Bioaccumulation of Mercury and its consequences on Biochemical parameters in Coriander (*Coriandrum Sativum*) plants

ABSTRACT

Mercury is a highly toxic heavy metal that poses severe environmental and ecological issues due to its ability to bioaccumulate in various ecosystems. It persists in the environment and is known to have serious effects on both plants and animals. This study investigates the bioaccumulation of Mercury and its consequences on biochemical parameters in Coriander (*Coriandrum Sativum*) plants. The experimental design involved four groups, with Group 1 serving as the control, received no mercury treatment, while Groups 2, 3, and 4 were subjected to mercury concentrations of 50, 100, and 200 mg respectively. These varying concentrations of mercury allowed for a detailed examination of the dose dependent effects of mercury exposure on plant growth and metabolic functions. The results demonstrated that mercury treatment caused a marked decline in crucial growth parameters, including germination percentage, root length, shoot length, fresh weight, dry weight and vigor index, all of which were significantly lower in the treated groups compared to the control plants, indicating that contamination of mercury hindered the overall growth and development of the plants. Biochemical analysis revealed that mercury exposure disrupted various metabolic processes, leading to oxidative stress and an imbalance in reactive oxygen species (ROS). Increased mercury concentrations were associated with a significant reduction in carbohydrate metabolism and protein synthesis, reflecting impairments in energy metabolism and overall physiological functions. The reduction in protein content was linked to enzyme inhibition, compromising protein synthesis pathways essential for cellular functions and stress tolerance. These findings highlight the phytotoxic effects of mercury and its detrimental effects on plant physiology, ultimately limiting growth potential and photosynthetic efficiency. This research contributes valuable insights into the ecotoxicological impacts of mercury contamination in plants and underscores the potential of plant-based phytoremediation strategies for mitigating heavy metal pollution in contaminated soils.

Key words: Coriander, Germination, Heavy metals, Mercury, Plants, Pollution.

INTRODUCTION

Soils can become contaminated with heavy metals (HMs) and metalloids from various sources, including waste disposal, wastewater irrigation, industrial emissions, fertilizers, petrochemical spills, mine tailings, manures, sewage sludge, pesticides, atmospheric deposition, coal residues, leaded gasoline and paints (Zhang et al., 2010). This contamination presents significant risks to human health and ecosystems through direct contact with the soil, decreased food quality, contaminated drinking water, disruption of the food chain, land tenure conflicts, and reduced agricultural productivity (McLaughlin et al., 2000).

Protecting and restoring soils contaminated by HMs requires proper characterization and remediation efforts. Environmental protection regulations, both at the national and international levels, depend on data regarding the chemical properties of environmental factors, especially those affecting the food chain (Kabata-Pendias and Pendias, 2001). Soil characterization is essential for understanding the speciation and bioavailability of HMs, while remediation necessitates knowledge of the sources, chemistry, and health impacts of contamination. Risk assessment plays a crucial role in managing contaminated sites efficiently, ensuring the protection of public health and ecosystems (Zhao and Kaluarachchi, 2002).

Understanding the environmental, chemical and health impacts of HMs is essential for evaluating their bioavailability, speciation, and remediation strategies. The fate and transport of HMs in soil are primarily determined by their chemical forms. Upon entering the soil, metals experience rapid adsorption like minutes to hours, followed by slower processes like days to years, which results in redistribution into different forms with varying mobility, bioavailability, and toxicity (Buekers, 2007). As awareness of soil contamination's effects on human and animal health increases, there is a growing focus on developing effective remediation technologies (Bolan et al., 2008).

Mercury (Hg) is a highly toxic HM ubiquitously distributed in the environment, existing in three chemical forms such as elemental mercury, organic mercury and inorganic mercury. Each form poses distinct toxicological risks. Human exposure to Hg occurs primarily through occupational and environmental pathways. Occupational exposure occurs in industries which involves Hg use, including mining and manufacturing processes, with additional risks from waste disposal and industrial effluents. Environmental exposure is mainly through the

consumption of Hg contaminated foods particularly sea fish, dental amalgams, drinking contaminated water and the use of Hg containing products like fluorescent lamps and batteries.

