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Effect of Pb Exposure on Germination and Morphological Characteristics of *Brassica nigra, Cicer arietinum* L. Seedlings

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***Abstract*:The increasing industrialization and modernization have resulted in introducing varieties of contaminants including heavy metals in soil, which directly and/ or indirectly negatively affect plant species. Lead (Pb) is a powerful toxicant that affects many physiological and metabiological processes in plants, leading to Pb toxicity. The present study was aimed to investigate the effects of various concentrations of Pb on the growth of *Brassica nigra* and to understand its bioaccumulation efficiency. Soils supplemented with Pb (10-100 ppm) were used to grow *Brassica nigra* and *Cicer arietinum* seeds under controlled conditions. The in vitro experiments proved that with increasing Pb concentration, there was retardation in the growth of the plants. The concentration of 100 ppm adversely affected the seed germination, root and shoot length, biomass, and chlorophyll content with noticeable effects on plant cells. However, the plants were able to accumulate a significant amount of Pb with increasing concentration. The results provided a hope to use such phyto- accumulators as an environment-friendly, cost-effective, and clean method for the treatment of metal-contaminated soils, and also contributing towards sustainable agriculture.**

Keywords*:Lead; phytotoxicity; germination; *Brassica nigra*; *Cicer arietinum

I. INTRODUCTION

Metals has been used by humans for centuries dating back to 7000 BC. In 1983, a total of 400,000-1,000,000 tons of mobilized Pb were disposed of with waste from metal extraction (Nriagu and Pacyna, 1988). In 2004, 3,150,000 tons of Pb were extracted from the earth's crust and brought into circulation in society (USGS, 2019). The element is present in various minerals in minute quantities excluding sulfide and lead glance (PBS) which are used to produce the metal around the world. Some of its common uses are for making paints and pipes as corrosion-resistant. It is used in car batteries and for soldering parts of electrical equipment. In laboratories and industries, it is used for the electrolysis process.

Natural Pb inputs include weathering and erosion of parent rocks that transfers a large quantity of metal to water bodies and land (Gadd, 2010). Pb has been used by humans for centuries but anthropogenic activities related to this metal have increased significantly in recent decades. These activities include mining, smelting, fuel combustion, synthetic fertilizers, and various industrial processes: building construction, Pb-acid batteries, bullets and shot, solder, pewter, and fusible alloys (Shotyk and Roux, 2005).

Lead is a cumulative poison that can be ingested, inhaled, or absorbed through the skin. It affects almost every organ in the body. Lead is toxic to the central nervous system, kidneys, and cardiovascular system, and even to the developing red blood cells at a concentration as low as 10 mg/dL. It is especially toxic to developing foetuses and children. It causes delayed development, diminished intelligence, and altered behaviour. Severe toxicity can cause sterility and abortion, as well as neonatal mortality and morbidity (Mukai et al., 2001). Of all the organs, the nervous system is the most affected target of Pb toxicity, both in children and adults. The toxicity in children is however of a greater impact than in adults. This is because their internal as well as external tissues are softer than in adults.

Chronic Pb exposure is found to reduce fertility in males. Increased Pb toxicity leads to the blood disorders and damage to the nervous system (Wani et al., 2015). The peripheral nervous system is seen to be affected in adults while the central nervous system is affected in children. A direct consequence of Pb exposure is Encephalopathy (progressive degeneration of certain parts of the brain) and its symptoms include irritability, dullness, headache, poor attention span, loss of memory, hallucinations, and muscular tremor. Repercussions of Pb exposure on the peripheral nervous system have also been observed in the form of peripheral neuropathy, involving reduced motor activity due to loss of myelin sheath which insulates the nerves, thus seriously impairing the transduction of nerve impulses, causing muscular weakness, especially of the exterior muscles, fatigue and lack of muscular co-ordination (Flora et al., 2012).

Lead exposure also affects the central nervous system of animals and inhibits their ability to synthesize red blood cells. Lead blood concentrations of above 40 µg/dl can produce observable clinical symptoms in domestic animals. Calcium and phosphorus can reduce the intestinal absorption of Pb. The US EPA report generalizes that a regular diet of 2-8 mg of Pb per kilogram of body weight per day, over an extended period of time, will cause death in most animals. Grazing animals are directly affected by the consumption of forage and feed contaminated by airborne Pb and somewhat indirectly by the up-take of Pb through plant roots. Invertebrates may also accumulate Pb at levels toxic to their predators. (Levin et al., 2021). Some evidence suggests that Pb can affect population genetics.

