

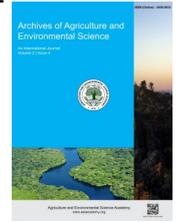


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ORIGINAL RESEARCH ARTICLE



Integrative approaches to understanding mercury toxicity in common bean (*Phaseolus vulgaris*) plants: Linking growth, biochemistry and antioxidant mechanisms

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ABSTRACT

Mercury is a widely studied toxic metal all over the world due to its ability to easily enter into the food chain. Even exposure to small quantities, causes adverse effects on various biochemical and physiological processes. This study aimed to investigate the integrative approaches to understanding mercury toxicity in common bean (*Phaseolus vulgaris*) plants by linking growth, biochemistry and antioxidant mechanisms. Experimental common bean plants were divided into 4 groups. Group 1 served as control, received normal nutritional and water support, while Group 2, 3 and 4 were treated with mercury concentrations of 50, 100 and 200 mg respectively. The results revealed that mercury treated common bean plants exhibited a significant decline in several key growth and physiological parameters, including germination percentage, root length, shoot length, fresh weight, dry weight and vigor index. Moreover, mercury treatment leads to reduction in carbohydrate and protein contents, as well as reduced activities of antioxidant enzymes like catalase and superoxide dismutase. These findings underscore the significance of further research to identify strategies for alleviating mercury-induced toxicity and enhancing plant resilience, with the goal of contributing to the development of sustainable agricultural practices that enhance the crop resilience in polluted environments.

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INTRODUCTION

Environmental pollution has become a significant challenge in today's modern society. Heavy metals (HMs) contamination is a well-known issue of environment that poses a serious risk to human's life. As the use of various metals in daily life and industries has increased, environmental problems caused by hazardous metal pollution have risen markedly (Gupta *et al.*, 2019). Agricultural waste, industrial effluents and sewage are the primary pollutants, contaminating the environment (Kumar & Chopra, 2013; 2014a,b). Most HMs pollution originates from industrial wastewater from mining, metal processing, pharmaceuticals, organic chemicals, tanneries, pesticides production, rubber and plastics manufacturing and the lumber and wood industries (Kumar *et al.*, 2020). Technological advancements

have improved the standards of life, has raised new environmental challenges in response to environmental safety, as urbanization and uncontrolled industrialization without good emission controls and pollution alleviation measures are now threatening human health and safety (Bennett *et al.*, 2003). HMs contamination has become a serious problem in various large parts of the developing world, especially in India and China (Meharg, 2004). Dietary substance contamination by chemicals and non-essential elements like HMs is known to have a range of harmful effects on both humans and animal health (D'Souza & Peretiakto, 2002).

HMs, that are available for plant uptake are those that exists as soluble forms in the soil solution or those that can be easily dissolved by root exudates (Blaylock & Huang, 2000). While certain HMs are necessary for their plant growth and development, elevated levels of these metals can become toxic to

plants. The mechanism that allows plants to accumulate essential metals also enable them to take up other nonessential harmful metals (Djingova & Kuleff, 2000). Since metals cannot be broken down, when its concentrations exceed optimal levels within plants, they adversely affect the plants both directly and indirectly. Direct toxic effects caused by elevated metal concentration which includes cytoplasmic enzymes inhibition and damage to cell structures due to oxidative stress (Assche & Clijsters, 1990). Additionally, the harmful impact of HMs on soil microorganisms can indirectly affect plant growth. HMs interference with microorganisms can hinder enzyme functions which is essential for plant metabolism. These direct and indirect toxic effects have led to reduced plant growth, even sometimes leads to plant death.

Mercury (Hg), is known as most toxic HM, has become significant part of the environment contaminant owing to human activities such as agriculture, mining, incineration, municipal wastewater discharge and release of industrial wastewater (Chen et al., 2012). Symptoms which are attributed to elevated level exposure to metallic Hg which includes vomiting, skin rashes, lung damage, diarrhoea, mucous membrane changes, nausea, renal dysfunction, increased heart rate or blood pressure and severe neurologic disorders (Asano et al., 2000). Main effects of Hg on human health and the environment are dependent on whether it is elemental Hg, inorganic Hg or organic Hg compounds. As a result, the risks linked with Hg exposure vary considerably depending on its form, which complicates the assessment of its toxicity.