Coriander (*Coriandrum sativum*) is an annual herb from the Apiaceae family, with all parts of the plant being edible. However, the fresh leaves and dried seeds (used as a spice) are the most commonly utilized in culinary practices. Coriander is recognized for its diverse biological properties, including antioxidant (Lakhera et al., 2015), anticancer (Tang et al., 2013), hypoglycemic (Eidi et al., 2009), hypolipidemic (Joshi et al., 2012), anti-inflammatory (Wu et al., 2010), analgesic (Batista et al., 2008), antihypertensive (Jabeen et al., 2009), antimicrobial (Zardini et al., 2012) effects.

Coriander serves as an effective model organism for investigating HMs toxicity, particularly Hg, due to its high sensitivity to metal contamination and the adverse effects of Hg on its growth and phytochemical properties. Consequently, coriander's response to Hg induced biochemical alterations and metal uptake provides significant insights into the mechanisms underlying HMs toxicity in plants. This research enhances our understanding of the potential risks posed by Hg contamination to plant health and its subsequent impact on human consumption through the food chain. By examining the bioaccumulation of Hg in coriander, researchers can develop remediation strategies to mitigate Hg pollution in agroecosystems, thereby improving food safety. Thus, our present study is aimed to investigate the bioaccumulation of Hg and its consequences on biochemical parameters in Coriander plants.

MATERIALS AND METHODS

The experimental protocol designed to achieve the objectives was executed in accordance with established standard operating procedures. Coriander seeds were procured from a local agricultural supply store in Puducherry, and mercuric chloride was employed to induce Hg toxicity in the plants.

Seed Sterilization

Uniform-sized coriander seeds were selected for the experiment. To prevent fungal contamination, the seeds were surface sterilized with a 0.1% mercuric chloride ($HgCl_2$) solution for 2-3 minutes. After sterilization, the seeds were promptly removed and thoroughly rinsed multiple times with sterile distilled water to remove any residual chemicals.

Polyethylene bag experiment

Polyethylene bag culture experiments were conducted to investigate the effects of Hg toxicity on *Coriandrum sativum* plants. The growth medium in the polyethylene bags consisted of artificially contaminated soil, with Hg concentrations of 50, 100, and 200 mg. Sterilized seeds were sown in each bag by creating 2 cm deep holes using a wooden stick. Each seed was subsequently covered with a thin layer of soil to ensure optimal conditions for germination process. Soil moisture content was maintained and adjusted regularly based on the soil's water holding capacity by using tap water to ensure proper hydration and support for seedling development.

Experimental design

Following the preliminary phase, *Coriandrum sativum* plants were assigned to four distinct treatment groups. Group 1, serving as the control, contained soil without any Hg treatment. In contrast, the plants in Groups 2, 3, and 4 were exposed to Hg concentrations of 50 mg, 100 mg, and 200 mg, respectively, throughout the experimental period. The plants were cultivated under controlled environmental conditions which includes relative humidity, average ambient temperature and natural photoperiod to simulate typical growth conditions.

Germination parameters

The germination percentage was calculated by dividing the number of germinated seeds on each day by the total number of seeds, then multiplying by 100 to obtain the daily germination rate. The cumulative germination percentage was determined by summing the daily percentages over the experimental period.

Germination rate = No. of Seeds germination/Total number of seeds

Germination %= Germination rate × 100

Root length, Shoot length, Fresh Weight and Dry weight

The root length, measured from ground level to the root tip, and the shoot length, measured from ground level to the shoot tip, were both quantified using a standard centimeter scale, while the fresh weight and dry weight of the entire plant were assessed using an electronic balance, following harvest and subsequent desiccation in a controlled environment.