Lead toxicity is not only limited to humans and animals but affects plants severely. The pores in a plant's leaves let in carbon dioxide needed for photosynthesis and emit oxygen. Lead pollution coats the surface of the leaf and reduces the amount of light reaching it. This results in stunting the growth or killing the plants by reducing the rate of photosynthesis, inhibiting respiration, and encouraging an elongation of plant cells influencing root development by causing premature aging (Pourrut et al., 2011). The downward movement of elemental and inorganic Pb compounds from soil to groundwater is very slow because of its slow mobility, and therefore, it is easily absorbed by plants and other organisms. Although the level of Pb is low in the upper parts of plants such as seeds, leaves, and fruits because of great accumulation in roots, it can be still, however, toxic to humans when edible parts are consumed (Clemens and Ma, 2016). Therefore, the pollution of soil and water is directly linked to the cross-contamination of the food chain (Dikilitas. et al., 2016; Küpper, 2017).

Though Pb can be removed physically by different methods (Oke et al., 2017, Sharma et al., 2018), more clean solutions are still missing in combating this toxic metal from the environment. Despite of the fact that Pb is toxic to most plant species, many plants have developed resistant mechanisms towards this metal. Plants have extensive root system which make them suitable for the phytoremediation purposes. They can easily be employed to tolerate and accumulate Pb and still show quick growth rate and high biomass yield. The present study focuses on the growth and germination of two plans *viz*. *Brassica nigra* (black mustard) and *Cicer arietinum* L.(chickpea) under Pb stressed environment and their ability to accumulate this metal (Das et al., 2021).

II. MATERIALS AND METHODS

Collection of soil

For in vitro experiments, the red-brown soil was collected from the greenhouse of KristuJayanti College, Kothanur, Bangalore, 560077. The collected soil carefully transferred to the laboratory and stored under 4 °C. Soil was sieved and physicochemical analyses was performed to understand its characteristics.

Selection of Plant material

In the light of the review done and keeping in mind the factors such as faster growth, availability, and temperature specification, *Brassica nigra* and *Cicer arietinum* L.were selected for the study. These two crops play an important role in Indian cuisine and they can grow easily in normal lab conditions. India is the largest producer of pulses and *Cicer arietinum* L. is one of them. The other reason for selecting these crops is that they are mostly grown in states that are also known for their textile and metal industries such as Rajasthan, Bihar, Uttar Pradesh, and Madhya Pradesh (Ali and Kumar, 2005; Chand et al., 2021).

Preparation of Pb Stock

One gram of $Pb(NO3)_2$was weighed and dissolved in 100 ml of sterilized distilled water in a standard flask to obtain a concentration of 100 ppm. The Pb stock solution was then stored in an air-tight container in the refrigerator until further use.

Experimental Design

The experiments were performed in triplicates for both the seedlings. About 10 g of sieved soil was added to 5 ml of distilled water in a boiling test tubes and were sterilized by autoclaving to kill all the microbial population. The Pb stock solution was diluted to obtain three different concentrations of 10 ppm, 50 ppm, and 100 ppm respectively and were added to the test tubes. Seeds of *Brassica nigra* and *Cicer arietinum* were surface sterilized using 0.1% $HgCl_2$and about 15 seeds were aseptically inoculated into the test tubes maintained at different concentrations. The tubes were incubated at 25°C ± 2°C and 16/8 h photoperiod for a period of 15 days. The uninoculated test tubes served as controls. After the completion of the experiments, the soil was analysed for any physicochemical changes.

The appropriately grown seedlings were carefully uprooted from the soil and washed thoroughly with tap water to remove traces of soil. The shoot and roots were separated, washed thrice with distilled water and analyzed (Pandey and Bhatt, 2016).

Analyses of Growth Parameters

Germination of seedlings

The seeds in each tube was constantly monitored every day to determine the effect of different Pb concentrations on the germination from the day of sowing. Further, the time taken by the seedling to grow in the presence of Pb was also studied.

Determination of the length and biomass of the seedlings

The seedlings were laid straight on a flat surface such as the working table and their length was measured using a centimetre scale separately for root and shoot. Further, fresh weight of the roots and shoots were measured separately using a weighing balance.

