., 2012; Naghavi et al., 2014; Sytar et al., 2016; Kulaz et al., 2021), few studies have been conducted in Pakistan. These studies were based on the evaluation of Pb accumulation in the seedling stage while limited studies were conducted to evaluate Pb exposure at the reproductive phase of soybean. The screening of a variety of soybeans with low Pb accumulation and tolerance efficiency in the reproductive stage is the need of time. Therefore, the current study was planned to investigate the growth, and physiological response of *Glycine max* under different lead treatments, and to evaluate the bioaccumulation and translocation of Pb in different soybean tissues at various levels of Pb.

Materials and Methods

Experimental setup

The current experiment was executed in a completely randomized design with three replicates of each treatment at the botanical garden of Allama Iqbal Open University (AIOU), Islamabad. The concrete pots (height: 25 cm, diameter 22 cm) were filled with 10 kg soils, which were collected from the surface depth of 0-10 cm from the experimental site of AIOU. The physicochemical properties of soil were measured by using the method developed by Lauber et al. (2008), and Allen et al. (1974) which are as follows; pH, 7.89; electric conductivity, 0.43 mS cm^{-1}; available P, 46.32 mg kg^{-1}; available K, 190 mg kg^{-1}; Pb concentration, 179.38 ppb. Seeds of Glycine *max* L. Merrill (cv. NARC-21) were obtained from Crop Sciences Institute (CSI), National Agricultural Research Centre (NARC), Islamabad. The seeds were thoroughly washed with distilled water before sowing. Each pot was homogenized with 2 seedlings by thinning. A Pb contaminated water @ 20, 40, 60, 80, 100 mg L^{-1} was prepared. The distilled water without any contamination was used as a control. Plants were exposed to treatments in three phases, (i) 65 days after sowing, (ii) 72 days after sowing, and (iii) 79 days after sowing. Plants were harvested 79 days after sowing and subjected to laboratory analyses for measuring different parameters.

Leaf gas exchange parameters and chlorophyll score

The leaf gas exchange parameters such as A, E, Gs, and Ci of the most expanded leaf in each treatment were determined by using a portable leaf gas exchange analyzer LCi T photosynthesis system (ADC BioScientific Ltd. UK) between 10:00-14:00 local time. The parameters were measured on day before harvesting the plants. The chlorophyll scores of the most expanded leaves were measured between 10:00-14:00 on the 79^{th} day of the experiment with a chlorophyll content meter (CCM-200, OPTI-SCIENCES).

Growth parameters

After harvesting, the plant was separated into the shoot, root, pods, and seeds to determine fresh and dry weights of each part of plants, number of matured pods, number of seeds per pod, and weight of 100 seeds. The samples of different parts plants harvested from each pot were dried at 70 °C for a period of 72 hours in a hot air oven.

Metal analysis

Allen et al. (1974) method was used for plants digestion, and amount of Pb in different plant's parts such as leaves and stem, roots, pods, and seeds were analyzed with an Atomic Absorption Spectrophotometer (PerkinElmer AAnalyst 200).

Determination of Pb factors for soybean

The bioaccumulation factor (BAF), bioconcentration factor (BCF), translocation factor (TF), and tolerance index (TI) were calculated by the methods proposed by Retamal-Salgado et al. (2017) using following formulae:

$$\mathrm{BAF} = \frac{\text{Pb conc.in aboveground tissue of the plant}}{\text{Pb conc.in soil}}$$

$$\mathrm{BCF} = \frac{\text{Pb conc.in the belowground tissue of the plant}}{\text{Pb conc.in soil}}$$

$$\mathrm{TF} = \frac{\text{Pb conc.in aboveground tissue of the plant}}{\text{Pb conc.in root}}$$

$$\mathrm{TI}\ (\%) = \frac{\text{Dry biomass of plant in Pb treatments}}{\text{Dry biomass of plant in control}} \times 100$$

Statistical analysis

The collected data was subjected to Statistix 8.1 (McGraw-Hill, 2008) to analyze the significance of experiment. One-way ANOVA was used to determine the difference in treatments, which was followed by the least significant difference (LSD) test at 5% ($p < 0.05$) to analyze the multiple comparisons among treatments.

