

Influence of Heavy Metal Stress on Physiological Parameters and Biochemical Responses of Green Gram (*Vigna Radiata* L.)

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ABSTRACT

This work examines the effects of various concentrations of lead Pb ($C_2H_3O_2$)₂ on the morphological characteristics of green gram plants. Lead Pb ($C_2H_3O_2$)₂ concentrations ranging from (control, 0.5, 0.10, 0.15, 0.20, 0.25, and 0.30 mg kg⁻⁵) to evaluate alterations in growth parameters such as root and stem length, number of leaves, root nodules, biomass, total leaf area and biochemical content on the 30th day. The results showed that all assessed traits consistently decreased in a dose-dependent manner as Pb concentrations rose. Pb exposure resulted in decreases in yield, morphological parameters, and biochemical content, at the highest level (0.30 mg/g). With the 0.30 mg/g lead Pb($C_2H_3O_2$)₂ treatment, green gram decreased, while all other metrics went up in the control group.

Keywords: Biochemical contents, lead acetate, morphological parameters, *Vigna radiata* L.

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Introduction

The right approach should be chosen for phytoremediation, to be applied satisfactorily. Among its most important kinds: phytoextraction, rhizofiltration, phytostabilization, phytofiltration which is a water-focused approach. Plant roots are used in rhizofiltration to directly absorb pollutants from water. Plant seedlings are used in blastofiltration to eliminate heavy metals like cadmium and lead. Both techniques provide effective and environmentally beneficial ways to purify water.

Environmental pollution continues to increase as cities grow endangering public health. To reduce the risk of diseases such as lung cancer, air pollution from household activities, industrial waste and automobile emissions must be monitored. Better transportation and interior air filtration are also required. Water contamination, essentially from industrial pollutants, put at risk drinking water quality. Some of the remedies include reducing wastewater output and improving treatment techniques. Garbage pollution is harmful since it leads to secondary contamination as well. Recycling

correctly is critical to maintaining the environment and public health.¹

Vigna radiata (L.) Wilczek, sometimes known as green gram, is a well-known and ancient leguminous crop in Asia due to its high nutritional content and crop-system adaptability. It is India's third most valuable pulse crop, occupying 4.07 mha of land and yielding a total of 1.90 million tonnes with a 477 kg/ha average yield. The major states for growing green gram include Andhra Pradesh, Maharashtra, Karnataka, Bihar, and Orissa. Green gram covers 1.85 lakh hectares in Tamil Nadu, where it produces and yields 516 kg/ha and 0.95 lakh tonnes, accordingly.²

Lead (Pb) is a toxic heavy metal is frequently found in industrial products, cosmetics and consumer goods. Lead acetate, sometimes known as the "sugar of lead" is a water-soluble form that is easily accessible increasing the risk of exposure. Lead may enter the body through the intake or inhalation of polluted dust, food, water, or air because it bonds to organic matter in soil. It accumulates in soft tissues, bones, and blood, serious health risks. Lead is easily absorbed and stored in plant tissues, influencing soil

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quality and plant growth even through it is not necessary for plants.³

Material and methods

Pot culture experiments

The source of the CO-6 green gram (*Vigna radiata* L.) seeds was Tamil Nadu Agricultural University (TNAU), located in Coimbatore, Tamil Nadu. The uniform seeds are picked for the experimental purpose. Source of Pb ($C_2H_3O_2$)₂ stock solution generated by dissolving the molecular weight of lead acetate and various concentrations viz., (Control, 0.5, 0.10, 0.15, 0.20, 0.25, 0.30 mg) of lead acetate the solutions were prepared freshly at the time of experimentation.

Morphological parameters

Root and stem length

The stem and root lengths of five randomly selected plants from each pot were calculated using a centimeter (cm) scale after the seedlings had grown for 30 days.

Fresh and dry weight

Five plants were chosen at random 30 days after germination in order to weigh them fresh using an electronic single-pan balance. The dry plant weight of the seedlings was then determined after they were in a hot air oven for 24 hours at 85°C.

Number of leaves

Five plants were randomly selected from each treatment at 30 days after seedlings growth, and each plant's total number of fully developed leaves was manually counted. After that, the average value was computed for analysis.

Total leaf area

At the 30 days of the seedling sampling. Five plant samples were taken, and the breadth and length of the leaf samples were calculated and noted. The kemp's constant was used to determine the total leaf area.