Determination of Survival Index

The survival index (SI) of the seedlings was calculated by dividing the number of plants that survived in each treatment by the total sample size multiplied by 100 to get a percentage value.

$$\mathrm{SI} = \frac{number\ of\ plants\ survived}{Sample\ size} X100$$

Determination of Tolerance Index

The tolerance index (TI) was measured by dividing the length of shoot/root of the plants exposed to Pb by that of control. The equation used was:

$$\mathrm{TI} = \frac{Mean\ length\ of\ root\ or\ shoot\ of\ lead\ treated\ plant}{Mean\ length\ of\ root\ or\ shoot\ of\ control\ plant} X100$$

Chlorophyll estimation

Total chlorophyll content in the leaf tissues of bot the seedlings grown in Pb stressed soil were estimated. About 1g of samples were cut into small pieces and homogenized with 10 mL of acetone (80% v/v) followed by centrifugation at 3000 rpm for 15 min. The absorbance was measured using a colorimeter at 663 nm and 645 nm, against 80% acetone as blank. Chlorophyll content was calculated using the equations given by Arnon (1949).

Chlorophyll a (mg/mL)=$12.7A_{663}$-$2.69\ A_{645}$
Chlorophyll b (mg/mL)=$22.9A_{663}$-$4.68A_{645}$
Total Chlorophyll = Chlorophyll a+ Chlorophyll b

III. RESULTS AND DISCUSSION

Physicochemical analyses of the soil samples

Results of the physicochemical analyses of soil samples revealed that the pH of the soil was close to neutral value (6.2) while the EC was recorded to be 0.1 dS/m. The soil was found to be rich in organic matter and nutrients, such as nitrogen, phosphorus, and potassium with considerable amounts of iron oxides. Further assessment of the soil samples did not reveal presence of any heavy metals.

The introduction of Pb in the soil changed its properties to some extent. The pH of the soil sample shifted towards alkaline values (7.1) with the considerable decrease in the organic matter and nutrients.

Germination of seeds

The rate of germination was recorded to be 90%, 83%, 80%, and 73% respectively for *B. nigra* seeds in 0, 10, 50 and 100 ppm Pb concentrations. Further, in *C. arietinum,* germination percent was found to be 80, 66, 53 and 40 respectively for 0, 10, 50 and 100 ppm Pb concentrations. The results clearly indicate that the germination percent decreased with the increasing Pb concentration (Fig 1).

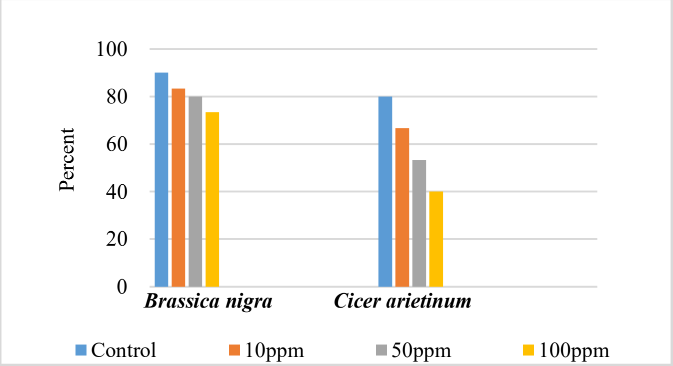

Fig 1. Effect of different lead concentrations on the germination of *Brassica nigra* and *Cicer arietinum*

Growth of the seedlings and determination of root and shoot length

After the successful completion of incubation period (15 days), plants were carefully uprooted from the soil and washed thoroughly with tap water to remove traces of soil. The seedlingswere judiciously observed and the effect of Pb on their growth was studied. It was evident from the results that the seedlings grown in the absence of Pb were completely normal, had a good growth and attained their full height when compared to the seedlings grown in the presence of various Pb concentrations (Fig 2).