Results

Pb stress on the growth of the plant

Subjecting soybeans to different concentrations of Pb led to a decrease in different growth attributes (Fig. 1). The Pb treatments resulted in a decrease in the number of pods, number of seeds, and 100 seed weight (Fig. 2). The most significant ($p < 0.05$) reduction in biomass of shoot, root, and pods was recorded at 100 mg L^{-1} Pb by 63.88 - 78.98% compared to control (Fig. 1). However, the reduction in dry biomass of shoot, root, and pod was not significantly different ($p > 0.05$) under 20 mg L^{-1} Pb treatment relative to control (Fig. 1B, D, F).

Pb stress on leaf gas exchange parameters

In our study, the Pb treatments significantly ($p < 0.05$) reduced the gaseous traits of soybean including A, Gs and E, thereby increased Ci (Fig. 3). The greater reduction in A (1.37 μmol mol $^{-1}$) by 58.55% was observed at 100 mg Pb L^{-1} treatment in comparison that the control treatment (Fig. 3A). However, the extent of the decrease in A under 20.0 mg Pb L^{-1} treatment was not significantly ($p > 0.05$) differed from the control treatment of this experiment (Fig. 3A). Additionally, 100 mg Pb L^{-1} treatment significantly ($p < 0.05$) reduced the stomatal conductance ($p < 0.05$) which reached 0.05 mol m^{-2}-s^{-1} compared to the control (0.17 mol m^{-2}-s^{-1}) (Fig. 3D).

Chlorophyll score

The effect of Pb on the chlorophyll score of plants is shown in (Fig. 4). The chlorophyll score was reduced with the increasing concentration of Pb treatments. The greatest reduction in chlorophyll score was (3.53) recorded at 100 mg Pb L^{-1} treatment which reduced by 65.13% compared to control (10.13). However, in this study, there was non-significant ($p > 0.05$) difference in the reduction in chlorophyll score exposed to 20 mg Pb L^{-1} treatment compared to the control.

Pb concentration in different tissues of the plant

Different plant tissues showed varied trends of Pb accumulation (Table 1). The maximum accumulation of Pb in plant tissues was in the following order root > stem > leaves > pods > seeds. Compared to the control, after 100 mg Pb L^{-1} treatment the greatest Pb content (1296.23 ppb) in root was recorded relative to the control. However, the extent of reduction of Pb content was non-significant ($p > 0.05$) under 20 mg Pb L^{-1} treatment relative to control.

Factors of Pb for soybean

The BAF, BCF, and TF were calculated in order to assess the ability of *Glycine max* to accumulate and extract Pb. The fig. 5 shows that the Pb accumulation was significantly ($p < 0.05$) influenced by the increasing levels of Pb treatments. Compared to control, the BAF value under 100 mg Pb L^{-1} treatment was significantly ($p < 0.05$) increased by 130.10% (Fig. 5A). Similarly, Pb treatments i.e., 20 and 40 mg Pb L^{-1} also enhanced the value of TF by 4.35 – 20.76% relative to control. However, reduction in the values of TF were recorded under 60 to 100 mg L^{-1} Pb treatments (Fig. 5B). Furthermore, the highest value of BCF (0.45 mg g^{-1}) was recorded in 100 mg Pb L^{-1} which increased by 133.74% compared to the control (Fig. 5C). *Glycine max* subjected to different levels of Pb treatments showed a significant effect on TI (Fig. 5D). Compared to the control, 20 mg Pb L^{-1} showed maximum tolerance index (TI %) (97.91%), while the minimum TI% (4.64%) was observed at 100 mg Pb L^{-1} treatment.

Correlation among different parameters

Pb stress negatively correlated (r ranged from -0.95 to -0.99) with A, E, Gs and chlorophyll scores (Table 2). However, a positive correlation was recorded between intracellular CO_2 concentration and Pb concentration.