Total leaf area = $B \times L \times K$

B- breadth, L- length and K-Kemp's constant (for dicot – 0.66).

Root nodules

Five plants were chosen at random from each treatment after the seedlings had grown for 30 days. The total number of nodules was manually counted after the roots were gently cleaned with tap water. After that, the average number of nodules per plant was noted for examination.

Biochemical analysis

Photosynthetic pigments

During the fifteenth day of activity, the photosynthetic pigments (chlorophyll and carotenoid contents) as well as the biochemical contents (non-reducing sugar, proline, total sugar, reducing sugar, amino acid, and protein) were measured.

Chlorophyll and carotenoid contents

Fresh leaf tissues of 500 mg were ground with 10 millilitres of 80% acetone in a mortar and pestle. The extract was centrifuged at 800 rpm for 10 minutes, and the supernatant was clear. The pellet was re-extracted further by adding another 10 ml of 80 percent acetone, centrifuging, and then combining the resultant supernatant with the first. Absorbance of the total extracts was quantified at 663, 645, and 480 nm in a UV spectrophotometer. Contents of chlorophyll and carotenoids were then estimated following methods and were presented as mg per gram of fresh tissue.^{4,5}

Estimation of protein

Plant sample of 500 mg was ground in a mortar and pestle with 10 millilitres of 20% trichloroacetic acid (TCA). The homogenate was spun at 800 rpm for a few minutes, and the obtained supernatant was discarded. The pellet was resuspended in 5 millilitres of 0.1 N NaOH, spun for 10 minutes, and the obtained supernatant was diluted with 10 millilitres of 0.1 N NaOH. This extract was used as the protein estimation source. In the assay, 1 millilitres of extract were pipette into a 10 millilitres test tube and added to 5 millilitres reagent C. The mixture was left in the dark for 15 minutes. 0.5 millilitre of Folin-phenol reagent was applied. The solution was then incubated in the darkness for a further 30 minutes, and absorbance at 660 nm was then measured using a UV spectrophotometer.⁶

Estimation of sugar:

Fresh leaf tissues weighing 500 mg were homogenised in a pestle and mortar using 10 millilitre of 80 percent ethanol and centrifuged at 800 rpm for 10 minutes. Save the supernatant. The ethanol was evaporated in a water bath at 50 °C, and the residue was stirred with distilled water to give a final volume of 20 millilitre. This extract was utilized to measure the quantity of reducing sugars. 1 millilitre of extract was pipetted into a 25-millilitre graduated test tube, then 1 millilitre of reagent C. It was boiled in a water bath at 100 °C for 15 minutes and then chilled, 1 millilitre of arsenomolybdate reagent was added. After shaking the mixture thoroughly, the solution was made up to 25 millilitres with distilled water.

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Absorbance was read at 520 nm in a UV spectrophotometer, and the reducing sugar content was measured on a g⁻¹ FW basis.⁷

Amino acid

500 mg of plant tissue was homogenized in a pestle and mortar with 10 millilitres of 80 percent ethanol. The homogenate was centrifuged for 15 minutes at 800 rpm, and the supernatant recovered was diluted to an end result of 10 millilitres with 80 percent ethanol. For the assay, 1 millilitre of extract were combined with 1 millilitre of ninhydrin reagent in a Folin-Wu tube and placed in a boiling water bath at 100°C for 25 minutes. When cooled under running tap water, the reaction mixture was diluted to 10 millilitre using the diluting solution. Absorbance of samples was subsequently read at 570 nm by a UV spectrophotometer.⁸

Estimation of Proline

Five hundred mg of plant material was homogenized with 10 millilitre 3 percent aqueous sulfosalicylic acid and filtered with Whatman No. 2 filter paper. The residue was washed twice with the same solution, and the combined filtrates were brought to 20 millilitres with 3 percent sulfosalicylic acid. A test tube was charged with 2 ml of extract, 2 millilitre of glacial acetic acid, 2 millilitres of acid ninhydrin reagent. The combination was incubated at 100 °C for one hour in a water bath and then cooled in an ice bath to stop the reaction. Four ml of toluene was added and vigorously shaken for 10-20 seconds prior to the toluene layer being decanted from the aqueous layer with a separating funnel. The absorbance of toluene layer was determined at 520 nm in a UV spectrophotometer (Hitachi U-2900) relative to a blank. Proline content was calibrated from a proline based standard curve and expressed as mg per gram fresh weight.⁹

Results

Morphological parameters

Stem and root length

Stem and root lengths reduced in an increasing manner with rising Pb concentration. The control plants retained the highest shoot length (22.3±1.11 cm) and root length (12.16±0.60 cm), while treatment with 0.30 mg Pb resulted in the lowest shoot (10.2±0.51 cm) and root (4.35±0.21 cm) lengths, reflecting vegetative growth inhibition.

