Effect of Lead stress on morphology and chlorophyll content of chickpea seedlings

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Abstract

Heavy metal contamination in soil, particularly lead (Pb), is a growing environmental issue worldwide including India. Pb, a toxic metal with no biological function, is known to adversely affect both plant and animal life by disrupting cellular functions and causing organ damage. Pb toxicity in plants results in reduced growth, metabolic disturbances and compromised photosynthetic activity.

In this study, morphological, biochemical and physiological changes in chickpea seedlings have been studied under varying concentrations of Pb. Chickpea seedlings showed a negative response to $Pb(NO_3)_2$ on morphological parameters as well as biochemical components. This indicates that lead is a toxic element for plant growth and development. Lead had a substantial impact on the chlorophyll a, b and total chlorophyll content.

Keywords: Abiotic stress, heavy metal stress, lead nitrate [$Pb(NO_3)_2$], chlorophyll, fresh weight, dry weight.

Introduction

Heavy metal contamination in soil, particularly lead (Pb), is a growing environmental issue worldwide including India. Pb, a toxic metal with no biological function, accumulates in the environment due to industrialization and anthropogenic activities. It is known to adversely affect both plant and animal life by disrupting cellular functions and causing organ damage. Pb toxicity in plants results in reduced growth, metabolic disturbances and compromised photosynthetic activity. Heavy metals in biological systems have been found to affect cellular organelles and components including cell membranes, mitochondria, lysosomes, endoplasmic reticulum and nuclei, as well as some enzymes involved in metabolism, detoxification and damage repair[6].

DNA damage and conformational changes leading to cell cycle modulation, carcinogenesis, or apoptosis have been caused due to metal ions interacting with cellular components such as DNA and nuclear proteins[1]. In the lignin cellulose biomass samples (Mbwazirume and Nakyinyika peels) acquired from bananas, the presence of lead was reported[7]. Lichens were used as bioindicators to determine the different isotopes of Pb since decades[3]. Pb-affected soils contain 400–800 mg kg^{-1} of lead whereas in industrialized areas, it may extend up to 1000 mg kg^{-1} of soil. The negative effects of Pb have been described on the growth and expansion of plants[4].

Material and Methods

Seed Germination: Chickpea seeds were grown hydroponically in nutrient solution containing varying concentrations of lead nitrate $Pb(NO_3)_2$ ranging from 0 to 100 µM in a plant growth chamber at 28°C and 80% relative humidity for 10 days to assess the effects on root, stem and leaf growth.

Lead treatment: Seedlings were treated with different concentrations of $Pb(NO_3)_2$. They were grown in separate dishes filled with nutrient solution DW, 0 µm, 25 µm, 50 µm, 75 µm and 100 µm of $Pb(NO_3)_2$.

Measurement of length: Seedlings were harvested on the 10th day for measurement of length. Leaves, roots and shoots were collected separately. Their length was then measured in centimeters with the help of a scale.

Determination of fresh weight: 10th-day-old seedlings were harvested for determination of fresh weight. Seedlings were blotted dry and their fresh weights were measured.

Determination of dry weight: After determining the fresh weight, these seedlings were incubated for 72 hours at 65 degrees centigrade and weights were measured.

Extraction of chlorophyll: One gram of fresh leaves was collected and ground with 20–40 ml of 80% acetone. The mixture was centrifuged at 5000–10000 rpm for 5 mins. This procedure was repeated with the supernatant till the residue becomes colorless. The absorbance of the solution was recorded at 645 nm and 663 nm against the solvent (acetone) blank.

Estimation of Chlorophyll Content: The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Total Chlorophyll: 20.2(A645) + 8.02(A663)
Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)

Results and Discussion

Morphological studies: Measurements of roots, stems and leaves of the chickpea seedlings were taken at different concentrations of $Pb(NO_3)_2$ on the 10th day which are presented in table 1 and fig. 1. The observation was that the length of the leaf, root and stem of gram seedlings decreased

with increased concentration of $Pb(NO_3)_2$ compared to control plants. There is about 13.7%, 34.4% and 22.8% decrease in root length at 25µm, 50µm and 100 µm respectively. Stem length was decreased by about 12.34% and 20.14% at 25 µm and 100µm $Pb(NO_3)_2$ respectively. There is also a significant decrease in leaf length with a significant increase in the concentration of $Pb(NO_3)_2$. This result is similar to the earlier findings of Jaishankar et al[4].