Table 1 Effect of different treatment of lead on accumulation of Pb in different tissues of soybean

Treatments	Leaves (ppb)	Stem (ppb)	Roots (ppb)	Pods (ppb)	Seeds (ppb)
Control	157.97±4.71 f	175±4.47 f	162.23±8.94 f	90.06±8.58 f	2.04±0.22 f
20 mg Pb L^{-1}	179.33±2.34 e	205.23±4.05 e	182.5±8.80 e	111.9±7.08 e	2.68±0.11 e
40 mg Pb L^{-1}	224.63±4.20 d	289.26±1.86 d	211.06±10.16 d	146.83 ±8.50 d	4.27±0.24 d
60 mg Pb L^{-1}	282.63±9.49 c	435.23±3.16 c	374.83±1.04 c	224.2±10.55 c	5.61±0.18 c
80 mg Pb L^{-1}	357.4±1.83 b	601.2±8.72 b	508.2±4.15 b	363.87± 22.39 b	10.98±0.23 b
100 mg Pb L^{-1}	497.16±11.8 a	793.4±11.04 a	762.90±45.60 a	667.56±12.75 a	16.79±0.24 a

Values represent Mean of three replicates ± SD. Different letters with each value represent treatments significance at $p < 0.05$.

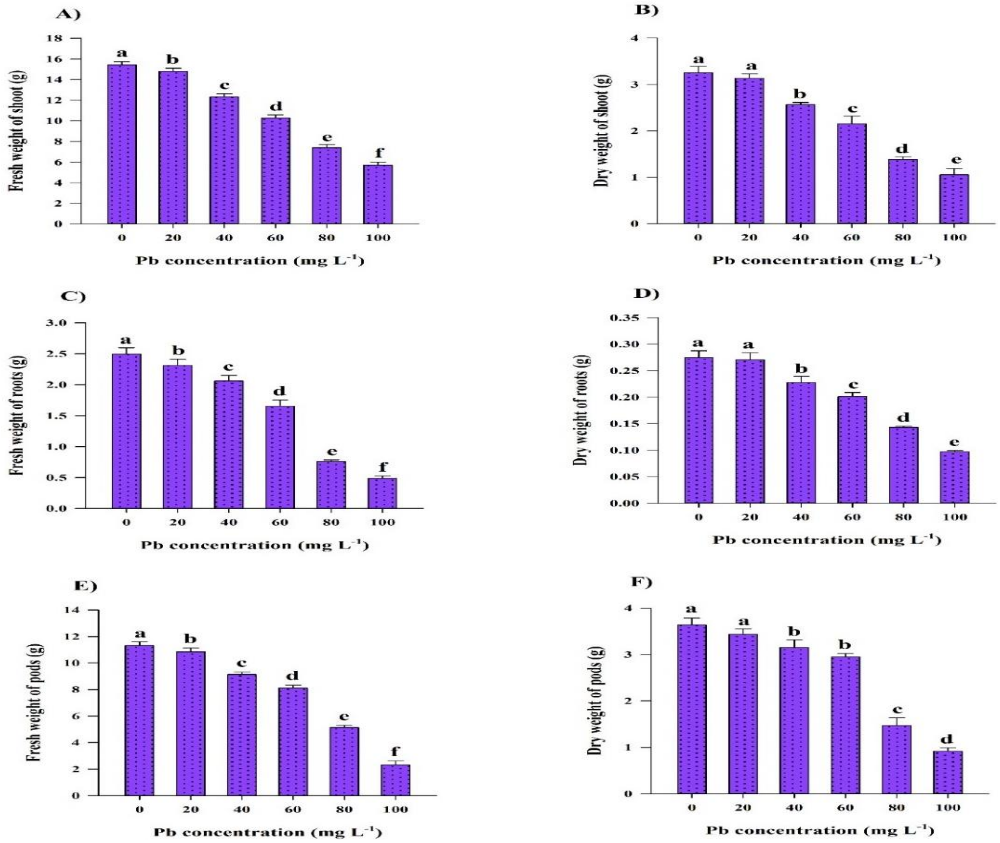

Fig. 1 Effect of Pb treatments on A) fresh weight of shoot, B) dry weight of shoot, C) fresh weight of root, D) dry weight of root, E) fresh weight of pods, and F) dry weight of pods of soybean. Bars represent mean of three replicates ± SD. Letters on each bar represent treatments significance at $p < 0.05$.