Common bean is the most widely consumed legume globally, and it is the most significant legume cultivated for direct human consumption, with commercial value exceeding that of all other legume crops combined (Porch et al., 2013; Kumari et al., 2022). It has more nutritional significance and it is a good source for proteins and calories. Furthermore, it exhibits various biological properties such as antioxidant (Atchibri et al., 2010), antihypertensive (Talha et al., 2017), anti-inflammatory (Reverri et al., 2015), antidiabetic (Gupta et al., 2014), anti-urolithic (Namburu et al., 2017), anticancer (Hangen & Bennink, 2002), analgesic (Yang et al., 2018), antiobesity (Finley et al., 2007), antihypercholesterolemic (Maruyama et al., 2008) effects. Understanding the effects of Hg on their growth, development and biochemical pathways is crucial for protecting agricultural productivity and assessing the potential health risks of consuming contaminated crops. This research can guide strategies for alleviating Hg exposure in agricultural systems, thereby promoting sustainable farming practices and safeguarding human health. Thus, our present study was aimed to investigate the integrative approaches to understanding Hg toxicity in common bean (*Phaseolus vulgaris*) plants by linking growth, biochemistry and antioxidant mechanisms.

MATERIALS AND METHODS

The experimental protocol designed to meet the objectives were carried out using standard procedures. Common bean seeds were obtained from agricultural shop in Puducherry. Mercury chloride was used to induce Hg toxicity.

Seed sterilization

Uniform sized seeds were chosen and surface sterilized with 0.1% mercuric chloride for 2-3 minutes to avoid infection of fungus. These seeds were immediately washed multiple times with distilled water.

Polyethylene bag experiment

Polyethylene bag culture experiments were conducted to investigate the effect of the HMs toxicity in common bean plants. The growth medium in the polyethylene bags consist of artificially polluted soil with mercuric chloride at concentrations of 50, 100 and 200 mg of mercury chloride. Sterilized seeds were sown in each bag, placed in 2 cm deep holes made with a wooden stick. Each seed was then covered with a small amount of soil for suitable supplement of germination conditions.

Experimental design

After the initial phase, the common bean plants were divided into four groups. Group 1, with soil served as control, not received Hg treatment. In contrast, groups 2, 3 and 4 were exposed to Hg treatments of 50, 100 and 200 mg respectively. The plants were cultivated under conditions of average temperature, natural photoperiod and relative humidity.

Observation and measurements

The germination percentage (%) was determined by dividing the number of seeds that germinated each day by the total number of seeds, then multiplying by 100 and summing the results. The root length, measured from ground level to the tip of the root, is calculated using a standard centimeter scale. The shoot length, measured from ground level to the tip of the shoot, is calculated using a standard centimeter scale. The fresh weight of the whole plant is determined using electronic balance. The dry weight of the whole plant is determined using electronic balance. For vigour index data were recorded on germination basis. Using the mean value of root length and shoot length, vigour index was calculated by the formula of Abdul-Baki & Anderson (1973).

Vigour Index = (Mean Shoot length + Mean root length) × Germination %

Estimation of carbohydrates

The carbohydrate content was estimated using the method described by Hedge & Hofreiter (1962). For sample preparation, 1 g of fresh leaves was ground with 50 ml of potassium hydroxide, then centrifuged for 15 minutes, discarding the residue. The supernatant was adjusted to a final volume of 100 ml. The optical density (OD) was measured at 640 nm against blank.

Estimation of proteins

The protein content was estimated using Lowry et al. (1951). For sample preparation, 1 g of fresh leaves was ground with 10ml trichloroacetic acid, then centrifuged for 15 minutes and the supernatant was discarded. Then, the pellet was centrifuged with 5 ml of sodium hydroxide and the pellet was also discarded. The supernatant was adjusted to a final volume of 100 ml for sample. The optical density was measured at 660 nm against blank.