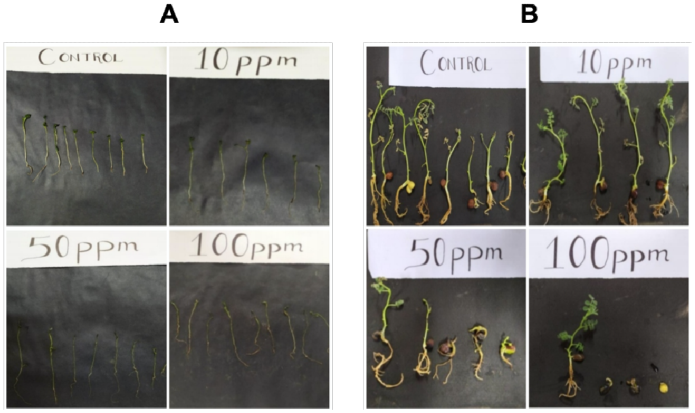

Fig 2. Growth of (A) *Brassica nigra* and (B) *Cicer arietinum* seedlings in the presence of control, 10 ppm, 50 ppm, and 100 ppm Pb concentrations

To determine the length of roots and shoots, they were separated and washed several times with distilled water. The mean length was separately measured for roots and shoots. In *B. nigra* the length of the root was recorded to be 5.38 cm, 5.84 cm, 5.0 cm and 4.23 cm, while for the shoots, it was 6.7 cm, 7.06 cm, 6.12 cm and 5.5 cm respectively for 0, 10, 50 and 100 ppm Pb concentrations (Fig 3). In *Cicer arietinum L*, the length of the root was calculated as 6.51 cm, 6.42 cm, 5.52 cm and 4.2 cm, while the length of the shoot was found to be 6.98 cm, 9.58 cm, 7.5 cm and 4.55 cm respectively for 0, 10, 50 and 100 ppm Pb concentrations (Fig 4). The results showed that the root and shoot length of the plants decreased significantly with the increase in Pb concentration.

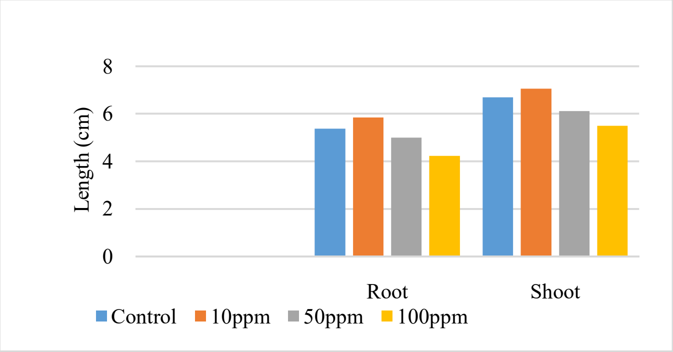

Fig 3. Effect of different concentrations of lead on *Brassica nigra* seedlings

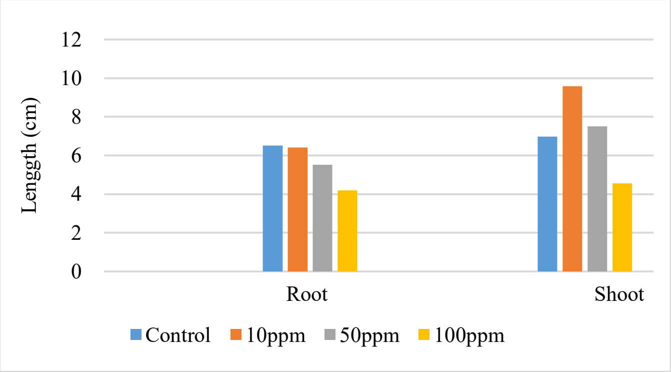

Fig 4. Effect of different concentrations of lead on *Cicer arietinum* seedlings

Determination of Biomass

The mean biomass of *B. nigra* roots was found to be 0.095 g, 0.116 g, 0.079 g, and 0.06 g, while the mean shoot biomass was 0.012 g, 0.0166 g, 0.021 g, and 0.03g respectively in the presence of 0, 10, 50 and 100 ppm Pb concentrations (Fig 5). The mean Biomass of *C. arietinum* roots was calculated to be 0.187 g, 0.36 g, 0.25 g and 0.14 g while the shoot biomass was 0.132 g, 0.458 g, 0.22 g and 0.14g for the different Pb concentrations (Fig 6).