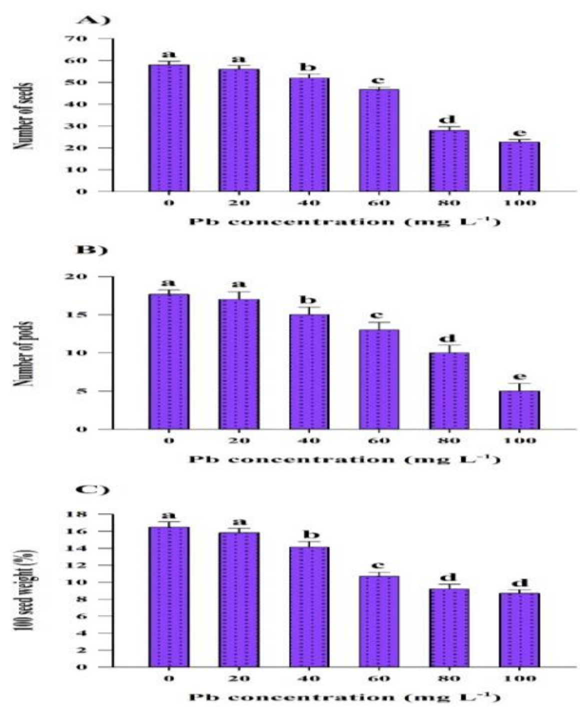

Fig. 2 Effect of Pb treatment on A) number of seeds, B) number of pods, and C) 100 seed weight of soybean. Bars represent Mean of three replicates ± SD. Letters on each bar represent treatment's significance at $p < 0.05$