Estimation of catalase

Leaves were homogenized in a 100 mM phosphate buffer (pH 7). The activity of catalase (CAT: EC 1.11.1.6) was assayed using the method described by Sinha (1972). The resulting colour solution was measured at 620 nm at 0 and 60 seconds. Standards ranging from 2-10 micromoles were taken and preceded as the test alongside a blank that contained only the reagent.

Estimation of superoxide dismutase

Leaves were homogenized in 100 mM sodium pyro phosphate buffer (pH 8.3). The activity of superoxide dismutase (SOD: EC 1.15.1.1) was assayed using the method described by Kakkar et al. (1984). Measure the intensity if the chromogen in butanol layer at 560 nm, again butanol as blank and system devoid of enzyme serves as control.

Statistical analysis

Results were expressed as means \pm standard deviation of six plants per group. Data were analyzed by one-way analysis of variance and any significant differences among treatment groups were evaluated using Duncan's multiple range test. Results were considered statistically significant when $P < 0.05$. All statistical analyses were performed using SPSS version 15.0 software package (SPSS, Tokyo, Japan).

RESULTS AND DISCUSSION

HMs contamination in soil and water has become most important concern in the world, with significant effects for both the public health and environment (Briffa et al., 2020). This widespread contamination of HMs in the environment is mainly due to anthropogenic activities such as industry, improper waste disposal, mining and agriculture. As a result, harmful HMs accumulate in ecosystems, presenting serious problem to human health and environment (Singh et al., 2022). These metals are non-biodegradable and persistent, leading to long term pollution and biological process disruption (Zaynab et al., 2022). Prolonged exposure of HMs at elevated levels can cause both acute and chronic health issues, affecting various organ systems (Singh et al., 2021). Addressing this pollution is necessary for maintaining the balance of ecosystem, ensuring food safety and safeguarding the health of public people.

Germination percentage, root length and shoot length

Table 1 shows the effect of Hg stress in common beans plant on germination percentage, root length and shoot length of different experimental groups. Results obtained from the experiments revealed that germination percentage of common bean plants was highest at control plant. However, at Hg concentra-

tion of 50 mg, seed germination was reduced. Further, seed germination of common bean plants was reduced with increasing Hg concentration of 100 and 200 mg suggesting that Hg having negative impact on seed germination. This alien with previous findings by Shourie, 2022 who also reported that significant reduction in seed germination was observed due to the toxic effect of HMs in *Jatropha curcas* L., respectively. The relatively smaller reduction in seed germination in response to Hg exposure in the soil may be attributed to its nutritive significance to the growth of plants at low concentrations. However, the decrease in seed germination of common beans exposure to Hg may be attributed to its toxicity being shows more pronounced solubility in acidic soils. Thus, Hg uptake by plants would be higher in acidic soil leads to greater reduction in the plant growth will occur respectively.

Although Hg is an essential micronutrient and component of many enzymes, at elevated concentrations it becomes toxic to plants, humans and microorganisms. Even at low concentrations Hg is potentially toxic, and at concentration above 200 mg in soil is considered hazardous to human health (USEPA, 2001). Overall, Hg affects seed morphology and also physiology, inhibits germination, seedling development, root elongation, plant growth, chlorophyll production, transpiration, protein and water content. These effects result from disruptions in chloroplast function, hindering electron transport chain (ETC) and Calvin cycle enzyme inhibition which aligns with previous findings by Caroli et al. (2020) who also reported that exposure to HMs affecting several aspects of plant biology. These metals can disrupt cellular ionic homeostasis, they also interfere with the structure and function of important cellular organelles such as mitochondria, nucleus, chloroplasts and vacuoles. In addition, HMs impact macromolecules such as carbohydrates, proteins, lipids and nucleic acids. The cumulative effect of these disruptions affects various biochemical, physiological and molecular processes in plants, ultimately impairing their growth and health. Additionally, it was observed that root length and shoot length of common bean plants were affected by Hg toxicity. Results shows that Hg concentrations of 50 mg, 100 mg and 200 mg in the growth media reduced the root length and shoot length of common bean plants. Generally, the observed significant effect of HMs on the root and shoot growth can be largely attributed to the accumulation of HMs in plant which primarily occur in the roots with slighter accumulation in stems and leaves. Similar experimental results were obtained in Vijay et al. (2024) study, where it was shown that Hg effectively reduces the growth and development of plants. The suppression of root elongation reduces the plant's capacity to absorb water and essential nutrients, while the reduction in shoot length adversely affects overall biomass production.