Biomass

Dry and fresh weight decreased with Pb concentration. Fresh weight decreased from 16.8±0.84 mg per gram in control to 2.67±0.13 mg per gram at 0.30 mg Pb, and dry weight was

decreased from 10.76±0.53 mg/g to 1.78±0.08 mg/g, indicating lower biomass formation under Pb stress.

Number of leaves

Leaf number declined gradually with increasing Pb concentrations. Control plants possessed 24±1.2 leaves, which decreased to 14±0.7 leaves at 0.30 mg Pb, suggesting a negative effect on leaf formation.

Total leaf area

Total leaf area declined from 6.5±0.32 cm in control to 2.3±0.11 cm at 0.30 mg Pb, reflecting decreased photosynthetic surface area due to Pb stress.

Root nodules

Root nodulation was adversely impacted by Pb exposure. The nodular count went down from 8±0.4 in control to 2±0.1 with the maximum Pb concentration, implying compromised nitrogen fixation.

Table 1: The effect of different concentrations of lead acetate mg/g on morphological parameters of *Vigna radiata L.* (mean ± SE, n=5, 30th day)

| Pb treatment | Shoot length (cm) | Root length (cm) | Fresh weight (mg/g) | Dry weight (mg/g) | Number of leaves | Total leaf area (cm) | Root nodules |
|--------------|-------------------|------------------|---------------------|-------------------|------------------|----------------------|--------------|
| Control | 22.3 ± 1.11 | 12.1 ± 0.60 | 16.8 ± 0.84 | 10.7 ± 0.53 | 24 ± 1.2 | 6.5 ± 0.32 | 8 ± 0.4 |
| 0.5 mg | 21.4 ± 0.07 | 11.1 ± 0.55 | 13.1 ± 0.65 | 8.77 ± 0.43 | 21 ± 1.05 | 6.1 ± 0.30 | 7 ± 0.35 |
| 0.10 mg | 19.6 ± 0.98 | 10.6 ± 0.53 | 12.3 ± 0.61 | 8.21 ± 0.41 | 20 ± 1 | 5.6 ± 0.28 | 5 ± 0.25 |
| 0.15 mg | 18.8 ± 0.94 | 9.21 ± 0.46 | 9.5 ± 0.47 | 6.36 ± 0.31 | 19 ± 0.95 | 4.3 ± 0.21 | 4 ± 0.2 |
| 0.20 mg | 16.3 ± 0.81 | 7.04 ± 0.35 | 5.04 ± 0.25 | 3.28 ± 0.16 | 16 ± 0.8 | 3.9 ± 0.19 | 3 ± 0.15 |
| 0.25 mg | 12.9 ± 0.64 | 6.52 ± 0.32 | 2.96 ± 0.4 | 1.97 ± 0.09 | 15 ± 0.75 | 2.5 ± 0.12 | 3 ± 0.15 |
| 0.30 mg | 10.2 ± 0.51 | 4.35 ± 0.21 | 2.67 ± 0.13 | 1.78 ± 0.08 | 14 ± 0.7 | 2.3 ± 0.11 | 2 ± 0.1 |

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Biochemical contents

Chlorophyll and carotenoid content

Carotenoid and chlorophyll contents reduced progressively with the rising concentration of Pb. Chlorophyll 'a' reduced from 1.93 ± 0.096 mg per gram fresh weight in control to 0.74 ± 0.037 mg g⁻¹ fresh weight at highest treatment. Chlorophyll 'b' also reduced from 0.96 ± 0.048 mg g⁻¹ fresh weight. The control of total chlorophyll also fell significantly from 2.89 ± 0.144 mg g⁻¹ fresh weight. Similarly, carotenoid content reduced from 0.94 ± 0.047 to 0.47 ± 0.023 mg g⁻¹ FW showing intense inhibition of photosynthetic pigments under Pb stress.