Vigour Index

The vigour index was determined based on germination parameters, with the root length and shoot length mean values being used to calculate the index according to the formula established by Baki and Anderson (1973). This calculation reflects the overall seedling growth and performance by integrating both morphological and physiological growth metrics.

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

Biochemical Estimations

Estimation of Carbohydrate

The carbohydrate content was determined quantitatively using the method described by Hedge and Hofreiter (1962). A volume ranging from 0.2 to 1.0 ml of the working standard carbohydrate solutions was accurately pipetted into separate test tubes, while 0.5 mL of the sample extract was placed in a different test tube. The volume in each test tube was then adjusted to 1 ml with distilled water. Following this, 4 ml of Anthrone reagent was added to each test tube. The test tubes were shaken to mix the contents thoroughly, then heated in a boiling water bath for 20 minutes. Afterward, the solutions were cooled, and the absorbance of the resulting green colored complex was measured at 640 nm.

Estimation of proteins

The protein content was determined using the Lowry method (1951). A range of 0.2 to 1.0 ml of the working standard protein solutions was accurately pipetted into a series of test tubes. Additionally, 0.2 ml of the sample extract was added to a separate test tube. Each of these test tubes was then filled with distilled water to a final volume of 1.0 ml, with 0.5 ml of distilled water serving as the blank. The contents of the tubes were thoroughly mixed and allowed to stand for 10 minutes. Following this, 0.5 ml of Folin-Cio calteu reagent was added to all the test tubes, and the mixtures were shaken well. The test tubes were then incubated at room temperature in the dark for 30 minutes, during which a blue colour developed. The absorbance of the resulting solution was measured spectrophotometrically at 660 nm.

Statistical analysis

The results are presented as means±standard deviation based on six plants per group. Data were analyzed using one-way analysis of variance (ANOVA), and any significant

differences between treatment groups were assessed using Duncan's multiple range test. Results were considered statistically significant when $P<0.05$. All statistical analyses were conducted using the SPSS software package, version 15.0 (SPSS, Tokyo, Japan).

RESULTS AND DISCUSSION

Germination percentage, Root length and Shoot length

Figure 1 illustrates the impact of Hg stress on the germination rate of coriander seeds, quantifying the germination percentage under various concentrations of Hg. Figure 2 presents the influence of Hg exposure on the growth parameters of coriander plants, specifically assessing root length and shoot length across different experimental conditions and Hg concentrations. These measurements were recorded on the 30th day after sowing. The application of a HMs mixture significantly inhibited seed germination, with the inhibitory effect increasing in correlation with the concentration of Hg in the coriander plants. The germination percentage of coriander was adversely affected by the varying concentrations of the HMs mixture. Hg, recognized as one of the most toxic HMs to biota, is particularly detrimental to root development, leading to significant inhibition of root elongation (Wang et al., 2013). A marked overall inhibition in both seed germination and seedling growth was observed in Hg treated coriander plants as compared to untreated control plants. Both root and shoot lengths were progressively reduced with increasing Hg concentrations in the growth medium. Elevated Hg levels severely suppressed plant growth, indicating a detrimental effect on plant morphology and physiological processes. These findings are consistent with previous studies, such as that by Muhammad et al. (2015), which reported a significant decrease in seed germination of mungbean exposed to high concentrations of Hg, providing evidence that excess Hg can be inhibitory to plant growth and development.

Figure 1: Effect of Mercury on Germination percentage in different experimental groups of Coriander plants.