Table 1. Effect of mercury in common bean plants on germination percentage (%), root length (in cm) and shoot length (in cm) of different experimental groups.

Groups	Germination percentage (%)	Root length (cm)	Shoot length (cm)
Control	90	13.98 \pm 1.13	32.43 \pm 2.4
Hg - 50mg	55	9.34 \pm 0.85	23.87 \pm 2.1
Hg - 100 mg	40	6.67 \pm 0.53	15.10 \pm 1.2
Hg - 200 mg	25	4.31 \pm 0.36	10.37 \pm 0.8

Values are expressed as mean \pm SD. Groups not sharing a common superscript letter differ significantly at $p < 0.05$. Duncan's multiple range test (DMRT).

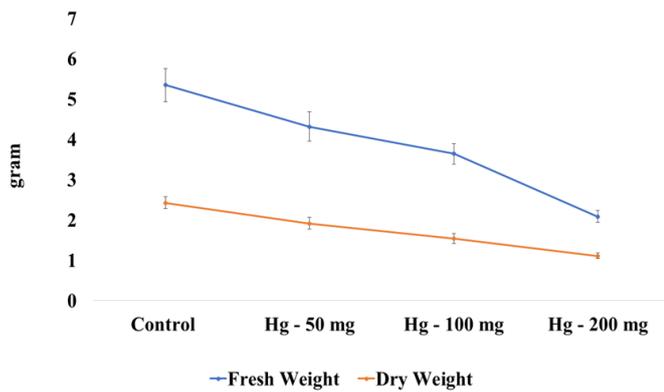


Figure 1. Effect of mercury on fresh weight and dry weight on different experimental groups of common bean plants. Values are expressed as mean \pm SD. Groups not sharing a common superscript letter differ significantly at $p < 0.05$. Duncan's multiple range test (DMRT).

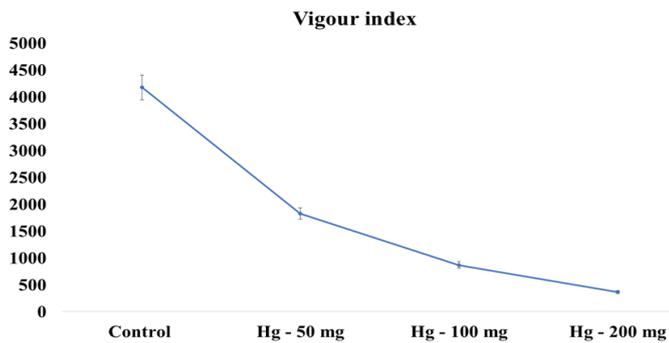


Figure 2. Effect of Hg on vigour index on different experimental groups of common bean plants.

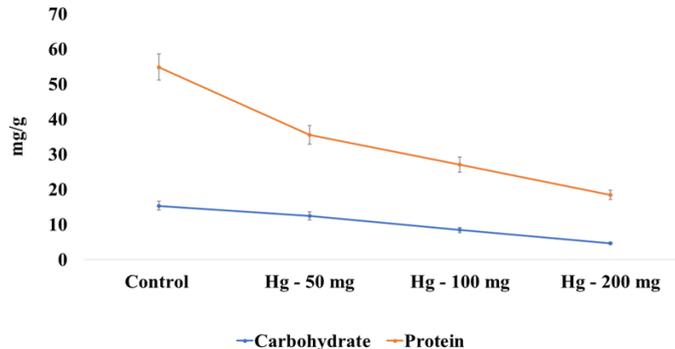


Figure 3. Effect of Hg on carbohydrate and protein contents on different experimental groups of common bean plants. Values are expressed as mean \pm SD. Groups not sharing a common superscript letter differ significantly at $p < 0.05$. Duncan's multiple range test (DMRT).

