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Study of effect of lead contamination on growth of *Brassica juncea*, *Parthenium hysterophorus* and *Azadirachta indica*

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ABSTRACT

Heavy metal contamination, especially from lead (Pb), poses a serious risk to environmental sustainability and agricultural output because of its toxicity, persistence, and capacity for bioaccumulation. This study assessed how different lead concentrations (0, 100, 200, 300, and 400 mg/kg) affected plant growth, physiological characteristics, and biochemical markers in order to comprehend tolerance mechanisms and toxicity responses. Growth measures such as plant height, root length, fresh weight, and dry biomass showed a concentration-dependent reduction, with 400 mg/kg showing the greatest suppression. Notably, lead stress had a particularly strong effect on root length, suggesting that roots are the main site of heavy metal toxicity. Furthermore, the amount of chlorophyll gradually decreased, indicating harm to the photosynthetic machinery and disruption of pigment production as a result of lead poisoning, which impairs chloroplast function and produces oxidative stress via reactive oxygen species (ROS). According to the research, lead exposure impairs physiological and morphological processes that are necessary for plant life. These effects are linked to oxidative damage, decreased cell division, poor water relations, and disturbed mineral feeding. Plant sensitivity to heavy metal pollution, even at modest levels, is highlighted by the steady drop in measured parameters, which suggests a dose-dependent harmful impact. All things considered, the study sheds light on how plants react to lead stress and highlights the necessity of tracking heavy metal pollution in agricultural soils in order to guide phytoremediation techniques and improve sustainable crop production.

Keywords: *Lead toxicity, Heavy metal stress, Plant growth, Chlorophyll content, Biomass reduction, Phytotoxicity, Oxidative stress, Environmental contamination.*

1. Introduction

The plants absorb elements from the soil of which some can be categorized as necessary for their life cycle, particularly small amounts of critical micronutrients including iron, manganese, molybdenum, copper, zinc, and nickel. Along with non-transition elements like aluminum that support growth but are not necessary, it also discusses other transition metals like silver, gold, and cobalt. It also emphasizes how non-essential elements, such as hazardous heavy metals like arsenic, cadmium, chromium, mercury, and lead, can be taken by plants, pointing out that even micronutrients can turn hazardous if absorbed in excess of certain thresholds[1]. Lead is found in the atmosphere, soil, water, and food, and it is particularly prevalent in urban areas. Because of its limited solubility, mobility, and relative immunity to microbial breakdown in the soil, lead has a tendency to collect in surface soil. Weathering and other pedogenic processes that affect the parent material of the soil, pollution from anthropogenic activities like mining, smelting, and waste disposal, or the use of hazardous and unethical agricultural practices like using sewage sludge and waste water to produce vegetable crops or grow vegetables close to highways and industrial areas are the main causes of lead in soil[2]. Due to the metal pollutant's bioaccumulation in plant tissues, lead is a prevalent environmental contaminant that causes a variety of disruptions in plant physiological systems. Excess Pb has a number of negative impacts on some plant species, including inhibition of chlorophyll synthesis, plant development, and seed germination[1]. Using plants to eliminate, stabilize, or detoxify environmental pollutants from soil, water, and air is known as phytoremediation, and it is a sustainable, economical, and environmentally benign method[3].

Heavy metal-tolerant and heavy metal-accumulating plants are useful instruments for cleaning up contaminated settings. Some plant species can accumulate heavy metals in their tissues and are tolerant to them. This phenomenon serves as the foundation for phytoremediation, an economical and environmentally beneficial method of eliminating pollutants from soil and water by employing plants. The processes of phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization are all included in phytoremediation. Numerous plant species, including *Azadirachta indica, Parthenium hysterophorus*, and *Brassica juncea*, have demonstrated promise in research on heavy metal accumulation and tolerance [4,5]. Due to its quick growth, high biomass output, and capacity to accumulate heavy metals including lead, cadmium, and chromium, *Brassica juncea*, or Indian mustard, is well known as a hyper-accumulator species[6]. A promising species for phytoremediation study, *Parthenium hysterophorus* is a resilient invasive weed that is well-known for its capacity to adapt to harsh environmental circumstances and its tolerance to pollutants[7]. A medicinal tree with considerable ecological significance, *Azadirachta indica* (neem) biomass can be used to remove different metals, dyes, and biocontaminants from both untreated and surface-modified materials[8]. Thus, the current study attempts to assess the impact of lead contamination on growth parameters of *Azadirachta indica, Parthenium hysterophorus*, and *Brassica juncea* grown under controlled conditions.

2. Materials and Methods

The experiment was carried out in a controlled atmosphere utilizing pot cultivation. Five treatment groups were created from the soil samples. A solution of lead nitrate was evenly mixed into the soil to generate lead contamination.