Table 2: The effect of different concentrations of lead acetate mg/g on biochemical contents of *Vigna radiata L.* (mean \pm SE, n=5, 30th day)

| Pb treatment | Chlorophyll 'a' (mg/g) | Chlorophyll 'b' (mg/g) | Total chlorophyll (mg/g) | Carotenoid (mg/g) |
|--------------|------------------------|------------------------|--------------------------|-------------------------|
| Control | 1.93 ± 0.096 96 | 0.96 ± 0.048 48 | 2.89 ± 0.144 144 | 0.94 ± 0.047 047 |
| 0.5 mg | 1.82 ± 0.091 91 | 0.91 ± 0.045 45 | 2.73 ± 0.136 136 | 0.85 ± 0.042 042 |
| 0.10 mg | 1.68 ± 0.084 84 | 0.84 ± 0.042 42 | 2.52 ± 0.126 126 | 0.83 ± 0.041 041 |
| 0.15 mg | 1.33 ± 0.066 66 | 0.62 ± 0.031 31 | 1.95 ± 0.097 097 | 0.78 ± 0.039 039 |
| 0.20 mg | 1.17 ± 0.058 58 | 0.58 ± 0.029 29 | 1.75 ± 0.087 087 | 0.66 ± 0.033 033 |
| 0.25 mg | 0.86 ± 0.043 43 | 0.43 ± 0.021 21 | 1.25 ± 0.062 062 | 0.50 ± 0.025 025 |
| 0.30 mg | 0.74 ± 0.037 37 | 0.41 ± 0.020 20 | 1.15 ± 0.057 057 | 0.47 ± 0.023 023 |

Protein

Protein content also decreased steadily with Pb treatment from 2.84 ± 0.142 mg per gram fresh weight in control to 0.95 ± 0.047 mg per gram fresh weight at 0.30 mg Pb. This reflects that Pb stress repressed protein synthesis considerably.

Sugar

Sugar contents fell steadily under Pb stress from 2.51 ± 0.125 mg per gram FW in control to 1.53 ± 0.076 mg per gram FW at 0.30 mg Pb. The decline reflects the inhibition of carbohydrate metabolism.

Proline

Proline content augmented progressively from 0.08 ± 0.004 mg per gram FW in control to 0.17 ± 0.008 mg per gram FW at 0.30 mg Pb. This

uptake is indicative of an adaptive osmoprotective response against Pb stress.

Amino acid

Free amino acids total rose progressively from 2.3 ± 0.115 mg per gram FW in control to 3.1 ± 0.155 mg/g FW at the maximum Pb concentration. This augmentation signifies augmented protein degradation under stress.

Table 3: The effect of different concentrations of lead acetate mg/g on biochemical contents of *Vigna radiata L.* (mean \pm SE, n=5, 30th day)

| Pb treatment | Protein (mg/g) | Sugar (mg/g) | Proline (mg/g) | Amino acid (mg/g) |
|--------------|------------------------|------------------------|------------------------|-----------------------|
| Control | 2.84 ± 0.142 42 | 2.51 ± 0.125 25 | 0.08 ± 0.004 04 | 2.3 ± 0.115 15 |
| 0.5 mg | 2.62 ± 0.131 31 | 2.26 ± 0.113 13 | 0.10 ± 0.005 05 | 2.4 ± 0.12 2 |
| 0.10 mg | 2.41 ± 0.120 20 | 2.17 ± 0.108 08 | 0.12 ± 0.006 06 | 2.6 ± 0.13 3 |
| 0.15 mg | 1.95 ± 0.097 97 | 2.09 ± 0.104 04 | 0.13 ± 0.006 06 | 2.7 ± 0.135 35 |
| 0.20 mg | 1.32 ± 0.066 66 | 1.85 ± 0.092 92 | 0.14 ± 0.007 07 | 2.9 ± 0.145 45 |
| 0.25 mg | 1.27 ± 0.063 63 | 1.62 ± 0.081 81 | 0.16 ± 0.008 08 | 3.0 ± 0.15 5 |
| 0.30 mg | 0.95 ± 0.047 47 | 1.53 ± 0.076 76 | 0.17 ± 0.008 08 | 3.1 ± 0.155 55 |

Discussion

According to the research, the kind of container that seeds are stored in has a significant impact on how quickly they deteriorate.¹⁰ A crop plant's essential germination phase is influenced by many different kinds of environmental conditions.¹¹