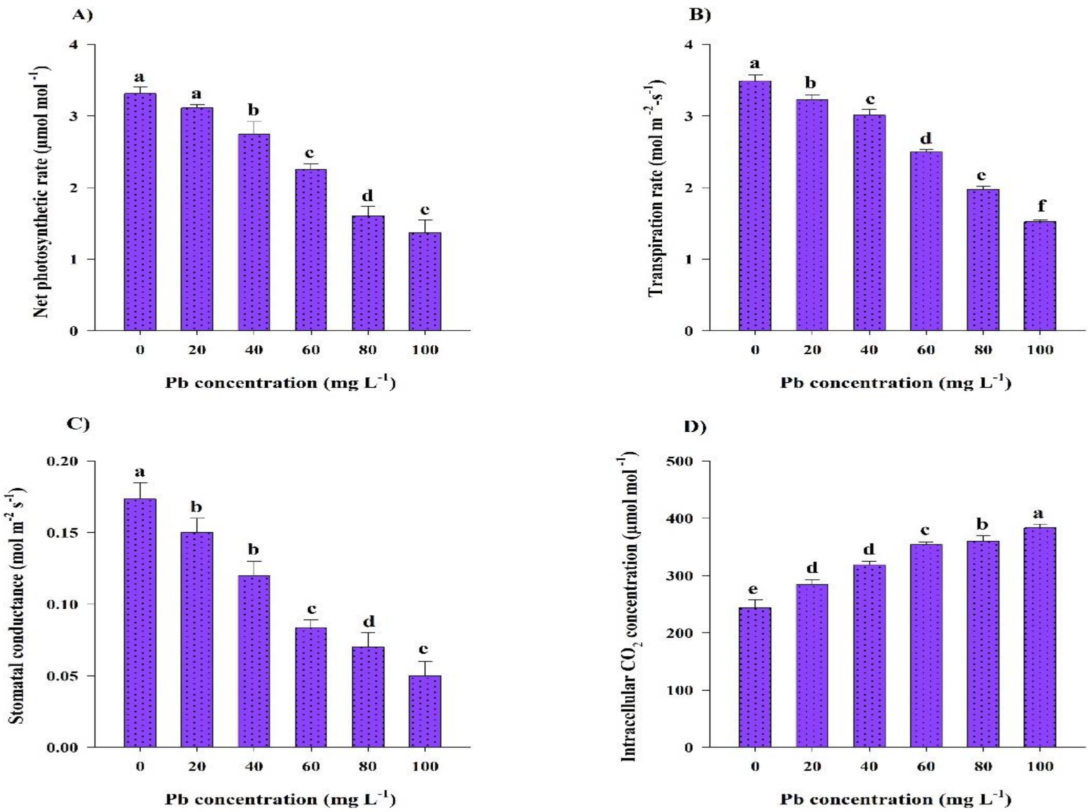

Fig. 3 Effect of Pb treatments on A) net photosynthetic rate, B) transpiration rate, C) intracellular CO_2 concentration, and D) stomatal conductance of soybean. Bars represent Mean of three replicates ± SD. Letters on each bar represent treatment's significance at $p < 0.05$.

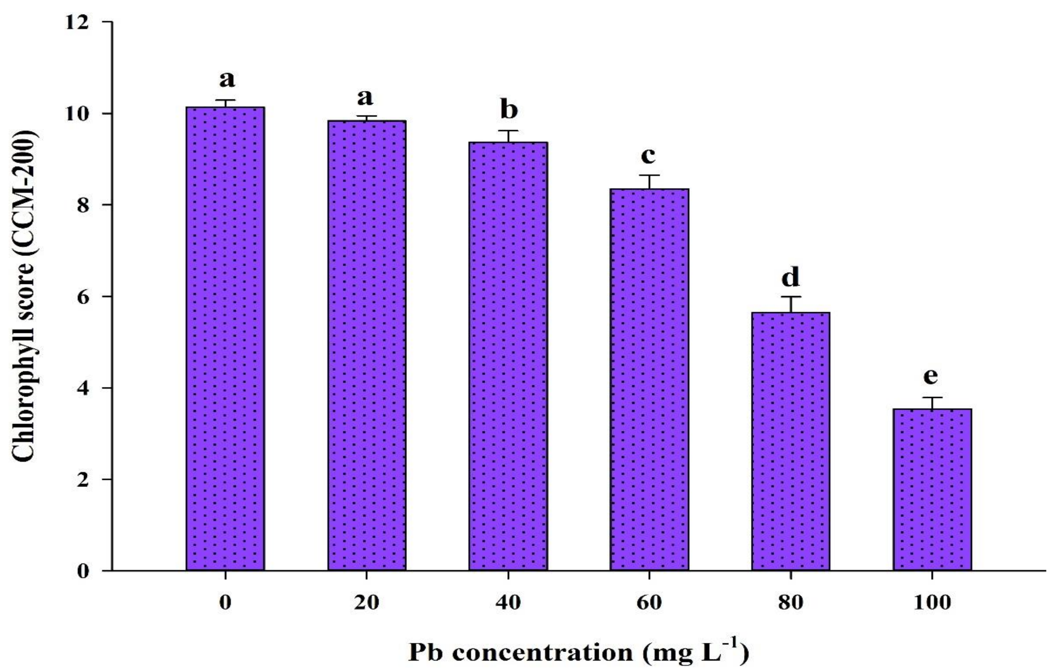

Fig. 4 Effect of Pb treatments on chlorophyll score of soybean plants. Bars represent Mean of three replicates ± SD. Letters on each bar represent treatment's significance at $p < 0.05$.

