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Toxicological effects of Mercury in Radish (*Raphanus Sativus*) plants – Biochemical Analysis

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Abstract **Original Research Article**

Mercury is one of the major toxic heavy metal, poses significant risks to both the environment and human health due to its persistence and its capability to accumulate within ecosystems. The primary route of mercury exposure is through contaminated soil, water and food sources, which can lead to substantial damage to living organisms. In particular, mercury exposure adversely affecting the kidneys, nervous system and other important organs. This study investigates the toxicological effects of Mercury in Radish (*Raphanus Sativus*) plants through biochemical analysis. The experimental plants were divided into four groups, Group 1, the control group, received no mercury treatment, while Groups 2, 3 and 4 were subjected to mercury concentrations of 50, 100 and 200 mg, respectively. The results demonstrated that mercury exposure led to a significant reduction in important growth parameters. Specifically, the germination percentage, root and shoot lengths, fresh and dry weight, and vigor index were all markedly decreased in the mercury treated groups compared to the control. Biochemical analysis revealed the mercury's clear negative impact on the metabolic processes in radish plants. Mercury at higher concentrations was associated with a notable reduction in carbohydrate and protein levels, reflecting the plant's impaired physiological functions. Additionally, the activities of key enzymic antioxidants, such as catalase and superoxide dismutase, were significantly reduced under mercury induced stress. By exploring the biochemical and physiological effects of mercury exposure, this research provides valuable insights into how mercury accumulation impacts plant health.

Keywords: Environment, Heavy metals, Mercury, Radish, Soil pollution, Toxicity.

INTRODUCTION

Heavy metals (HMs) have been shown to affect various cellular organelles and components, including the cell membrane, nuclei, endoplasmic reticulum, mitochondria, lysosomes and enzymes involved in metabolism, detoxification, and damage repair (Wang *et al*., 2001). These metal ions can interact with cellular components such as nuclear proteins and DNA, leading to DNA damage and conformational changes that may disrupt the cell cycle, induce apoptosis and promote carcinogenesis. (Beyermann and Hartwig, 2008). HMs, once released from different sources, can enter soil, water, vegetation water, with their movement largely influenced by its density. Once deposited in these systems, since metals cannot be broken down and remain persistent in the environment, posing significant risks to human health through ingestion, inhalation and skin absorption. Acute exposure to these metals may result in symptoms such as anorexia, dermatitis, nausea, vomiting and gastrointestinal issues. Chronic exposure can also impair mental and central nervous functions (Gybina and Prohaska, 2008), kidneys (Reglero *et al*., 2009), livers (Sadik, 2008), lungs (Kampa and Castanas 2008), alter blood composition (Cope *et al*., 2009), and other vital organs (Lindemann *et al*., 2008) in the body.

In the environment, Mercury (Hg) is one of the highly toxic heavy metal (Castro-González & Méndez-Armenta, 2008) and is persistent pollutants that can bioaccumulate in humans, animals and fishes (Chang *et al*., 2009). Mercury salts and organomercury compounds are among the most harmful substances present in the environment. Mostly, terrestrial plants are less sensitive to Hg's harmful effects, it can still disrupt photosynthesis and oxidative metabolism by interfering with the mechanism of electron transport in mitochondria and chloroplasts. Hg and its compounds are collective toxins, and even lower concentration can pose significant health issues. Exposure to high levels of metallic, organic or

inorganic Hg can cause permanent damage to the kidney, brain and developing fetus (ATSDR, 2003b). The toxicity and its consequences are significantly influenced by the type of Hg compound and its redox state. Hg contamination in the environment is primarily caused by various industries, including painting, petrochemicals and mining, as well as agricultural sources like fungicidal sprays and fertilizers. The primary symptoms of Hg poisoning are renal and neurological disorders, as Hg can easily cross the blood-brain barrier, affecting the functions of brain (Resaee *et al*., 2005).