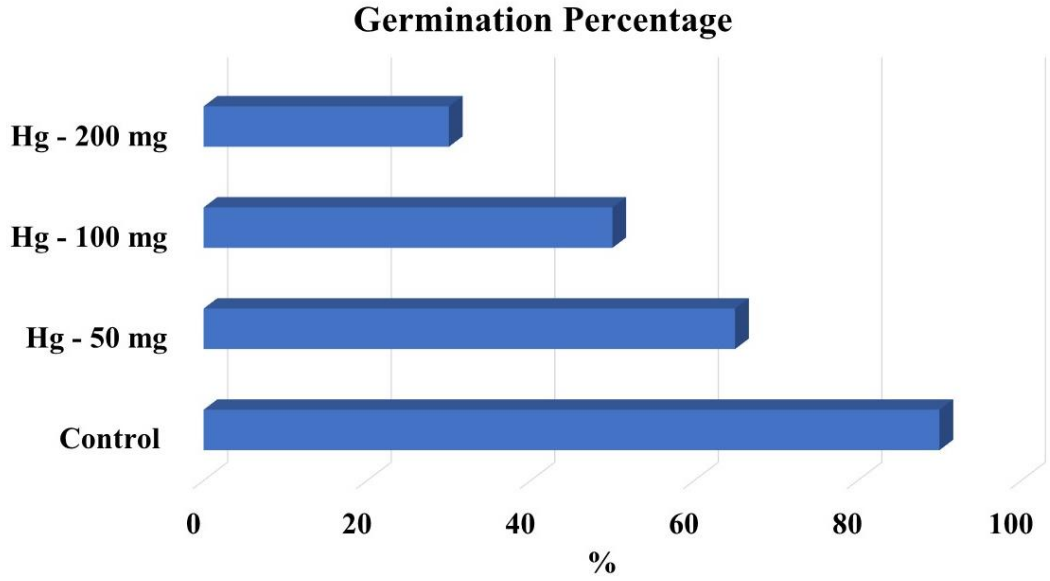
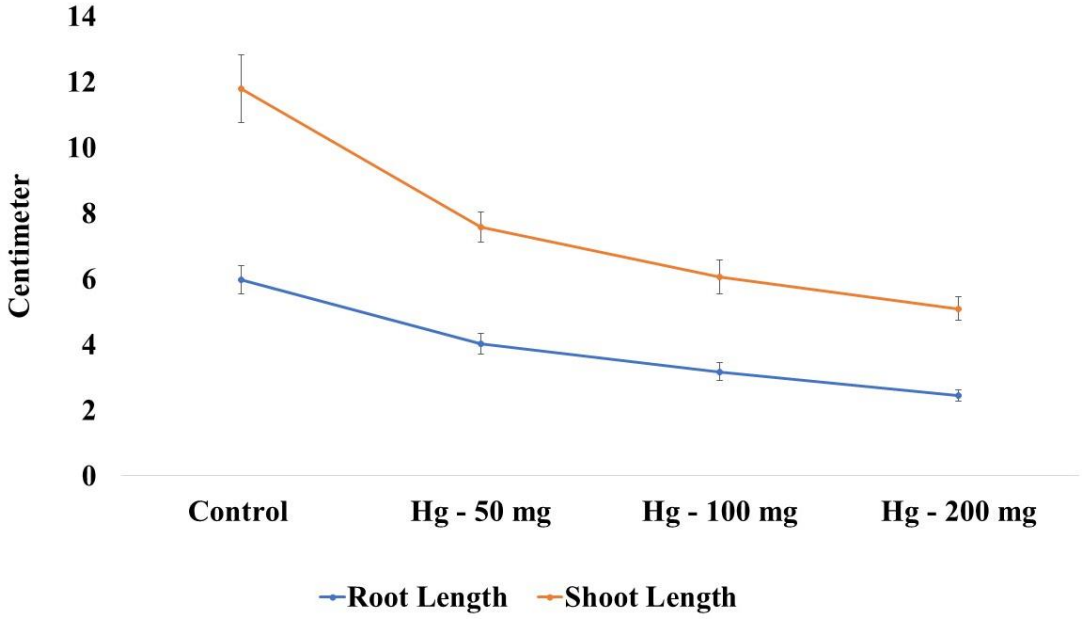


Figure 2: Effect of Mercury on Root length and Shoot length in different experimental groups of Coriander plants.