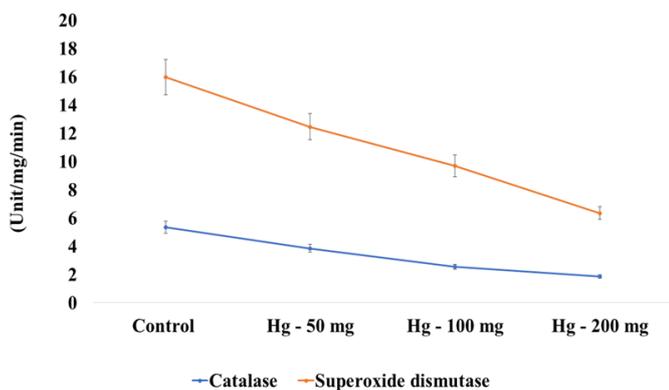


Figure 4. Effect of mercury on enzymic antioxidants status on different experimental groups of common bean plants. Values are expressed as mean \pm SD. Groups not sharing a common superscript letter differ significantly at $p < 0.05$. Duncan's multiple range test (DMRT).

Fresh weight, dry weight and vigour index

Figure 1 illustrates the effect of Hg induced stress on fresh weight, dry weight, while figure 2 shows the effect of Hg on vigour index on different groups of common bean plants. These observations were recorded at 30th day after sowing. The dry weight of the seedling has the most substantial positive indirect influence on seedling vigour through seed vigour index followed by fresh weight of the seedling, absolute seedling water content, radicle length, radicle elongation rate, seedling length, hypocotyl elongation rate and hypocotyl length. These findings suggest that seedling vigour is a complex character influenced by multiple parameters and it plays an important role in seed technology. Understanding the direct and indirect effects of these traits can help in designing effective selection strategies and identifying the important parameters to target for improvement. The fresh weight, dry weight and vigour index of common bean plants were significantly affected at all Hg concentrations as compared to the control group. The results concluded that elevated concentrations of Hg inhibited growth, induced structural alterations and significantly reduced the biochemical and physiological activities, ultimately disrupting the plant's internal structure (Mohapatra et al., 2021). It has been already reported that plants can lead to toxicity symptoms when it is exposed to HMs in soil such as seed germination inhibition, plant height reduction, decreased root and shoot growth (Vijay et al., 2024).

Carbohydrates and protein contents

Figure 3 illustrates the effect of Hg on total carbohydrate and protein contents on three groups of tested common bean plants. These observations were recorded at 30th day after sowing. Hg severely affects plant growth results in morphological alterations in medicinal and food crops. It induces lipid peroxidation, and reactive oxygen species (ROS) generation which activate signaling molecules that generate bioactive compounds. It also causes chromosomal aberrations, disrupting the metabolism of phytochemicals and reduces chlorophyll pigment levels, thereby suppressing photosynthesis. This inhibition, coupled with interference in uptake of minerals and activities of enzymes further exacerbates the plant's overall stress (Chakraborty & Choudhury, 2023) results in decreased carbohydrate synthesis and a decline in carbohydrate levels. Our findings showed that Hg treated common bean plants exhibits significant reduction in carbohydrates content as compared to normal control plants. This observation is consistent with the previous study by Duan et al. (2020), who found that HMs exposure leads to diminished carbohydrate levels in *Glycine Max*. Ultimately, Hg stress lowers water potential and causes cell death, further exacerbating growth, development and metabolic disturbances.

