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Elucidation of the Impact of Lead Chloride on Growth Characteristics and Chlorophyll Content in Two Varieties of Sunflower (*Helianthus annuus* L.)

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Abstract. Lead (Pb) is one of the common environmental stressors around the globe. To assess the effects of lead chloride on growth and physiological attributes of two varieties (Hysun-33 and FH-701) of sunflower (*Helianthus annuus* L.), a pot experiment was laid down with different levels of lead chloride. Pb (50, 100 or 150 ppm $PbCl_2$/Kg of soil) fed to soil significantly affected the growth traits and photosynthetic pigments as compared to control (0 ppm) in both the varieties. In present findings, exposure of high Pb levels induced reduction in biomass (fresh and dry weights), root and shoot length and leaf traits (leaves per plant, leaf area, leaf lengths and leaf width) with the maximum readings found in control. The plant biomass decreased non-linearly in plants with higher doses of Pb. Root and shoot lengths declined up to 36.2 and 30.34% in Hysun-33, respectively. However, leaf length, leaf width, leaf area and leaves per plant reduced from 1.5 to 33%, 3.8 to 22.1%, 5.2 to 48.2% and 17.5 to 45.6% in Hysun-33, while 5.8 to 20.5%, 6.8 to 28.8%, 12.3 to 43.5% and 15.5 to 40% in FH-701 respectively. All photosynthetic (main and accessory) pigments showed a reduction proportional to the increased Pb levels. The maximum drop in chlorophyll a (24.1%), chlorophyll b (42.07%), total chlorophyll (28.26%) and carotenoids (15.82%) was observed in FH-701 at highest Pb levels. The phytotoxicity of Pb on sunflower was dependent on varietal potential of sunflower and Pb concentration.

Keywords: carotenoids, chlorophyll, impact, lead, morphological, sunflower cultivars

Introduction

Salt stress and heavy metal contamination are one of the growing ecological concerns to earth's ecosystems since the past decade and is rising due to anthropogenic activities such as automobiles, use of fertilizers, mining, urbanization and fast industrialization (Toscano *et al*., 2023; Saini and Dhania, 2020). Cash and food crops are affected to varying degrees by abiotic stresses such as salt stress, temperature, drought and heavy metal adulteration. Salt and heavy metal stresses are closely linked to each other. Sometimes, salts of different heavy metals such as lead (Pb), chromium (Cr) or cadmium (Cd) act as their chief sources which reduce the plant production and inflict detrimental impacts on health of living beings (Liu *et al*., 2018). The potential for bioconcentration of trace metals and their salts in the food chain is one of the most pressing health and ecological concerns of modern era. The heavy metals can move from industries to soil and then enter the food chain though uptake by plants and influence animals and humans (Zaynab *et al*., 2022). Heavy metal stress causes a decline in crop production, stem length, root length, dry weight, chlorophyll synthesis, soil quality and food quality (Hussain *et al*., 2021; Kotecha *et al*., 2019). Plants need energy from photosynthesis but metal ions disrupt the structure of photosynthetic pigments, leading to a decrease in quantum efficiency and loss of light energy (Maes *et al*., 2022; Cabrita *et al*., 2018).

Lead chloride ($PbCl_2$) is one of the sparingly water-soluble salt of Pb, which occur naturally in shape of the mineral cotunnite. Pb is a non-essential hazardous heavy metal which is very persistent in nature. It is a common pollutant in agricultural soils and produces negative impacts on plant growth, metabolism and development, especially by affecting photosynthesis and reducing the levels of chlorophyll pigments in the plant tissues (Tan

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et al., 2022). The main sources of Pb contamination in the environment are anthropogenic, which include burning of fossil fuel, fertilizers utilization, Pb batteries, electroplating and smelting (Engwa *et al.*, 2019; Gottesfeld *et al.*, 2018). Lead chloride can negatively affect plant growth and development by disturbing physiological and biochemical processes in plants. Pb is reported to disrupt antioxidant enzymes production in plants (Zulfiqar *et al.*, 2019) and inhibits various enzymes vital for chlorophyll biosynthesis (Souahi, 2021). Moreover, Pb restricts seedling growth, germination rates (Anwar *et al.*, 2022) and cell elongation (Zheng *et al.*, 2021). It causes alterations in membrane permeability and hormonal levels, which can lead to water imbalance, altered mineral nutrition and other physiological disturbances.