Radish (*Raphanus sativus*) an edible root vegetable belonging to the family *Brassicaceae,* was domesticated in Asia during pre-Roman antiquity. Radishes are cultivated and consumed globally, predominantly in their raw form as a crisp, pungent salad ingredient. They exhibit considerable variability in terms of flavor, size, color and maturation duration. Radishes usually having rapid germination and swift growth, with smaller cultivars also reaching harvestable maturity within approximately one month itself, while larger varieties, such as daikon, require several months for full development. Due to its easy cultivation and fast harvest cycle, radishes are often favored by novice horticulturists. Radish is known to possess many biological properties such as antioxidative (Xiaoling *et al*., 2001), anticancer (Khalid *et al*., 2018), antimicrobial (Kim *et al*., 2010), cardioprotective (Jin and Kyung, 2001), nephroprotective (Salah-Abbes *et al*., 2008), antidiabetic (Ouyang *et al*., 2016) and antiviral (Strack *et al*., 1985) effects.

Radish plants are frequently utilized in studies of HMs toxicity due to its rapid growth and high sensitivity to environmental stressors, and their ability to bioaccumulate metals within their tissues. These traits render them valuable bioindicators for investigating the HM effects on both plant health and the surrounding ecosystem. Radishes have the capacity to absorb and concentrate HMs from polluted soil, providing an ideal model for exploring the metal uptake, translocation and potential pathways of detoxification mechanism. Furthermore, radish plants are widely employed in the research of phytoremediation to assess their potential for the removal or immobilization of toxic HMs from polluted soils, presenting a promising, eco-friendly approach to alleviating environmental contamination. Therefore, the present study is aimed to evaluate the toxicological effects of Hg exposure in Radish plants through biochemical analysis.

MATERIALS AND METHODS

The experimental protocol deduced in order to fulfill the objectives were carried out with standard procedures in plant toxicity studies. Radish seeds were sourced from a reputable agricultural supply store in Puducherry. Mercury chloride, a well established toxicant, was utilized to induce Hg toxicity in the plants.

Seed Sterilization

Uniform sized radish seeds were carefully selected to minimize variability in seed size and ensure consistency in the experimental results. To prevent fungal contamination and microbial growth, the seeds were surface sterilized using a 0.1% mercuric chloride solution for 2-3 minutes. After the treatment, the seeds were promptly washed several times with sterile distilled water to thoroughly remove any remaining mercuric chloride and prevent any potential phytotoxic effects. This step ensured that the seeds were free from external contaminants, maintaining the integrity of the experimental conditions.

Polyethylene Bag Experiment

Polyethylene bag culture experiments were conducted to investigate the effects of Hg toxicity on radish plants. The growth medium in the polyethylene bags consisted of soil artificially contaminated with Hg at concentrations of 50, 100, and 200 mg. To plant the sterilized seeds, 2 cm deep holes were made in the soil using a wooden stick, with one seed sown in each hole. The seeds were then lightly covered with a small amount of soil to ensure proper conditions for germination. Soil moisture content was maintained by regularly adjusting the level of water to match the soil's water holding capacity using tap water. The Hg concentration was carefully chosen based on previous studies to achieve appropriate exposure levels for the experiment.

Experimental Design

After the initial phase, the radish plants were separated into four distinct groups. Group 1, which served as the control, consisted of bags with soil that were not treated with Hg. In contrast, groups 2, 3 and 4 were exposed to Hg concentrations of 50, 100 and 200 mg, respectively. The plants were cultivated under controlled conditions, with relative humidity, natural photoperiod, and average temperature maintained throughout the experiment.

Germination Parameters

Germination percentage (%) was calculated by dividing the seed germination on each day by total number of seed × 100 and finally adding the total percentage.

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

Germination %= Germination rate × 100

Root Length and Shoot Length

The root length is measured from the ground level to the tip of the root, and the shoot length is measured from the ground level to the tip of the shoot, both using a standard centimeter scale.

Fresh Weight and Dry Weight

The fresh weight and dry weight of the entire plant are measured using an electronic balance.

Vigour Index

The vigour index was calculated based on germination data, using the mean values of root length and shoot length, according to the formula proposed by Baki and Anderson (1973).

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

Biochemical Estimations

Estimation of Carbohydrates

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

Estimation of Proteins

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

Enzyme Assays

Estimation of Catalase

Catalase (CAT: EC 1.11.1.6) activity was measured using the method of Sinha (1972). For sample preparation, leaves were homogenized in 100 mM phosphate buffer (pH 7). The optical density (OD) was then recorded at 620 nm.