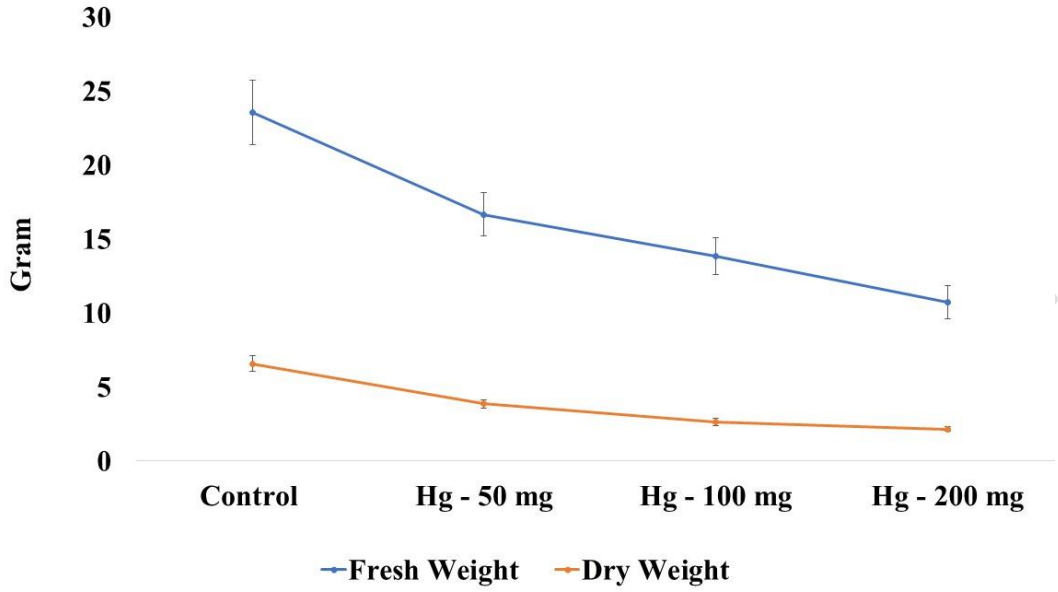
Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Fresh weight, dry weight and vigour index

The findings from the figures and studies referenced illustrate the detrimental effects of Hg exposure on the growth and development of coriander plants. Figure 3 highlights the negative impact of Hg stress on both fresh and dry weight in experimental coriander plants, showing significant reductions in these parameters as Hg concentrations increase. Specifically, the fresh and dry weights of the plants were significantly lower under Hg toxicity as compared to the control group, suggesting that Hg exposure inhibits normal plant growth and biomass accumulation. Figure 4 further examines the effect of Hg on the vigour index, which combines different growth measures of seedlings. The vigour index was also significantly reduced in Hg treated plants, supporting the conclusion that higher Hg concentrations suppress the overall plant's growth. These observations were recorded 30 days after sowing, providing an early indication of the phytotoxic effects of Hg.