Photosynthesis serves as a foundational process in biological systems, providing the primary energy source for the entire trophic chain. Chlorophyll pigments play a vital role in photosynthesis. Changes in pigment levels can have cascading effects on plant growth, development and their responses to stresses. Therefore, changes in chlorophyll pigments can be used as indicators of plant stress caused by various environmental factors, including heavy metal toxicity. Higher concentrations of Pb limit chlorophyll synthesis because of excessive absorption hinders the uptake of magnesium and iron by plants. It can hinder the supply of carbon dioxide by interfering with the stomatal opening and closing mechanism and damages cell membranes, further impeding CO_2 uptake (Rasool *et al.*, 2020; Huang *et al.*, 2019). Pb poisoning harms photosynthesis by decreasing the photosynthetic apparatus's affinity for protein S- and N-ligands (Winska-Krysiak *et al.*, 2015). It may adversely alter mitochondrial structure, leading to a lower number of mitochondrial cristae and hence lowering oxidative phosphorylation (Gassowska *et al.*, 2016; Malecka *et al.*, 2001).

Sunflower (*Helianthus annuus* L.) is a deep-rooted, quick-growing oil seed crop with a high biomass production. Sunflower seeds are not only used in oil production but also used for medicinal, feedstock, fodder, dyes for textile industry and for ornamental use. Sunflower can tolerate a wide range of stresses, however, their yield drops when grown on marginal soils (Skoric, 2009). Toxic heavy metals may accumulate in sunflower roots and can be transported to the leaves or edible parts such as flowers and seeds (Qamar *et al.*, 2020). Sunflower exhibits a wide range of genetic diversity in its various cultivars and varieties. These genetic differences can result in variations in the plant tolerance to environmental stressors such as heavy metal toxicity and some varieties may show better adaptability to heavy metal stress than others. Varietal differences in heavy metal tolerance have been documented in numerous studies, with some cultivars showing higher resistance and better adaptability to heavy metal toxicity than others (Tan *et al.*, 2023; Farid *et al.*, 2017). The aim of this study was to evaluate the effect of lead chloride on growth and physiological traits of sunflower and to assess variation of Pb tolerance among two varieties of sunflower (*Helianthus annuus* L.).

Material and Method

Plant cultivation, treatments and growth conditions. Healthy seeds of two varieties, Hysun-33 (V1) and FH-701 (V2) of sunflower (*Helianthus annuus* L.) were acquired each from local grain market and Ayub Agricultural Research Institute, Faisalabad respectively. The seed purity was ensured through physical inspection, where debris and weed seeds were removed. Additionally, germination and wet sieving tests were conducted, affirming a robust seed viability. The seeds were first soaked for five minutes in a 0.1% $HgCl_2$ (w/v) solution before being given a 20 min soak with distilled water for surface sterilization. This process effectively reduced the risk of fungal or bacterial infections during germination and seedling growth. Selected healthy seeds were sown in plastic cups with washed mixture of sand and cocopeat irrigated with half-strength hoagland solution. Four to six selected uniform sized seedlings at four-leaf stage were then transplanted to properly labelled equal sized pots each with 7 Kg of soil after five days of germination. The seedlings then underwent a triple rinse process, including a rinse with regular tap water, followed by two rinses with distilled water. Four treatments of lead chloride ($PbCl_2$) at three levels (50, 100, 150 ppm) and a control (0 ppm) were applied 3 days after transplantation into larger pots. Four combinations of treatments of Pb were designated as T1, T2, T3 and T0. This experiment employed a 2×4 factorial design for research. Tap water was used to irrigate the plants, which was characterized by an electrical conductivity of 900.6 μS/cm, calcium and magnesium ions of 6.03 meq/L, sodium concentration of 4.2 meq/L, bicarbonate content of 6 meq/L, and chloride concentration of 1.6 meq/L. The plants were cultivated in a semi-controlled greenhouse maintaining a temperature of 21 ± 3 °C with a daily light-dark cycle of 14 h of light and 10 h of darkness. Fresh plant samples were collected on maturity by carefully uprooting from soil avoiding any damage and sectioned into root, stems and leaves. Collected plant samples were rinsed with distilled water, blotted with tissue paper and placed in plastic bags, labelled and brought to the laboratory.

Determination of morphological parameters. Morphological or growth traits such as fresh and dry weights of shoot and root, shoot and root lengths and leaf features such as number of leaves per plant, leaf width, length and area were measured by randomly selecting three plant samples per treatment and tagging them for analysis after harvesting. Fresh weights of tagged roots and shoots were estimated immediately after uprooting the plants at harvest using electric balance. Dry weights of same roots and shoots were calculated with the help of electric balance after oven drying at 65 °C for three days. The root length was measured from the stem base to the root tip by using a measuring scale and shoot lengths were estimated by the aid of measuring scale from base to top. Leaf number in one plant was manually counted. Leaf lengths and widths for tagged plants were measured by using a measuring scale. The area of leaf was measured according to Shi *et al*. (2019) for triangular leaves.