Effect of lead on fresh weight and dry weight: Table 2 and fig. 2 demonstrate the dry weight and fresh weight in chickpea seedlings. It was observed that there is a decline in fresh weight at higher concentrations of lead, while dry weight increased significantly as compared to the control. It is in accordance with similar reports of other heavy metal exposure also.

Effect of lead on chlorophyll pigment: Total chlorophyll was found to be decreasing as the concentration of $Pb(NO_3)_2$ increases in the samples. Treatment at 25 µm and 100 µm recorded a significant decline of 49.53% and 49.62% respectively. In chlorophyll. Chlorophyll a and b also declined at 100µm conc. of $Pb(NO_3)_2$ in comparison to the control. The results were in accordance with the previous studies. Chlorophyll b is more adversely affected as compared to chlorophyll a by Pb toxicity[5]. Contamination of plants with lead increased the activity of chlorophyllase, which led to chlorophyll breakdown[2].

Conclusion

The present result allows us to conclude that chickpea seedlings showed a negative response to $Pb(NO_3)_2$ on morphological parameters as well as biochemical components. This indicates that lead is a toxic element for plant growth and development. Lead had a substantial impact on the chlorophyll. Uptake of lead is not only toxic, but it also affects the uptake of other essential elements which may cause a deficit of other nutrients in plants.

Table 1
Effect of lead on length of root, stem and leaves.

Treatment	Primary root	Stem	Leaves
0µm	6.13cm±3.302 100%	14.72 cm±1.23 100%	0.63 cm±0.160 100%
25 µm	5.29 cm±3.045 86.29%	12.90 cm±1.86 12.34%	0.54 cm±0.155 85.71%
50 µm	4.02 cm±2.035 65.6%	12.56 cm±0.87 14.66%	0.54 cm±0.196 85.71%
75 µm	4.24 cm±2.433 69.2%	10.79 cm±1.20 26.65%	0.45 cm±0.133 71.43%
100 µm	4.73 cm±2.776 77.2%	11.75 cm±4.08 20.14%	0.52 cm±0.251 82.54%

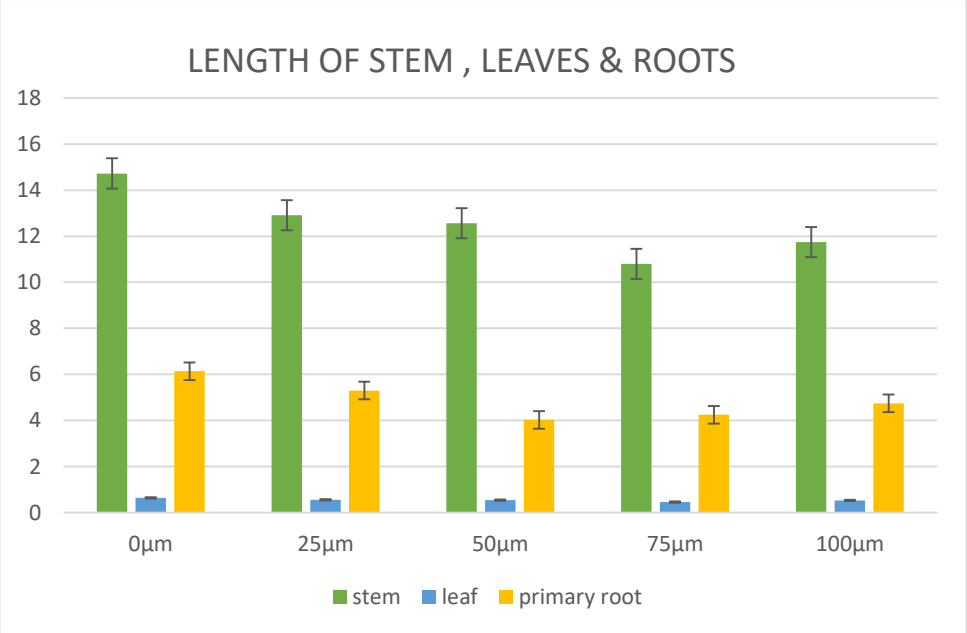

Fig. 1: Effect of lead on the length of root, stem and leaves

Table 2
Lead effect on fresh wt. and dry wt.