Table 1: Experimental Treatments with Different Lead Concentrations in Soil

Treatment	Lead concentration (mg/kg soil)
T0	Control (0 mg/kg)
T1	100 mg/kg
T2	200 mg/kg
T3	300 mg/kg
T4	400 mg/kg

The growth parameters like plant height, root length, fresh weight, dry weight and chlorophyll content were measured over a period of time.

2.1. Plant Height (cm): At regular intervals (15, 30, 45, and 60 days following planting), the height of the plants was measured using a measuring scale from the soil surface to the apical tip. At harvest, final height measurements were taken.

2.2 Root Length (cm): The plants were gently removed without causing any damage to their roots. To get rid of soil particles, the roots were cleaned under running water. Using a ruler, the length of the roots was measured from the base of the stem to the tip of the longest root.

2.3. Fresh weight (gm): Blotting paper was used to remove extra water as soon as the plants were harvested, and a digital balance was used to weigh the entire plant samples to calculate the fresh biomass.

2.4. Dry Weight (gm): Plant samples were dried for 48–72 hours at 70°C in a hot air oven until they reached a consistent weight. Analytical balances were used to record dry biomass.

2.5. Chlorophyll Content (mg/g fresh weight): The chlorophyll content was estimated using Arnon's method. Ten milliliters of 80% acetone were used to grind 0.1 grams of freshly chopped, finely chopped leaves. After that, it was centrifuged for five minutes at 5000–10000 rpm. After transferring the supernatant, the process was repeated until the residue was colorless. In comparison to the solvent (acetone) blank, the solution's absorbance was recorded at 645 and 663 nm[9].

The readings were noted, and the calculations were done using the following formulas:

Using the following formula, calculate the amount of chlorophyll present in the extract (mg chlorophyll per gram of tissue):

Chlorophyll a (mg/g) = $12.7\ (A_{663}) - 2.69\ (A_{645}) \times V / 1000 \times W$

Chlorophyll b (mg/g) = $22.9\ (A_{645}) - 4.68\ (A_{663}) \times V / 1000 \times W$

Total chlorophyll (mg/ g) = $20.2\ (A_{645}) - 8.02\ (A_{663}) \times V / 1000 \times W$

Where,

A= Absorbance at specific wavelengths

V= Final volume of chlorophyll extract in 80 % acetone

W= Weight of the fresh tissues extracted

3. Results and Discussion:

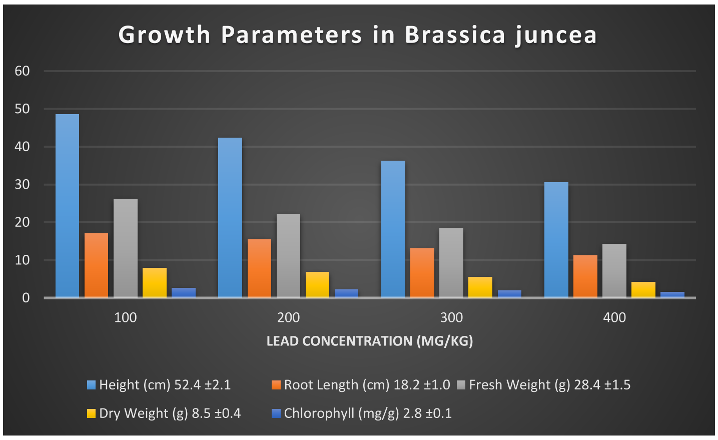
3.1 Growth of *Brassica juncea*: The information displayed in Table 2 illustrates how rising lead concentrations affect *Brassica juncea* growth performance. All growth indicators, including plant height, root length, fresh weight, dry weight, and chlorophyll content, showed a distinct downward trend as lead contamination levels increased in comparison to the control. In the absence of heavy metal stress, the control plants showed normal physiological functioning by recording the maximum plant height. However, plant height gradually declined as lead content rose from 100 mg/kg to 400 mg/kg. Lead-induced suppression of cell division and elongation is the cause of this decrease, which eventually impacts shoot growth. Stunted growth is another effect of lead exposure, which disrupts hormonal balance and nutritional absorption.

Because they come into direct contact with contaminated soil, roots are more vulnerable to the harmful effects of heavy metals. A build-up of lead in root tissues can harm root membranes, decrease water absorption, and prevent the growth of new root cells. In addition to limiting nutrient uptake, the decrease in root growth also results in decreased shoot growth. As lead levels rose, fresh and dry biomass dramatically declined. The overall metabolic disruption brought on by lead exposure is reflected in the loss of biomass. Heavy metals decrease the build-up of organic matter by interfering with respiration, photosynthesis, and enzymatic processes. Reduced synthesis of structural elements and poor carbon assimilation are indicated by the drop in dry weight.