Estimation of Superoxide Dismutase

Superoxide dismutase (SOD: EC 1.15.1.1) activity was measured using the method of Kakkar *et al*., (1984). Leaves were homogenized in 100 mM sodium pyrophosphate buffer (pH 8.3). The color formed at the end of the reaction was extracted into the butanol layer and its absorbance was measured at 560 nm.

Statistical Analysis

The results are presented as means±standard deviation for six plants per group. Data were analysed using one-way analysis of variance (ANOVA), and significant differences among treatment groups were assessed with Duncan's multiple range test. Results were considered statistically significant when $P<0.05$. All statistical analyses were conducted using the SPSS version 15.0 software package (SPSS, Tokyo, Japan).

RESULTS AND DISCUSSION

Germination Percentage, Root Length and Shoot Length

Seed germination and early seedling growth are highly sensitive to alterations in the environmental conditions (Seregin and Ivanov, 2001). As such, the germination performance and growth rate of seedlings are often used to explore the plant tolerance ability to metal stress (Peralta *et al*., 2001). Figure 1 illustrates the effect of Hg stress on germination percentage (%), while Figure 2 shows the impact of Hg on root length (cm) and shoot length (cm) in the different experimental groups of radish plants. These observations were recorded on the 30th day after sowing. Soil is a vital, non-renewable resource necessary for seed germination, growth and plant survival, thereby supporting all life forms on the earth. The results indicate that Hg treatment reduced the water uptake, moisture content and relative water content in the germinating seeds. Previous studies have shown that HMs can impair the digestion and mobilization of food reserves such as carbohydrates and proteins in germinating seeds (Li *et al*., 2005). The data revealed that as the Hg concentration increased to 50 mg, 100 mg and 200 mg, the germination percentage decreased to approximately 55%, 40% and 25%, respectively. On the other hand, 90% seed germination was observed in control group.

In plants, the roots are the first organ to encounter toxic heavy elements and typically accumulate elevated concentrations of metals than the shoots (Rout *et al*., 2001). Inhibition of root elongation is often the earliest visible symptom of HMs toxicity. This reduction in root elongation can result from either the inhibition of root cell division or a decrease in cell expansion within the elongation zone (Fiskesjo, 1997). Since inhibition of root elongation is often the first observable effect of HMs stress, root length can serve as a crucial index for assessing plant tolerance to metals (Han *et al*., 2007).

The response of roots and shoots to HMs has been extensively studied in radish plants. Group 1, the control group, exhibited the highest root and shoot lengths compared to the Hg treated groups. The longest roots and shoots were observed in the control plants, while increasing the Hg concentrations progressively reduced both the root and shoot lengths in radish plants. Higher Hg concentrations induced significant morphological alterations, with root and shoot elongation showing a strong sensitivity to higher Hg levels. These results suggest that elevated Hg concentrations negatively impact germination percentage, root and shoot length. Furthermore, Hg exposure in radish plants severely restricts seedling growth and development. At high concentrations, Hg inhibits root and shoot growth, which directly affects the plant's capability to absorb water and nutrients, ultimately leading to stunted growth.

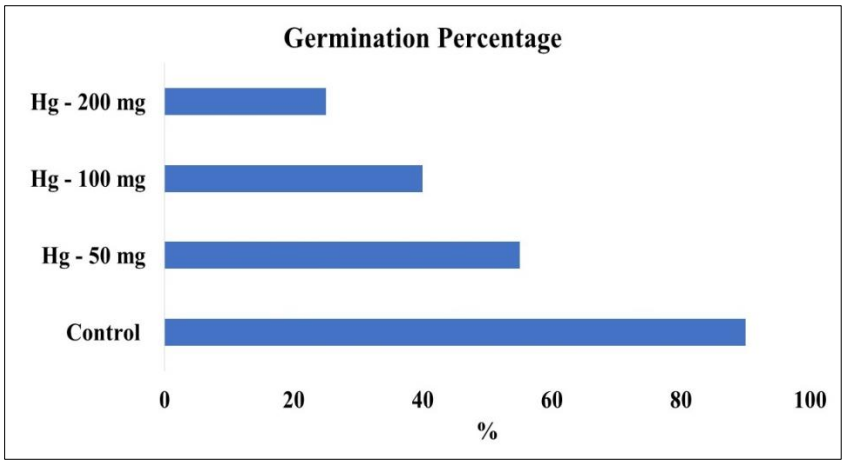

Figure 1: Effect of mercury on germination percentage (%) in different experimental groups of radish plants.