Conc.	Fresh weight	Dry weight
0µm	9.00 ± 2.9 100%	1.37 ± 0.10 100%
25 µm	7.81 ± 2.31 86.78%	1.39 ± 0.08 101.46%
50 µm	7.48 ± 2.36 83.11%	1.33 ± 0.05 97.08%
75 µm	7.26 ± 2.24 80.67%	1.33 ±0.07 97.08%
100 µm	8.82 ± 4.14 98.00%	1.45 ± 0.3 105.84%

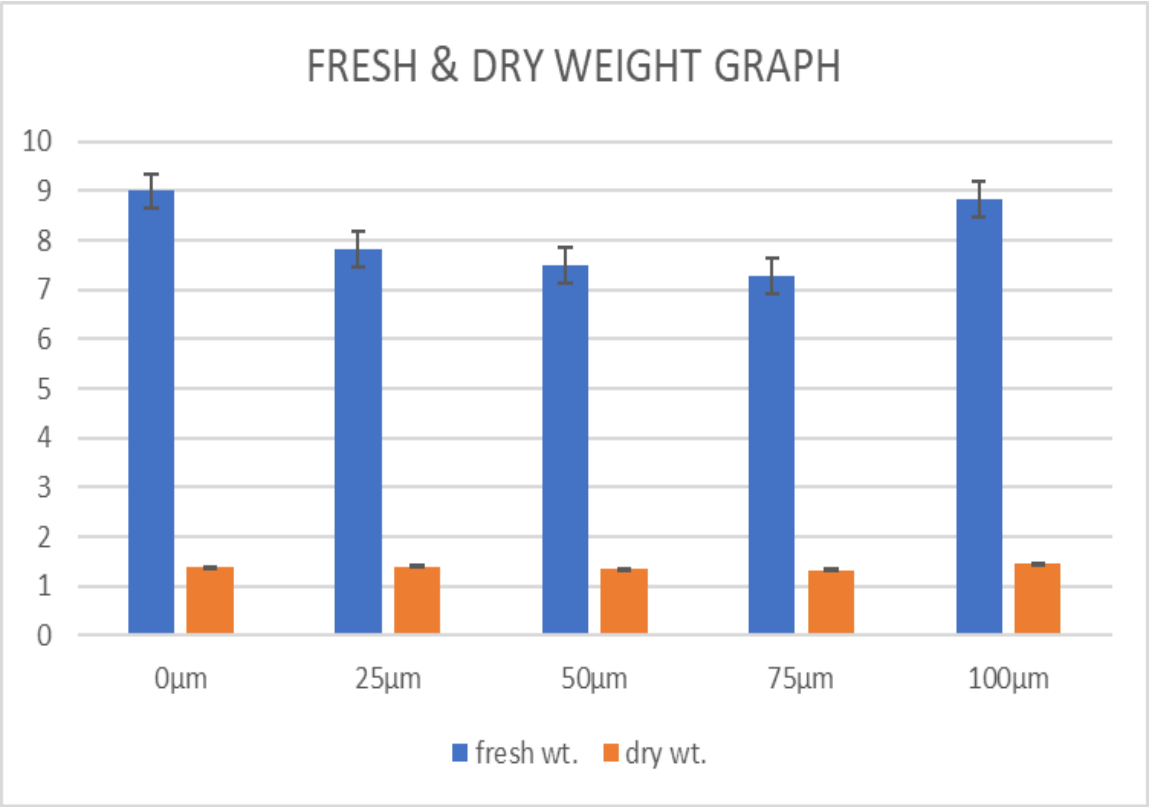

Fig. 2: Effect of lead on fresh wt. and dry wt.

Table 3
Lead effect on chlorophyll content

Treatment	Total chlorophyll	Chlorophyll a	Chlorophyll b
0µm	5.33±6.08 100%	3.47 ± 3.29 100%	1.92 ± 1.61 100%
25µm	2.69±1.42 50.47%	0.67 ± 1.06 19.31%	5.88 ± 6.33 306.25%
50µm	4.01±5.82 75.28%	2.50 ± 3.01 72.05%	1.50 ± 1.73 78.12%
75µm	5.55±7.39 104.12%	3.26 ± 3.85 93.95%	2.33 ± 2.16 121.35%
1ooµm	2.68±3.49 50.38%	1.48 ± 1.51 42.64%	1.30 ± 1.27 67.71%