As the concentration of lead increased, the amount of chlorophyll likewise decreased, suggesting that the photosynthetic apparatus was harmed. Reduced pigment levels and photosynthetic efficiency are the results of lead's disruption of chloroplast structure and inhibition of enzymes involved in chlorophyll production. Overall, the findings show that *Brassica juncea* is susceptible to high lead concentrations; nonetheless, its capacity to endure contaminated environments points to a moderate tolerance and potential for phytoremediation uses.

Table 2: Growth parameters of Brassica juncea

Lead (mg/kg)	Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll (mg/g)
0	52.4 ±2.1	18.2 ±1.0	28.4 ±1.5	8.5 ±0.4	2.8 ±0.1
100	48.6	17.0	26.2	7.9	2.6
200	42.3	15.4	22.1	6.8	2.2
300	36.2	13.1	18.4	5.5	1.9
400	30.5	11.2	14.2	4.2	1.5



Graph 1: Growth parameters in Brassica juncea

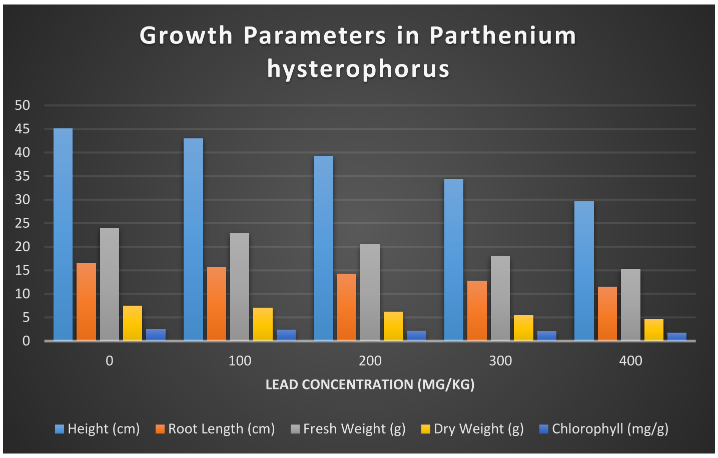
3.2 Growth of *Parthenium hysterophorus* : *Parthenium hysterophorus's* reaction to varying degrees of lead contamination is seen in Table 3. *Parthenium hysterophorus* exhibited comparatively less decline in growth parameters than the other species under investigation, indicating a greater capacity to withstand lead stress. The drop in plant height was less pronounced than that seen in *Brassica juncea*, although it did occur progressively as the lead concentration rose.

This suggests that *Parthenium* has adaptive mechanisms that enable it to continue growing in the face of heavy metal stress. Metal sequestration in vacuoles, antioxidant enzyme activation, and the synthesis of metabolites linked to stress are a few examples of such systems.

Although it was a minor loss, root length also decreased as lead levels rose. Even in contaminated environments, *Parthenium's* large root system may help improve nutrient uptake. One key component of roots' survival is their capacity to withstand lead build-up without suffering significant harm. The values of both fresh and dry biomass gradually decreased as the concentration of lead increased. Better metabolic stability was shown by the fact that the overall biomass decline was less than that of other species. The plant may be able to withstand environmental stress because of its invasiveness and ecological flexibility. Even at increasing lead concentrations, the amount of chlorophyll only slightly decreased, suggesting comparatively constant photosynthetic activity. The preservation of chlorophyll under stressful circumstances points to effective defenses against oxidative damage. According to these findings, *Parthenium hysterophorus* is more resistant to lead pollution and could be a good option for phytoremediation of contaminated soils.

Table 3: Growth in Parthenium hysterophorus

Lead (mg/kg)	Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll (mg/g)
0	60.2	20.4	30.1	9.2	3.0
100	57.8	19.3	28.6	8.7	2.9
200	53.4	17.8	26.4	8.0	2.6
300	49.1	16.2	23.5	7.1	2.4
400	44.6	14.8	20.3	6.4	2.1



Graph 2: Growth parameters in Parthenium hysterophorus

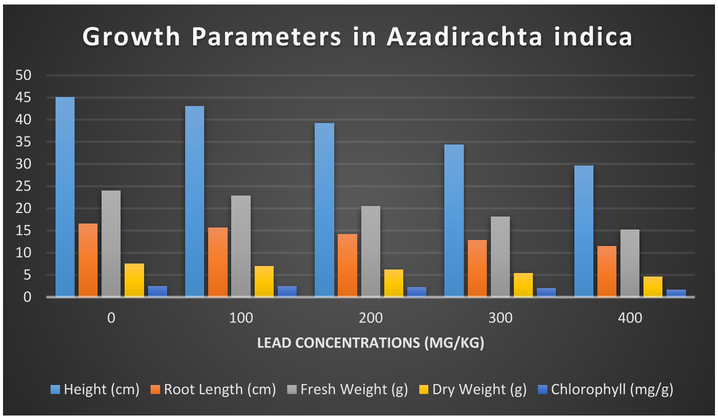
3.3 Growth of *Azadirachta indica-:* The impact of lead contamination on *Azadirachta indica* growth metrics is shown in Table 4. Plant height, root length, biomass, and chlorophyll content all decreased as lead concentrations increased, just like in the other species.