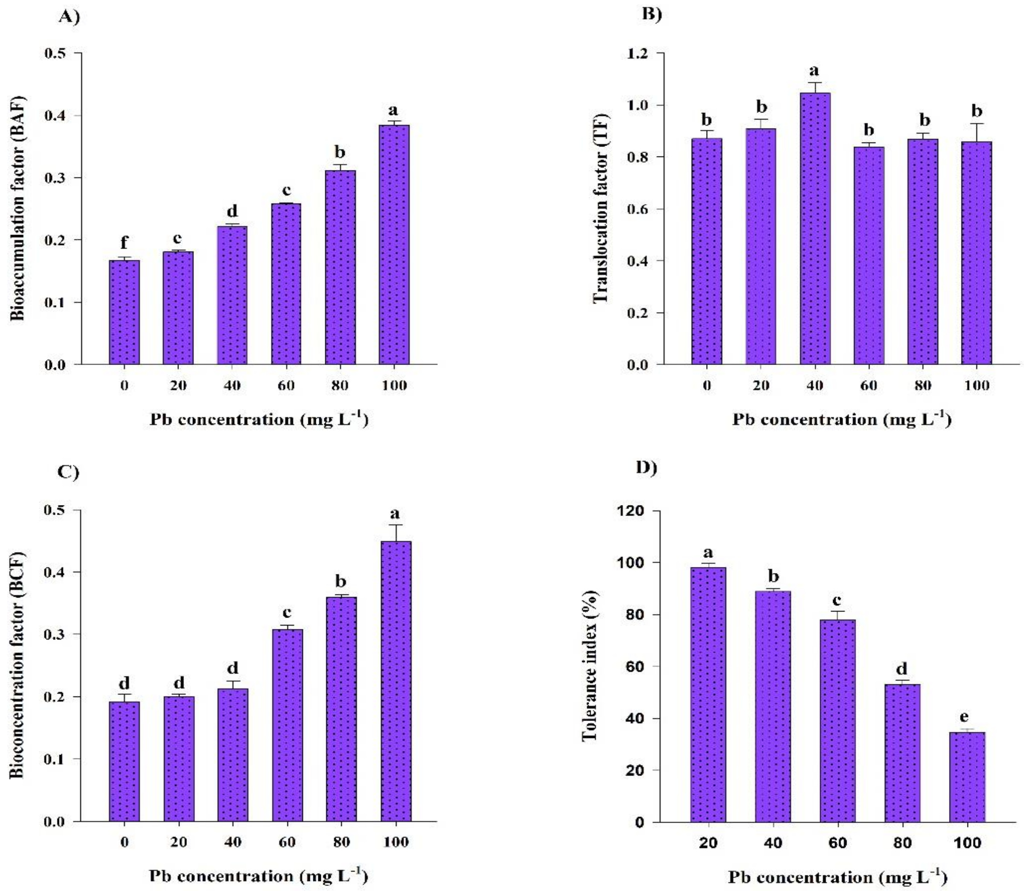

Fig. 5 Effect of Pb treatments on A) bioaccumulation factor (BAF), B) translocation factor (TF), C) bioconcentration factor (BCF), and D) tolerance index (TI) of Soybean. Bars represent mean of three replicates ± SD. Letters on each bar represent treatment's significance at $p < 0.05$

Table 2 Pearson`s correlation among Pb content and Physiological parameters of soybean

	Pb con.	A	E	Ci	Gs	Chl
Pb con.	1					
A	-0.95	1				
E	-0.98	0.99	1			
Ci	0.99	-0.98	-0.99	1		
Gs	-0.91	0.98	0.97	0.95	1	
Chl	-0.99	0.96	0.98	0.98	0.90	1

Pb con. = Lead concentration; A = Net photosynthetic rate; E = Transpiration rate; Ci = Intracellular CO_2 concentration; Gs = Stomatal conductance; Chl = Chlorophyll score

Discussion

Pb toxicity affects the growth and biomass of plants (Rani et al., 2024). Pb retards the tolerance level, and dry biomass of shoot (Gupta et al., 2024). In the current study, Pb reduced the fresh and dry biomass of plants significantly ($p < 0.05$) which is consistent with the result reported by Kulaz et al. (2021). The most significant reduction ($p < 0.05$) was recorded in the root dry biomass (74.52%) at 100 mg Pb L^{-1} treatment (Fig. 1) which might be due to Pb-induced disruption in the cell wall and spindle formation that results in a reduction of root volume (Wei et al., 2025). Additionally, a great reduction in the number of pods, seeds number, and 100 seed weight was recorded in the maximum concentration of 100 mg Pb L^{-1} treatment (Fig. 2). The reduced growth yield might be due to a decrease in photosynthesis. Pb hinders the growth of plants by disrupting the formation and levels of nutrients and modulating the structure of proteins, such as regulatory proteins and transporters (Pirzadah et al., 2020). Furthermore, the lowest Pb treatment (20 mg Pb L^{-1}) had the highest tolerance index. Pb-induced reduction in the growth of the plant might be linked with photosynthesis (Rahman et al., 2024).