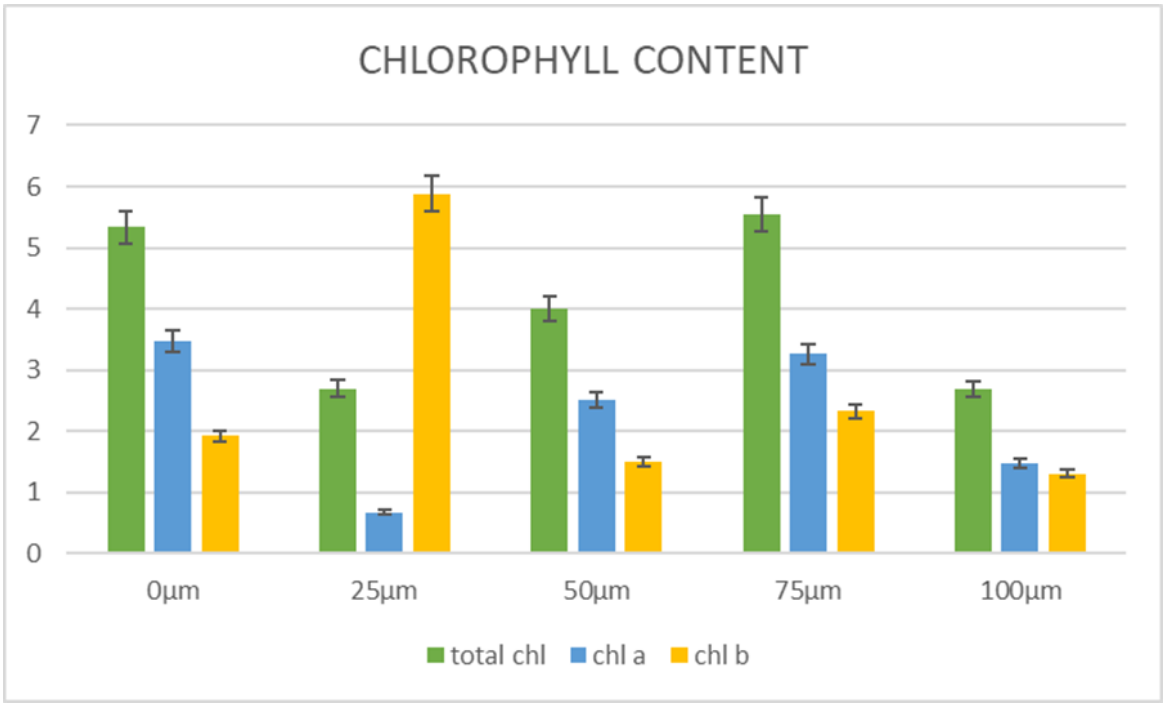

Fig. 3: Effect of lead on chlorophyll content

References

1. Chang L.W., Magos L. and Suzuki T., Toxicology of metals, Lewis, Boca Raton **(1996)**

2. Drążkiewicz M., Chlorophyllase: occurrence, functions, mechanism of action, effects of external and internal factors *Photosynthetica*, **30(3)**, 321-331 **(1994)**

3. Gupta N., Gedam V.V., Moghe C. and Labhasetwar P., Investigation of characteristics and leaching behavior of coal fly ash, coal fly ash bricks and clay bricks, *Environmental Technology & Innovation*, **7**, 152-159 **(2017)**

4. Jaishankar M., Tseten T., Anbalagan N., Mathew B.B. and Beeregowda K.N., Toxicity, mechanism and health effects of some heavy metals, *Interdisciplinary Toxicology*, **7(2)**, 60 **(2014)**

5. Vodnik D., Jentschke G., Fritz E., Gogala N. and Godbold D.L., Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings, *Physiologia Plantarum*, **106(1)**, 75-81 **(1999)**

6. Wang S. and Shi X., Molecular mechanisms of metal toxicity and carcinogenesis, *Molecular and Cellular Biochemistry*, **222**, 3-9 **(2001)**

7. Yusuf A.A. and Inambao F.L., Characterization of Ugandan biomass wastes as the potential candidates towards bioenergy production, *Renewable and Sustainable Energy Reviews*, **117**, 109477 **(2020)**.

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