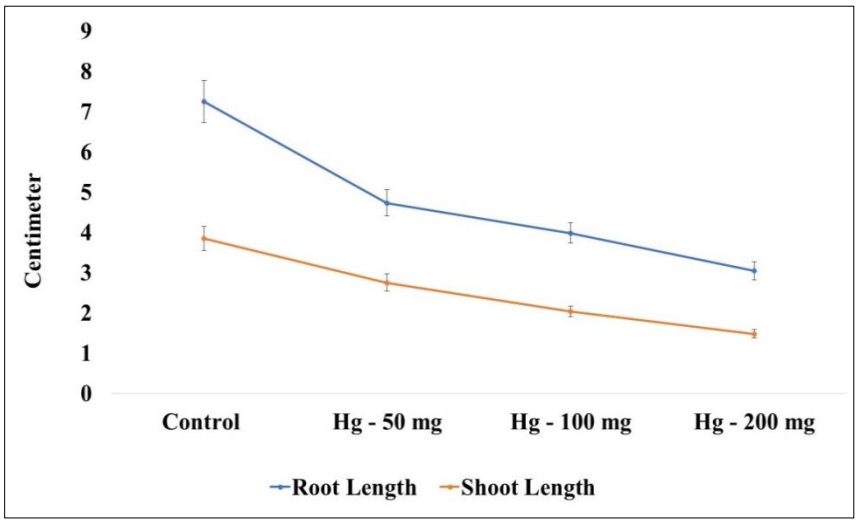

Figure 2: Effect of mercury on root length and shoot length in different experimental groups of radish plants.

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

Table 1 presents the impact of Hg stress on the fresh weight, dry weight, and vigour index of radish plants at different Hg concentrations, recorded on the 30th day after sowing. The effects of Hg contaminated soils on these parameters showed distinct differences between the control and Hg treated plants. A significant reduction in fresh weight, dry weight, and vigour index was observed in the Hg exposed radish plants compared to the control group. This decline was directly correlated with increased concentration of Hg. The high sensitivity of plants to Hg can be attributed to the fact that the root, being the major site of HMs absorption, is the first to encounter Hg. Excessive concentration of Hg has been reported to impair seedling establishment and root growth by inhibiting root cell division, elongation, and cell cycle progression (Rellen-Alvaraz *et al*., 2006). Additionally, elevated concentration of Hg leads to increased production of hydrogen peroxide (H_2O_2) and lipid peroxidation, causing oxidative stress and further compromising the growth of plants.

Table 1: Effect of mercury on fresh weight, dry weight and vigour index on different experimental groups of radish plants

Groups	Fresh weight (g)	Dry weight (g)	Vigour index
Control (C)	135.53±11.45	25.64±1.93	997.2±63.34
Test (T1)	100.32±8.32	18.43±1.23	411.4±34.54
Test (T2)	79.09±5.43	13.42±0.08	240.4±17.32
Test (T3)	67.32±3.93	9.90±0.64	112.75±13.73

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 3 illustrates the impact of Hg on the total carbohydrate and protein contents in three groups of radish plants, with data recorded on the 30th day after sowing. Carbohydrates are produced by green plants through the process of photosynthesis, which involves the conversion of carbon dioxide and water. These carbohydrates serve as energy source for the body, such as glucose, and as a storage form of energy, like starch in plants. Additionally, sugar signaling plays a crucial role in plant defense responses under both biotic and abiotic stress. According to the data presented in figure 3, carbohydrate content was significantly reduced in radish plants treated with varying concentrations of Hg compared to the untreated control plants, which correlates with the previous study by vineeth *et al*., 2015. The substantial decrease in carbohydrate contents in Hg treated radish plants may be attributed to the inhibition of chlorophyll biosynthesis, leading to reduced carbohydrate levels (Kupper *et al*., 1998).