Leaf area (cm^2) = 1/2 × Leaf length × Leaf width

Quantification of photosynthetic pigments. The amount of chlorophylls (Chl) and carotenoids (Car) was estimated according to Arnon (1949) and Davies (1976). About 0.5 g of fresh leaf tissue was sliced into 0.6 cm pieces, ground with pestle and mortar and submerged in 5 mL of acetone (80%) at 10 °C overnight under dark conditions in refrigerator. Samples were centrifuged at 14000 rpm for 5 min and supernatant was gathered in new tube. Supernatant absorbance readings were determined in UV-Vis spectrophotometer, Model (Hitachi-220 Japan) at 645, 663 and 480 nm. Estimation of chlorophylls a and b, total chlorophylls and carotenoids in mg/10 g fresh weight were done by using the formulae:

$$\text{Chl a (mg/10 g)} = [12.7(OD_{663}) - 2.69(OD_{645})] \times 1/10 \times V/1000 \times L.W$$

$$\text{Chl b (mg/10 g)} = [22.9(OD_{645}) - 4.68(OD_{663})] \times 1/10 \times V/1000 \times L.W$$

$$\text{Chl t (mg/10 g)} = [20.2(OD_{645}) + 8.02(OD_{663})] \times 1/10 \times V/1000 \times L.W$$

$$\text{Carotenoids (mg/10 g)} = [OD_{480} + 0.114(OD_{663}) - 0.638(OD_{645})/2500] \times 1/10 \times 100$$

where:

V was volume of used acetone and L.W was leaf weight.

Statistical analysis. Data was analyzed by performing a two-way analysis of variance (ANOVA) and post-hoc Tukey test for comparing the means using Minitab 20 and SPSS 25. Means of data were estimated by using triplicate measurements with the help of MS Excel 2016. Each data was represented as mean ± standard deviation.

Results and Discussion

Morphological attributes. Growth impediment can serve as a crucial indicator for metal-induced stress tolerance in plants. Pb is non-essential metal for plant growth and the effects of its exposure on growth can vary depending on factors such as plant species, cultivars, organs and metabolic processes (Khan *et al*., 2021). Excess lead chloride levels in soil negatively affected growth indices as compared to control plants in both the varieties (Table 1). This declining trend may be due to fact that heavy metal becomes highly toxic to plant at higher levels (Alengebawy *et al*., 2021).

Biomass production has been used as an estimate for tolerance of crops to soils that have been supplemented with metals. In current study, the biomass of sunflower plants under elevated lead chloride levels did not vary significantly at lower doses but significant drop was observed in plants with higher concentrations of Pb. Sunflower varieties grown at various concentrations of lead chloride exhibited inhibition of fresh shoot weights with the exception of lower Pb concentrations whereas decrease in root fresh weight was significant in V2 (FH-701). Elevated Pb concentrations reduced dry root weight of both varieties and dry shoot weight of FH-701 but its lower levels had no considerable effects on dry shoot weights up to 150 ppm in V1 (Table 1). Both varieties varied in terms of root and shoot dry mass which is due to the unique threshold levels for different varieties of sunflower (Rizwan *et al*., 2016).

Fresh root weights exhibited a reduction of up to 19.53% in V2 under the influence of T3 (150 ppm) lead stress, while fresh shoot weights experienced a 4.66% decrease. In contrast, V1 displayed greater resistance to lead stress at lower concentrations, manifesting in a notable increase in fresh root weight. However, a simultaneous decline of up to 19.68% in fresh shoot weight was observed for V1 under T3 (150 ppm) conditions. The increase in fresh root weight for V1 could be attributed to phyto-remediation ability of some sunflower varieties (Kumar *et al*., 2020). Reduction in root dry mass was assessed to fall up to 51.88 and 27.5% in V1 and V2, respectively when compared to the respective controls. Similarly, the shoot dry mass decreased as compared to the control, falling up to 9.44% in V1 and 3.7% in V2, in agreement with the work presented by Saleem *et al*. (2018). Inhibition of fresh and dry mass under Pb stress was also reported in rice (Akhtar *et al*., 2017) and in canola (Kanwal *et al*., 2014) which slightly differed from our results as sunflower showed slight resistance in reduction of fresh and dry biomass at lower levels of Pb stress. Lower external Pb levels restrict its translocation from the root to the leaves but high Pb concentrations disrupt the endodermis barrier in plants leading to changes in biomass (Pourrut *et al*., 2011).