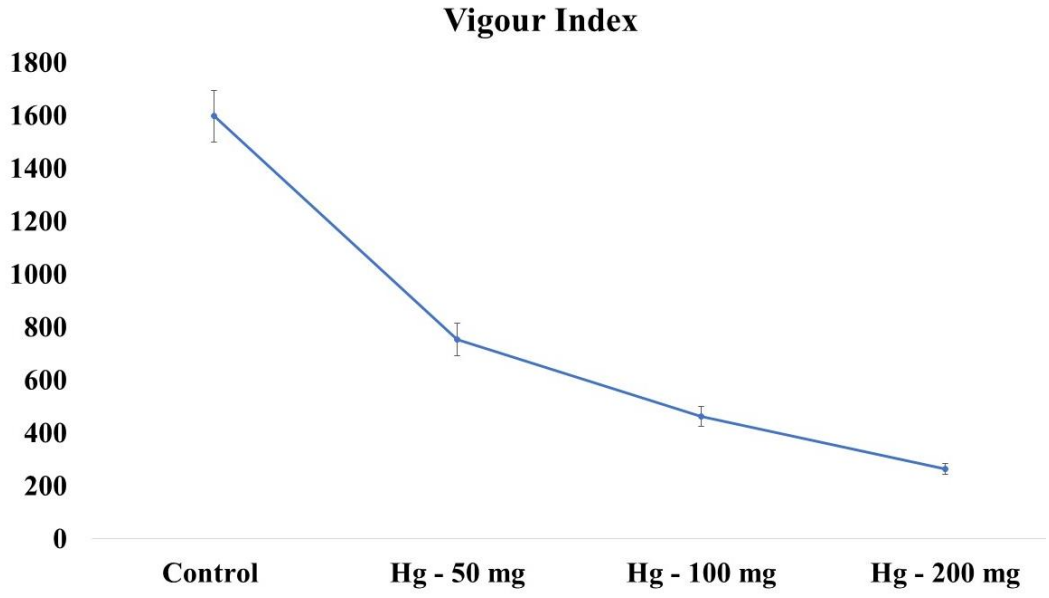
The underlying physiological mechanisms behind these significant reductions can be attributed to the cellular process disruption. As cited by Pirselova et al. (2012), Hg exposure can inhibit the production of important growth regulators like auxin and cytokinin, which are necessary for proper regular cell division and overall plant's development. Without proper cell division, the plants fail to accumulate biomass efficiently, which results in reduced fresh and dry weights. Furthermore, the results align with the findings of Gandhi et al. (2020), suggesting that while there may be an initial increase in dry weight at early growth stages due to accumulation of metals, the prolonged exposure to high Hg levels (such as the 200 mg concentration) leads to greater stunted growth and phytotoxicity. This effect was more pronounced in the plants tested with the highest concentration of Hg, in comparison to the lower doses of 50 mg and 100 mg, as well as the control group. Finally, these observations correlate with previous studies, such as the work of Vijay et al. (2024), which similarly reported that increasing concentrations of Hg led to a significant reduction in growth parameters like fresh weight, dry weight and vigour index in common bean plants. This reinforces the conclusion that Hg has a significant negative impact on plant growth and development.

Figure 3: Effect of Mercury on Fresh Weight and Dry Weight in different experimental groups of Coriander plants.



Values are expressed as mean±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 Vigour index in different experimental groups of Coriander plants.