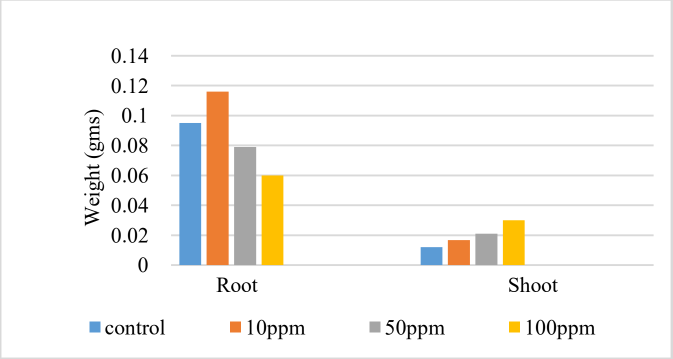

Fig 5. Effect of different concentrations of lead on the biomass of *Brassica nigra* seedlings

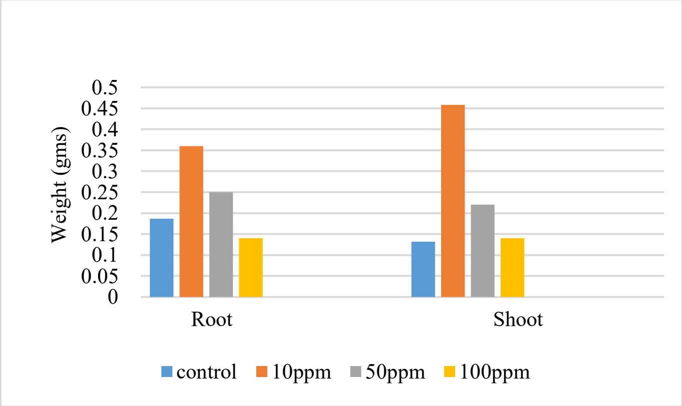

Fig 6. Effect of different concentrations of lead on the biomass of *Cicer arietinum* seedlings

Survival Index

The survival index (SI) was calculated by dividing the number of plants that survived in each treatment by the total sample size multiplied by 100 to get a percentage value. The survivability index of *B. nigra* was determined to be 90%, 83%, 80% and 73% while that of *Cicer arietinum* was calculated to be 80%, 66%, 53%, and 40% respectively, in the presence of 0, 10, 50 and 100ppm of Pb concentrations. (Fig 7, 8).

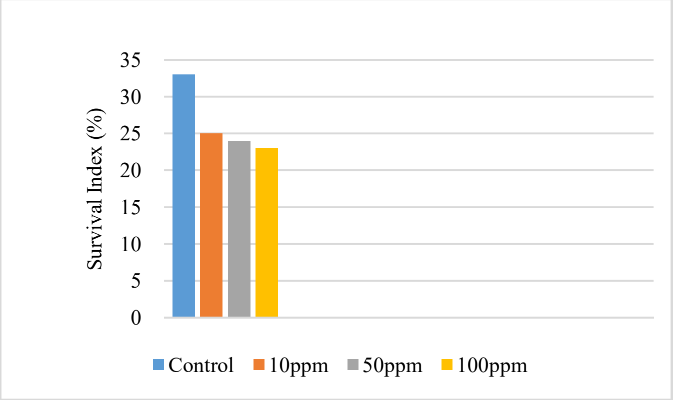

Fig 7. The survival index of *Brassica nigra* in different concentrations of Pb

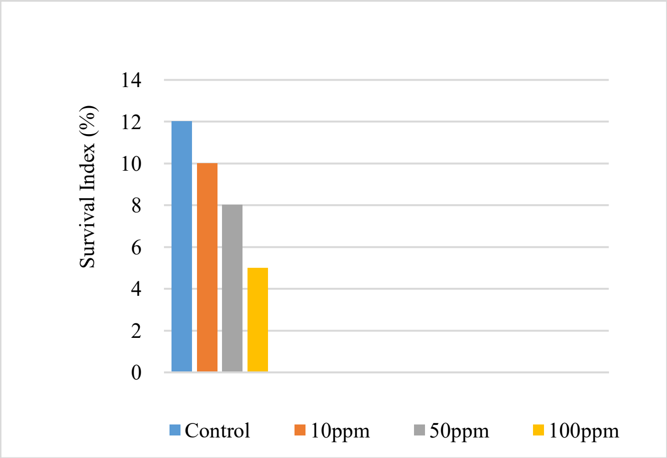

Fig 8. The survival index of *Cicer arietinum* in different concentrations of Pb

Tolerance index

The tolerance index of *B. nigra* root was found to be 45%, 21% and 20%, while that of shoot was reported to be 82%, 79% and 77%, respectively in the presence of 10 ppm, 50 ppm and 100 ppm of Pb concentration (Fig 9). The average TI of *C.arietium*roots resulted in 98%, 84% and 79% values while the shoots showed 73%, 65% and 37% tolerance towards 10 ppm, 50 ppm and 100 ppm Pb concentrations (Fig 10).