The shoot and root length are often taken as an indicator of heavy metal toxicity in plants (Sen *et al*., 2013). The imposition of increasing levels of Pb stress extensively suppressed root and shoot lengths in both varieties of sunflower but magnitude of reduction was concentration dependent and varied from variety to variety (Table 1). The maximum shoot and root length was determined in control (T1), while minimum was noted in T3 for both varieties. In present findings, root lengths declined from 3 to 36.2% and 6.3 to 30.9% for V1 and V2 under increasing Pb levels, respectively. This showed that V2 was less affected by Pb levels in T3 (150 ppm) as compared to V1. Similarly, shoot lengths decreased from 4.7 to 30.3% and 4.4 to 16.7% for V1 and V2, respectively showing V2 to be less affected by increasing Pb concentrations. These results are in agreement with the investigations done by Altaf *et al*. (2021). The decrease in root and shoot length under Pb stress may be attributed to the inhibition in cell elongation (Kozhevnikova *et al*., 2009) or to reduced mitosis (Kaur *et al*., 2014). Similar changes in root and shoot lengths under Pb toxicity were documented by Fahr *et al*. (2013).

Leaves are termed as food making factory of plant. Gradual increase in lead chloride levels significantly decreased all leaf characteristics such as leaf number per plant, leaf lengths, leaf widths and leaf area of both

Table 1. Means of growth attributes in two varieties of sunflower (*Helianthus annuus* L.) with different levels of Pb

Parameters	Varieties	Lead chloride levels (ppm)			
		T0	T1	T2	T3
Fresh root weight (g)	V1	1.89±0.08a	2.02±0.02a	1.71±0.05b	1.64±0.01b
	V2	1.28±0.05a	1.15±0.03a	1.1±0b	1.03±0.05b
Fresh shoot weight (g)	V1	12.4±0.04a	13.2±0.26ab	10.66±0.32bc	9.96±0.09c
	V2	11.16±0.13a	10.76±0.04ab	10.7±0.02bc	10.64±0.06c
Dry root weight (g)	V1	1.06±0.01a	0.6±0.03b	0.65±0.03b	0.51±0.01b
	V2	0.41±0.04a	0.36±0.02b	0.33±0b	0.29±0.02b
Dry shoot weight (g)	V1	2.33±0.03a	2.44±0.04a	2.47±0.04a	2.11±0.03b
	V2	1.09±0.05a	1.07±0.03a	1.12±0a	1.05±0.01b
Root length (cm)	V1	13.85±0.12a	13.43±0.22a	11.33±0.17b	8.84±0.09c
	V2	12.53±0.26a	11.74±0.17a	10.9±0.2b	8.66±0.13c
Shoot length (cm)	V1	67.43±0.97a	64.29±1.01a	58.81±1.05b	46.97±0.9c
	V2	59.03±0.95a	56.46±0.87a	53.37±0.78b	49.19±0.44c
Number of leaves per plant	V1	19±1a	15.67±0.58b	13±0c	10.33±1.15d
	V2	15±1a	12.67±0.58b	10.67±0.58c	9±1.73d
Leaf length (cm)	V1	8.01±0.11a	7.89±0.06a	6.19±0.19b	5.33±0.12b
	V2	5.02±0.09a	4.73±0.04a	4.6±0.04b	3.99±0.14b
Leaf width (cm)	V1	5.3±0.14a	5.1±0.06b	4.7±0.06c	4.13±0.12d
	V2	3.99±0.09a	3.72±0.1b	3.56±0.03c	2.84±0.17d
Leaf area (cm^2)	V1	21.23±0.61a	20.13±0.09a	14.57±0.61b	11±0.31c
	V2	10.02±0.05a	8.79±0.3a	8.19±0.13b	5.66±0.2c

Each reading reflects the means±SD of three replications; non-shared letters are significantly different at P≤0.05.

varieties of sunflower (Table 1). This in turn caused hinderance in overall plant growth but the magnitude of this reduction varied in both varieties due to physiological differences. However, leaf length, leaf width, leaf area and number of leaves per plant reduced from 1.5 to 33%, 3.8 to 22.1%, 5.2 to 48.2% and 17.5 to 45.6% in V1, while 5.8 to 20.5%, 6.8 to 28.8%, 12.3 to 43.5% and 15.5 to 40% in V2 respectively. The maximum growth of leaves (Leaf length, leaf width, leaf area and number of leaves per plant) was noticed in control (0 ppm). The stunting of leaf growth by increasing levels of Pb was also observed by many earlier researchers in castorbeans (Zeng *et al*., 2021), cotton (Bharwana *et al*., 2014) and wheat (Parveen *et al*., 2022).