Plants exposed to greater lead concentrations had discernible decreases in growth, whereas the control group experienced the greatest growth. Lead toxicity-induced suppression of meristematic activity and disturbance of hormonal control may be linked to the decline in plant height. Higher lead concentrations caused a considerable decrease in root length, demonstrating how sensitive root tissues are to heavy metal stress. Reduced growth may arise from lead build-up in the roots, which can affect membrane permeability and root respiration [10].

As lead concentrations rose, fresh and dry biomass steadily declined. Reduced photosynthetic efficiency and metabolic stress are indicated by a decrease in biomass. *Azadirachta indica* plants, on the other hand, showed some tolerance by surviving even at greater lead levels. As the quantity of lead increased, the amount of chlorophyll decreased, suggesting that the structure of the chloroplasts was damaged and that the synthesis of pigment was inhibited. In contrast to *Brassica juncea*, the reduction was modest, indicating that neem has superior defense mechanisms. Overall, *Azadirachta indica* showed a moderate resistance to lead contamination. Because of its woody form and slower growth rate, it may be more useful for phytostabilization than active phytoextraction.

Table 4: Growth in Azadirachta indica

Lead (mg/kg)	Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll (mg/g)
0	45.1	16.5	24.0	7.5	2.5
100	43.0	15.6	22.8	7.0	2.4
200	39.2	14.2	20.5	6.2	2.2
300	34.4	12.8	18.1	5.4	2.0
400	29.6	11.5	15.2	4.6	1.7



Graph 3: Growth parameters in Azadirachta indica

4. Conclusion

Rapid industrialization, urbanization, mining, agricultural inputs, and inappropriate waste management have all contributed to heavy metal contamination becoming one of the most significant environmental issues in the world. Because of its persistence, inability to biodegrade, and capacity to accumulate in biological systems, lead (Pb) is regarded as one of the most hazardous heavy metals[11]. Significant threats to human and animal health arise from lead pollution in soil, which has an impact on plant development, soil fertility, microbial activity, and eventually makes its way into the food chain[12].

Numerous physiological and metabolic systems in plants are disrupted by lead. It lowers food intake, interferes with photosynthesis, slows enzyme activity, changes membrane permeability, and causes oxidative stress by producing reactive oxygen species (ROS)[13]. Stunted growth, chlorosis, decreased biomass, root damage, and poor seed germination are morphological signs of lead toxicity[14]. Because they are the main location where metals accumulate and interact with soil pollutants, roots are especially vulnerable.

Using plants to eliminate, stabilize, or detoxify environmental pollutants from soil, water, and air is known as phytoremediation, and it is a sustainable, economical, and environmentally benign method[3]. Heavy metal-tolerant and heavy metal-accumulating plants are useful instruments for cleaning up contaminated settings. Numerous plant species, including *Azadirachta indica, Parthenium hysterophorus,* and *Brassica juncea*, have demonstrated promise in research on heavy metal accumulation and tolerance[4,5].

Due to its quick growth, high biomass output, and capacity to accumulate heavy metals including lead, cadmium, and chromium, *Brassica juncea*, or Indian mustard, is well known as a hyperaccumulator species[6]. A promising species for phytoremediation study, *Parthenium hysterophorus* is a resilient invasive weed that is well-known for its capacity to adapt to harsh environmental circumstances and its tolerance to pollutants[7]. A medicinal tree with considerable ecological significance, *Azadirachta indica* (neem) has a moderate tolerance to heavy metal stress and aids in environmental restoration and soil stabilization [15]. Evaluating the effectiveness of phytoremediation requires an understanding of how lead pollution affects plant development metrics. Important markers of plant health and stress tolerance under heavy metal exposure include growth metrics as plant height, root length, biomass accumulation and chlorophyll content [16]. Owing to its cost-efficiency, energy- efficiency, and satisfying method for cleaning up polluted sites, phytoremediation has gained rapid acceptance as a solution to environmental contamination. It is also applicable to a variety of contaminants, such as heavy metals, radionuclides, and organic solvents like chlorinated solvents, polycyclic aromatic hydrocarbons, pesticides, insecticides, explosives, and surfactants [17-18]. Thus, the goal of the current study is to assess how lead contamination affects the growth performance of particular plant species cultivated in controlled environments with different lead contents, including a control soil sample. Additionally, the study intends to evaluate their potential for phytoremediation using growth responses and tolerance.

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