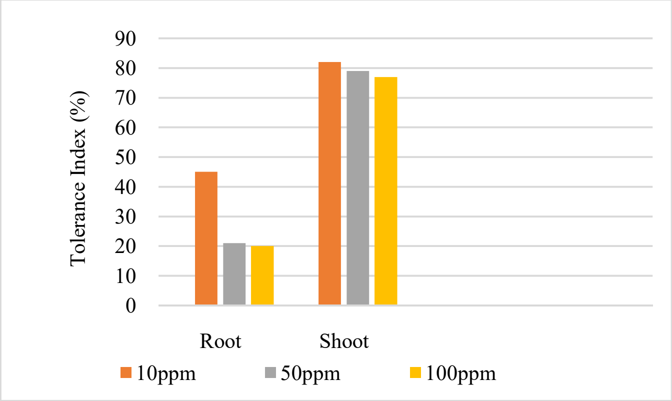

Fig 9. The tolerance index of *Brassica nigra* in different concentrations of Pb

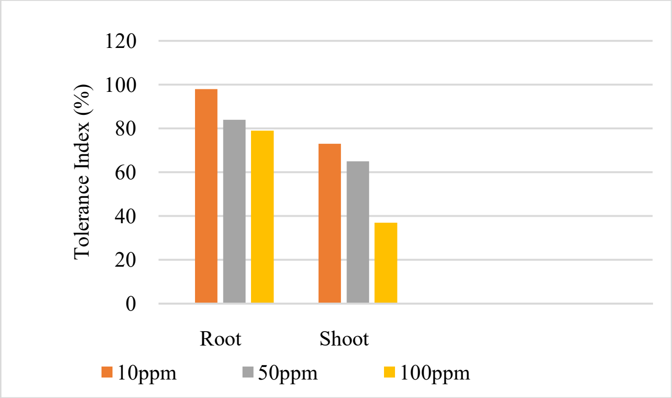

Fig 10. The tolerance index of *Cicerarietium* in different concentrations of Pb

Chlorophyll Estimation

The total chlorophyll content of *B. nigra* was recorded to be 4754 mg/ml in control, 4718 mg/ml in 10 ppm, 4581 mg/ml in 50 ppm and 4387 mg/ml in 100 ppm Pb respectively (Fig 11). Further, the total Chlorophyll content of *C. arietinum* was 2.29 mg/ml in control, 1.93 mg/ml in 10 ppm, 1.29 mg/ml in 50 ppm and 0.92 mg/ml in 100 ppm of Pb respectively (Fig 12)