Physiological traits. The total chlorophyll content has been shown to be one of the important indicators to assess the tolerance of plants to heavy metals. Lead chloride treatments significantly reduced the photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) in both varieties of sunflower in concentration dependent pattern (Table 2). Chlorosis and leaf curling was clearly visible in plants with higher levels of Pb (150 ppm). The highest concentration of chlorophyll a and b were found in control treatments (T0) in both varieties and minimum concentration was found in plants treated with 150 ppm in both varieties respectively. Similarly, carotenoids exhibited a similar trend, showing their highest concentration in the control (0 ppm) and gradually decreasing along the concentration gradient, although this decline was comparatively milder at lower doses of Pb when compared to other parameters. The maximum total chlorophyll was found in control treatments (T0) in both varieties and minimum concentration was found at plants treated with 150 ppm in both varieties, respectively.

Results showed reduction in chlorophyll a (9.48-13.39%), chlorophyll b (36.5-42.45%), total chlorophyll (18.39-22.97%) and carotenoids (0.12-12.73%) under lead stress in V1 (Hysun-33), while chlorophyll a (2.08-24.1%), chlorophyll b (5.53-42.07%), total chlorophyll (2.91-28.26%) and carotenoids (0.83-15.82%) in V2 (FH-701) under Pb stress. These reductions were concentration and varietal dependent. However, carotenoid and chlorophyll content diminished significantly more at a higher Pb level (T3). Small irregular spike at 50 ppm for chlorophyll b in V2 was an exception in trend which could be due to genetically induced tolerance. The reduction in photosynthetic pigments by gradual rising of Pb concentration is also reported in earlier investigations by various researchers (Al-Jobori and Kadhim, 2019; Afaj *et al*., 2017) which corroborates with the present study on chlorophyll and carotenoid contents. Similar findings were revealed by Altaf *et al*. (2021) who reported decrease in a and b up to 44% and 48% under Pb stress respectively. Lead-induced reductions in leaf pigments may be attributed to the inhibition of chlorophyll synthesis, primarily due to the reduced uptake of magnesium (Mg). Pb also affects the structure, and physiology of photosynthetic apparatus and induces chlorophyll degradation by enhancing chlorophyllase activity and reduced uptake of magnesium (Baryla *et al*., 2001; Stefanov *et al*., 1995).

Conclusion

The growth and photosynthetic pigments decreased with rising levels of lead chloride in both varieties of

Table 2. Means of various photosynthetic pigments including chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in two varieties of sunflower (*Helianthus annuus* L.) with different levels of Pb

Parameters	Varieties	Lead chloride levels (ppm)			
		T0	T1	T2	T3
Chlorophyll a (mg/10 g f.w)	V1	17.1±0.15a	15.48±0.07ab	15.37±0.08b	14.81±0.16c
	V2	18.8±0.2a	18.41±0.14ab	16.38±1b	14.27±0.18c
Chlorophyll b (mg/10 g f.w)	V1	8.41±0.32a	5.34±0.19b	5.12±0.06b	4.84±0.13c
	V2	5.61±0.12a	5.3±0.06b	5.39±0.04b	3.25±0.16c
Total chlorophyll (mg/10 g f.w)	V1	25.51±0.44a	20.82±0.13b	20.49±0.08b	19.66±0.26c
	V2	24.4±0.06a	23.68±0.04b	21.81±0.04b	17.53±0.1c
Carotenoids (mg/10 g f.w)	V1	8.25±0.09a	8.24±0.07a	7.42±0.12b	7.2±0.07c
	V2	7.27±0.04a	7.21±0.03a	6.31±0.1b	6.12±0.05c

Each reading reflects the means±SD of three replications. Non-shared letters are significantly different at $P \leq 0.05$

sunflower. The highest Pb level (150 ppm) exhibited greatest decline in morphology and growth of sunflower while lower levels were tolerated by sunflower varieties. The measure of phytotoxicity of Pb and their tolerance was dependent on varietal potential of sunflower varieties. Although we didn't pinpoint a specific tolerance threshold in this study, these findings suggest that FH-701 (V2) can endure higher lead levels while Hysun-33 (V1) can display more resistance to lead stress at lower concentrations. Future research endeavors should aim to determine precise tolerance thresholds for these sunflower varieties and explore sustainable crop management strategies.

Conflict of interest. The author declare that they have no conflict of interest.

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