Heavy metal contamination is an environmental issue worldwide, having a negative impact on plant growth. High concentrations of heavy metals are toxic to plants and can hinder their development. WHO states that the allowed level of heavy metals in plants is 0.2 μ g/g. Lead levels in soil sample analysed ranged from 0.43 to 0.48 μ g/g, whereas the control soil had 0.03 μ g/g. Almost all the soil samples collected revealed lead concentration more than the recommended limit by WHO. Lead in soil contamination is a common issue; it will deposit over time in the bones, aorta, kidneys, liver, and spleen. Lead can be absorbed by human beings mainly via food (65%), water (20%), and air (15%).¹²

The length of both root and shoot exhibited a slight increase at lower concentrations of heavy metals, but showed a significant decline as the

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concentration increased. Previous observations under lead and cadmium stress are consistent with this pattern.¹³⁻¹⁴ The shorter shoot, which restricts the availability of nutrients to the developing shoot, may be caused by the deactivation of enzymes involved in food mobilisation and the reduction of meristematic cell activity. Similar to this, the buildup of heavy metals in root tissues prevents cell elongation and mitotic cell division in the meristematic zone, which shortens the root.

The current investigation found that fresh and dry weights gradually decreased as lead acetate concentrations increased; finger millet under nickel and arsenic stress showed a similar tendency.¹⁵ Similarly, *Vigna mungo* treated with zinc sulphate showed reduced fresh and dry weights of roots and shoots.¹⁶ The root system is the main organ affected by heavy metal buildup, which hinders plant growth. These results demonstrate the detrimental effects of heavy metal poisoning on plant development and are in line with the current investigation. High quantities of metals like arsenic and nickel limit seedling growth, which lowers dry and fresh biomass.

In the current investigation, *V. radiata* subjected to higher lead acetate concentrations exhibited a considerable decrease in both total leaf area and leaf number, but lower concentrations only slightly altered the plant, suggesting partial tolerance. There have been reports of similar suppression of leaf growth under heavy metal stress as a result of decreased cell expansion and disrupted nutrient uptake.¹⁷ Additionally, the effect of lead acetate on root nodulation was concentration-dependent, showing a modest increase at lower doses and a decrease at higher values. *Cicer arietinum* showed a similar tendency under other heavy metals, including Zn, Cd, Cr, Ni, and Pb,¹⁸ suggesting that while greater concentrations negatively impact plant growth and nodulation, lower levels may momentarily promote physiological responses.

The current investigation showed that lead acetate treatment significantly reduced the amount of chlorophyll in *V. radiata*, demonstrating a dose-dependent effect. There have been reports of a comparable decrease in chlorophyll as a result of disruption of pigment production and stability under Pb stress.¹⁹ Total plant performance and photosynthetic efficiency are negatively impacted by this decrease. Similar outcomes were also noted in *Asplenium scolopendrium* under lead stress,²⁰ indicating that elevated Pb concentrations surpass

tolerance limits and disrupt physiological systems connected to pigment.

V. radiata protein and sugar content reacted to lead acetate treatment in a concentration-dependent way, gradually decreasing at higher levels and slightly increasing at lower values. ²¹*Chlorella* sp. showed a similar drop in protein content under Pb stress as a result of reduced metabolic activity, suggesting a trend similar to the current findings. Similarly, *Zea mays* showed decreased sugar accumulation under increasing Pb stress.²² These findings imply that while lower Pb concentrations may momentarily increase metabolic activity, greater concentrations interfere with regular biochemical processes, which eventually affects plant growth and development.

Proline and free amino acid levels in *V. radiata* showed a similar dose-dependent trend, rising at higher lead acetate concentrations and falling at lower concentrations. The current results are corroborated by a similar rise in proline and amino acids under cadmium and lead stress in *Phoenix dactylifera*.²³ As antioxidants, osmolytes, and metal chelators, this buildup is linked to the breakdown of proteins, the activation of proteolytic enzymes, and stress-induced synthesis. Under increased heavy metal exposure, these metabolites support cellular integrity and metabolic balance, suggesting a protective response.

Conclusions

The present investigation clearly establishes that lead toxicity at various levels modifies basic physiological and biochemical processes in *Vigna radiata* and inhibits the overall performance of the plant. While the induction of some defense mechanisms, such as proline and amino compound accumulation, is initiated in the plant, these adaptive mechanisms are not enough to neutralize the stress caused at higher levels of Pb. All changes observed indicate that *Vigna radiata* is prone to Pb contamination, particularly when the exposure is above its tolerance level. These results strengthen the rationale for better management practices and the breeding or remediation strategies in the future to reduce the impact of heavy metal pollution in the crop-growing environment.

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