Photosynthesis is essential for plants' survival under stressful environment (Qiao et al., 2024). However, elevated Pb absorption by plants reduced the photosynthetic rate, stomatal conductance, and chlorophyll content (Rasool et al., 2020). In the current study, the maximum decrease in net photosynthesis (56.25%) was observed from plants under 100 mg Pb L^{-1} treatment (Fig. 3A) which resulted in reduced transpiration rate, stomatal conductance, and ultimately increased intracellular CO_2 concentration (Fig. 3). This reduction in photosynthetic rate might be due to disruption in electron transport processes caused by Pb accumulation which results in a decrease in the energy efficiency of photosystem II (Rizvi et al., 2020; Kaur et al., 2014; Madhu et al., 2020). The reduced photosynthetic rate, and chlorophyll synthesis, lead to CO_2 deficiency which results in the closing of stomata (Khan et al., 2015). The efficiency of photosynthesis is determined by photosynthetic pigments (Khalofah & Farooq, 2023). The toxicity of Pb in plants results in a reduction in photosynthesis, uptake of nutrients, and water balance (Dogan et al., 2018). In the current study, the chlorophyll score was reduced by 3.5 to 63.75% with the increasing level of Pb from 20 to 100 mg Pb L^{-1} (Fig. 4). This might be explained by the fact that Pb toxicity hinders the growth irregularity in the functioning of plants by inhibiting guard cells, resilience of cell walls, and development of chlorophyll pigments, (Kurtyka et al., 2018).

Lead (Pb) acts as a substitute nutrient which is taken up by plants through xylem vessels mediated by specific proteins in divalent free cations (Sharma & Dubey, 2005; Engwa et al., 2019). In the current study, most of the Pb accumulated in the root, followed by stems, leaves, pods, and seeds as BAF value ranged from 0.21 to 0.43 (Fig. 5). This might be due to the reason that Pb is mainly translocated in the root due to Casparian stripe-induced blockage, thus root system accumulates a large portion of Pb than the aerial parts of the plant (Dogan et al., 2018; Kiran & Parsas, 2017). The plants that retain most of the Pb in the root networks are classified as excluders whereas hyperaccumulator plants accumulate an abundant level of Pb in the shoot without affecting the metabolism of the plant (Collin et al., 2022). Transfer factor is different for species based on genotypes and physiochemical properties of soil (Rahman et al., 2024). In the current study, BAF and BCF were found to be <1, which indicates the non-hyperaccumulating ability of plants. This increase in BAF and BCF might be due to a greater accumulation of Pb as a substitute nutrient through the xylem from the soil. Greater TF under 20 – 40 mg Pb L^{-1} treatments indicates that most of the Pb accumulated in the shoot than aerial part of the plant, however after achieving the threshold limit at 40 mg Pb L^{-1} treatment, TF non-significantly ($p > 0.05$) decreased relative to the control. This might be due to the reason that the root system of soybeans could be damaged under heavy Pb contamination which causes excessive storage of Pb contents in the root system.

Conclusion

The current study indicates that increasing Pb concentration resulted in a decrease in the growth and physiological parameters of the plant. The Pb (100 mg L^{-1}) had a maximum bio-accumulation factor (BAF), bioconcentration factor (BCF), and low translocation factor (TF) indicating the Phyto stabilization efficiency of the plant. Maximum Pb level had a low tolerance index (TI) indicating the weak tolerance of *Glycine max,* which was consistent with reduced photosynthetic pigments, photosynthetic rate, stomatal conductance, and transpiration rate. It was concluded that the *Glycine max* L. Merrill has a low tolerance against Pb toxicity and may pose a health risk to humans because seeds of *Glycine max* were found to accumulate considerable levels of Pb.

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