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

Carbohydrates and protein contents

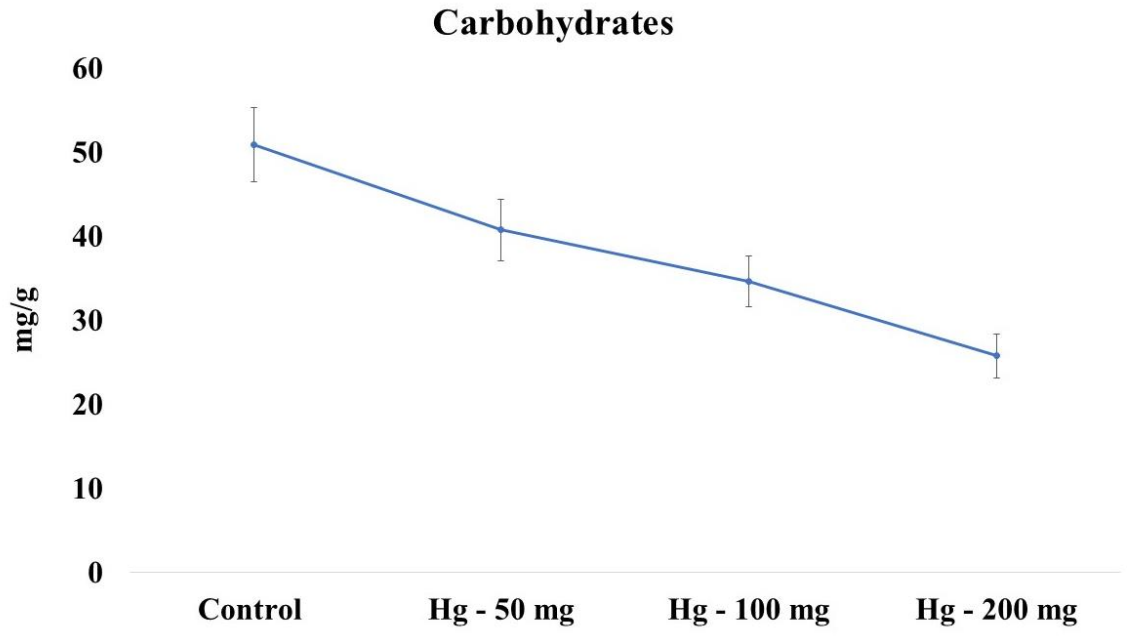
Figure 5 illustrates the effect of Hg exposure on the total carbohydrate content, examining the alterations in the metabolism of carbohydrates in coriander plants subjected to varying concentrations of Hg. Figure 6 highlights the impact of Hg stress on the concentrations of proteins, evaluating alterations in protein biosynthesis across different experimental groups of coriander plants. These biochemical analyses were performed on the 30th day after sowing to assess the cumulative physiological responses to Hg exposure. Biochemical analysis provides a comprehensive understanding of the plant's phytochemical and metabolic reprogramming, elucidating the changes in primary and secondary metabolites triggered by HMs toxicity, thus offering insights into the phenotypic plasticity observed under Hg exposure.

Carbohydrates are important essential biomolecules which plays a significant role in plant defense mechanisms against various abiotic stresses. They function as protectants for cellular macromolecules, particularly proteins, and act as osmoprotectants by stabilizing cellular structures under stressful conditions. Notably, Hg is readily absorbed by plant roots and negatively affected the plant growth and productivity by disrupting important physiological and biochemical processes, including nutrient uptake, enzyme activity and cellular respiration (Ahmad et al., 2018). The results from the present study demonstrate a significant reduction in carbohydrate contents in *Coriandrum sativum* plants exposed to Hg stress, compared to the untreated controls. This suggests that the presence of HMs, particularly Hg, impairs carbohydrate biosynthesis and accumulation. More pronounced decline in the levels of carbohydrate was observed in seedlings subjected to various Hg concentrations, indicating a detrimental effect of Hg exposure on carbohydrate metabolism in coriander. Since, Hg has long been recognized for its inhibitory effects on energy generating processes and metabolic pathways in plants, possibly through ionic imbalances and osmotic stress that disrupt enzymic functions and ion transport mechanisms (Greenway & Munns, 1980). Additionally, the significant reduction in photosynthetic efficiency due to Hg stress likely contributed to decreased carbohydrate synthesis, which, in turn, led to lower carbohydrates concentrations and a subsequent decline in growth rate and biomass accumulation, as previously reported (Soni and Thanki, 2014).

Proteins are fundamental macromolecules essential for cellular structure, function, and regulation, playing a significant role in nearly all biological processes. They are highly susceptible to disruption by different environmental stressors, including HMs. Elevated concentrations of HMs have been shown to adversely affect the synthesis of proteins, leading to