Additionally, results from our study reveals that protein contents were significantly decreased in Hg treated plants when compared to control plants with more pronounced loss observed at higher Hg concentrations. Vijay et al. (2024) also reported a significant reduction in protein levels with increasing Hg exposure. Higher cellular concentrations of Hg lead to oxidation of biological macromolecules like proteins results from

harmful effects of ROS generated during normal cellular metabolism (Cyboran-Mikolajczyk *et al.*, 2022). This observed decline in protein contents at increasing concentrations of Hg in common beans plants may be due to the process of enhanced protein degradation likely caused by increased activity of protease under stress conditions. It is well known that excessive ROS results in oxidative stress, which damages the biomolecules such as Proteins, lipids and DNA (Hu *et al.*, 2020). Moreover, the toxic consequences of Hg may induce protein fragmentation and lipid peroxidation through ROS generation, further contributing to the reduced protein content.

Enzymic antioxidants (catalase and Super oxide dismutase)

For equilibrium maintenance in response to more synthesis of ROS under stressful conditions, plants generate enzymic antioxidants and osmolytes. When plants experience a decline in their photosynthetic machinery, ROS accumulate in the cells, damaging the functions of mitochondria and chloroplast which ultimately leads to cell death (Khalid *et al.*, 2022). Figure 4 shows the effect of Hg stress on enzymic antioxidant status on different groups of experimental common bean plants. Compared to control plants, activity of catalase enzyme was decreased in Hg tested common bean plants in our study. This reduction in catalase activity under various concentrations of Hg, likely contributed to the increased hydrogen peroxide (H₂O₂) production in Hg affected common bean plants. The decline in catalase activity at high Hg stress may be due to enzyme synthesis inhibition process. Superoxide dismutase is another important enzyme for protecting cells against oxidative damage. It catalyzes the conversion of O₂⁻ to H₂O₂ and O₂. In our study, we have observed a decline in SOD activity in common bean plants when compared to control plants. This decrease in SOD activity may also be attributed to decreased binding of cofactor metal ions which is necessary for enzymic activity, as elevated Hg concentrations may inhibit the uptake of essential metal ions required for enzymic activity. Additionally, HMs caused a reduction in concentrations of total protein, likely due to proteolysis and decreased antioxidant capacity (Georgiadou *et al.*, 2018). Furthermore, metal ions may bind to the enzyme's active site, impairing its function. A high concentration of HMs entered the cells, disrupting the function of the antioxidant system (Ben *et al.*, 2023). The excess ROS generated under HMs stress conditions disrupts the metabolic pathways and inactivate enzymic antioxidants. This increased production of ROS can trigger cell death by apoptosis process through mechanisms such as enzyme activity inhibition, lipid peroxidation, protein oxidation or nucleic acids damage (Fujii *et al.*, 2022).

Conclusion

In conclusion, the present findings confirm that Hg exert toxic effects in common bean plants as evidenced by decrease in growth parameters such as germination percentage, root length, shoot length, fresh weight, dry weight and vigour index under Hg stress as compared to control plants. Additionally, carbohy-

drates and protein contents were reduced and the levels of antioxidant enzymes including catalase and super oxide dismutase were decreased in Hg treated plants than in untreated controls, further highlighting the harmful impact of Hg toxicity on common beans. These findings offer valuable insights into how Hg affects plant physiology, particularly by disrupting growth and metabolic processes. The observed significant reductions in growth parameters may be linked with Hg's interference with cellular functions such as synthesis of protein, activity of enzymes and cellular respiration. Moreover, depletion in the levels of enzymic antioxidants suggests that the plant's defense system is overwhelmed, resulting in increased oxidative damage. Looking forward, these findings highlight the need for future research aimed at mitigating the effect of Hg toxicity on plants.

DECLARATIONS

Author contribution statement

Conceptualization: VM; Methodology: VM.; Software and validation: VM; KD and JJ; Formal analysis and investigation: VM; Resources: VM; Data curation: VM; Writing—original draft preparation: VM; Writing—review and editing: VM, KD and JJ; Visualization: VM; Supervision: VM; Project administration: VM; Funding acquisition: VM. All authors have read and agreed to the published version of the manuscript.

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Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

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Supplementary data: Not available.

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