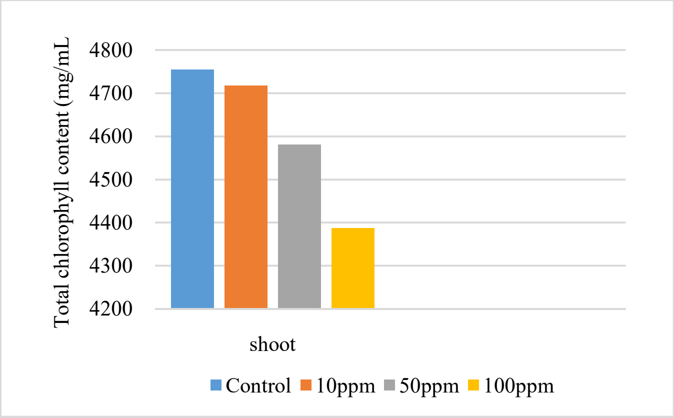

Fig 11. Chlorophyll content in the shoots of *Brassica nigra*

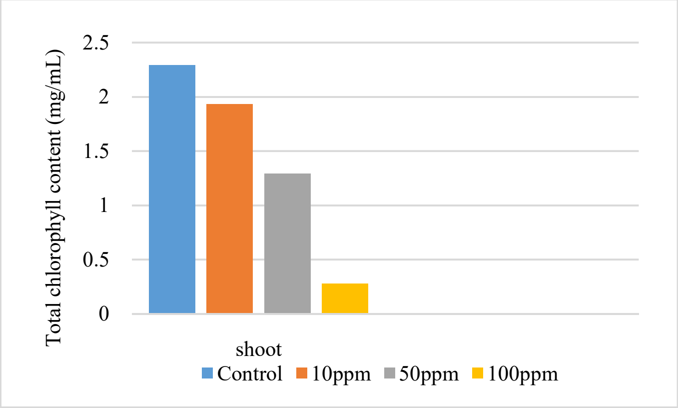

Fig 12. Chlorophyll content in the shoots of *Cicer arietinum*

Contamination of soil by heavy metals is one of the major factors contributing to poor soil quality, increased bioaccumulation and loss of biodiversity (Wieczorek et al.,2023). Lead is one of those heavy metals which possesses severe threat to all living organisms including lower and higher plant species. It is evident from the previous studies that Pb have three main mechanisms of toxicity in plants: inhibition of photosynthesis, oxidative stress, and DNA damage (Küpper, 2017; Kumar and Prasad, 2018). Further, the higher concentrations of Pb uptake in plants also causes defects in mitosis. The alarming environmental impacts of Pb has initiated many studies in the areas of risk assessment and mitigation to overcome its negative environmental effects.

Many plant species have been known today which can tolerance and accumulate convenient amount of Pb from their surrounding environments and helps in remediation (Debela et al., 2022; Duan et al., 2022). The present study was aimed to understand the Pb tolerance capacity of two Indian plant species *viz*. *Brassica nigra* and *Cicer arietinum* L. The results obtained in this study showed that the plants were tolerant to lower concentration (10 ppm) of Pb which actually catalysed the germination process and promoted the biomass and length of the plants. It was clearly evident from the study that *B. nigra* had a faster germination in 10 ppm Pb concentration, followed by the control. However, the growth of the plants decreased when the concentration was increased to 50 ppm and 100 ppm. Further, in *C. arietinium*,the germination followed a usual pattern, where fastest growth was observed in control, followed by 10 ppm, 50 ppm and 100 ppm. This implies that Pb plays different roles in different plant species and enables or impairs their growth in different ways (Cândido et al., 2020).

The close observation of the length of roots and shoots of both the plants indicated that root and shoot of *B. nigra* were the longest at 10 ppm and the shortest at 100 ppm, while the root of *C. arietinium* grew the longest in the control, while the shoot was longer than the rest at 10 ppm. Study of the biomass revealed that surprisingly the biomass of plants grown in 10 ppm Pb concentration was the highest in both the shoot and the root which was found to be constant in both *B. nigra* and *C. arietinium*, followed by the control, 50 ppm and 100 ppm Pb concentrations. Although a small amount of Pb promoted germination, length and biomass, it hindered the survival of all planted seeds. The tolerance of *B. nigra* and *C. arietinium* seedlings to different Pb concentrations was observed which followed the same pattern in both root and shoot, i.e. as the concentration increased, the tolerance of the plant decreased. Increased concentration of Pb also induced colour changes in the affected plants especially in the basal portion of the stem and the leaves. Similar findings have been reported by Huang et al. (2014) and BedabatiChanu and Gupta (2016).

From the present study, it can be understood that plants can tolerate and survive in the lower concentration of Pb, however, the presence of the toxic metals do alter their internal characteristics and functions (Cândido et al., 2020). The overall survivability depends upon different functional aspects of plants such as chlorophyll content, antioxidant reactions, and stress. The study clearly indicated that Pb had an adverse effect on the chlorophyll content of the tender shoot and leaves, which was further deteriorated with the increasing Pb concentration. The study also suggests the difference in plant sensitivity towards Pb concentration in soils. The overall soil characteristics play a very crucial role increasing or decreasing the availability of Pb for plants (Sidhu et al., 2018). Lead phytoremediation constitutes an important area of study to understand the fate of this metal not only in plants but also the living organisms which are directly or indirectly associated with plant products.

IV. CONCLUSION

This study leads to the conclusion that Pb contaminated soils pose a threat to all living organisms, including animals and plants. Although, plants can tolerate the lower concentrations of Pb, both physiological and metabolic properties are impaired as the concentration increases. Both *Brassica nigra* and *Cicer arietinum* play an important role inIndian agriculture and hence more attention needs to be paid to the soil in which crops are grown. Undoubtedly, Pb has devastating effects on all forms of life and therefore we need to find alternative methods of disposing Pb waste. More studies need to be conducted in this direction to understand the uptake of Pb by crops and the cellular damage it causes in plants, and to find innovative ways to remove Pb contamination from agricultural soil.

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