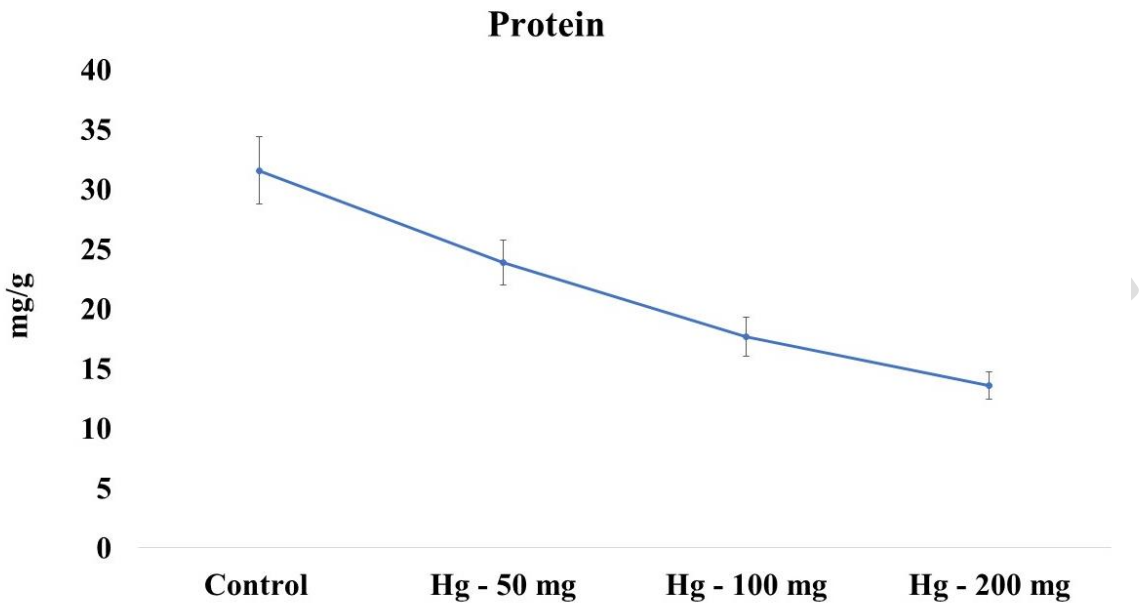
a reduction in protein contents (Tandon and Gupta, 2002). In the present study, results indicate that protein levels in *Coriandrum sativum* plants were significantly diminished under Hg exposure, compared to the control group. This reduction may be attributed to the inhibition of protein biosynthesis, a well documented effect of HMs toxicity (Samantary, 2002). Furthermore, Hg exposure could impair the activity of key enzymes involved in nitrogen assimilation, such as nitrate reductase, which plays an important role in amino acid and protein synthesis (Vajpayee et al., 2000). These findings are consistent with previous studies by Vijay et al. (2024), who observed a significant decrease in both carbohydrate and protein contents in pumpkin plants subjected to increasing Hg concentrations. The observed decline in protein content under Hg stress suggests an overall disruption of cellular metabolism, which could hinder growth and physiological function.

Figure 5: Effect of mercury on carbohydrate contents in different experimental groups of Coriander plants.



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

Figure 6: Effect of mercury on protein contents in different experimental groups of Coriander plants.



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

CONCLUSION

Based on the experimental results, the following conclusions can be drawn: Exposure to Hg stress significantly impaired various growth parameters of coriander plants, including germination percentage, root length, shoot length, fresh weight, dry weight and vigour index, when compared to the control group. The observed reductions in these growth parameters suggest that Hg toxicity inhibits essential physiological processes such as seed germination, root and shoot development, and overall plant biomass accumulation. Additionally, Hg stress led to a marked reduction in the levels of both total carbohydrates and protein content, indicating a disturbance in metabolic processes and synthesis of proteins. This decline in carbohydrate and protein levels reflects the compromised energy storage and biosynthetic capabilities of the plants under Hg exposure. Furthermore, the decreased vigour index under Hg stress suggests an overall decline in the plant's ability to grow and establish, further highlighting the detrimental effects of Hg on coriander plant health. These findings underscore the toxic impact of Hg on plant growth and metabolism, potentially limiting agricultural productivity in contaminated environments.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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