Proteins are complex molecules made up of smaller chemical compounds called amino acids. They play important roles in various processes, including maintaining intracellular structure, facilitating membrane transport, catalyzing chemical reactions (as enzymes) and generating energy through electron transport. However, proteins have a finite lifespan and must be continually synthesized from mRNA to support ongoing plant growth and development. The data presented in the table demonstrate that Hg, at varying concentrations, significantly reduced the levels of protein contents in radish plants compared to control plants, thereby impairing plant growth and development. The reduced protein content in HMs treated plants subsequently inhibited the synthesis of proteins. The observed decline in protein levels with increasing Hg concentrations in radish plants may be due to enhanced proteolysis, a process driven by elevated activity of protease enzyme (Palma *et al*., 2002) which is known to increase under the conditions of toxicity. Additionally, it is likely that Hg exposure triggered the process of lipid peroxidation and protein fragmentation induced by the toxic effects of reactive oxygen species (ROS), which contributed to the significant reduction in protein content. The depletion of proteins, which are necessary for the activity of enzymes, metabolic regulation and cellular architecture, suggest that Hg toxicity disrupts important cellular processes which include metabolic homeostasis, protein turnover and redox balance.

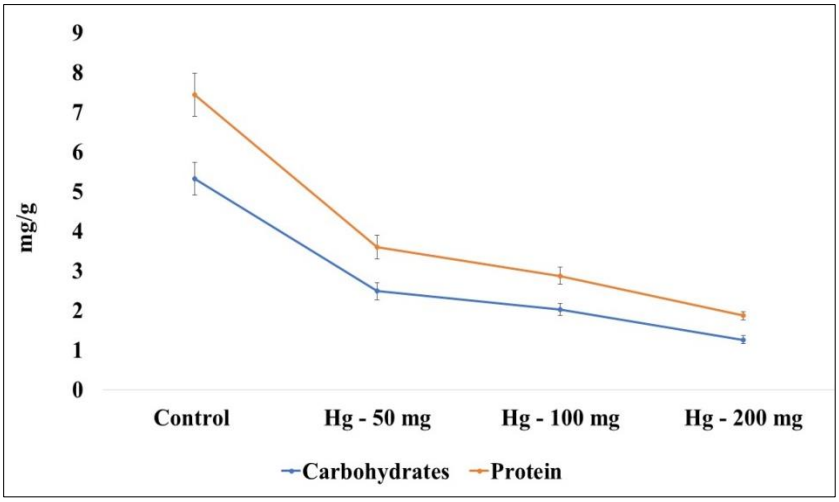

Figure 3: Effect of mercury on carbohydrate and protein contents in different experimental groups of radish plants

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).

Enzymic Antioxidants (Catalase and Super Oxide Dismutase)

Plant cells possess a sophisticated antioxidant defense system that effectively mitigates ROS through redox homeostasis. Reactive species such as free radicals and H_2O_2 are known to induce cellular damage, particularly to membrane structures, often through lipid peroxidation (Hendry *et al*., 1992). Consequently, the upregulation of antioxidant enzyme activities, particularly catalase (CAT) and superoxide dismutase (SOD), in hyperaccumulator plants may be regarded as an important adaptive mechanism against the oxidative stress and toxicity induced by contamination of HMs. Exposure of plants to abiotic stress factors, which includes drought, limited water availability, and HMs contamination, triggers an increased the production of ROS and free radicals, which are byproducts of cellular metabolism under stress conditions (Gill and Tuteja, 2010). The accumulation of these ROS and free radicals can disrupt cellular homeostasis, leading to oxidative damage to proteins, nucleic acids and lipids, ultimately results in cellular dysfunction and death. In response to

this oxidative challenge, plants have evolved a highly coordinated antioxidant defense system. This system is composed of both enzymic antioxidants and non-enzymic antioxidants. These antioxidant molecules play a crucial role in mitigating oxidative stress by scavenging ROS, thereby protecting cellular macromolecules and increasing plant tolerance to environmental stresses, including HMs toxicity (Gratao *et al*., 2005).

The major function of CAT is to metabolize H_2O_2 generated in the peroxisomes during the process of photorespiration, where glycollate is converted to glyoxylate by glycolate oxidase (Kendall *et al*., 1983). Catalase is also essential for the detoxification of H_2O_2 synthesized during the fatty acid metabolism, particularly in germinating seeds (Holtman *et al*., 1994). In the present study, higher CAT activity was observed in control radish plants. However, a significant reduction in catalase activity was recorded in radish plants treated with different concentrations of Hg, as shown in Figure 4. This decrease in activity is consistent with previous findings by Vijay *et al*., (2024), who also reported diminished catalase activity in fenugreek plants exposed to Hg. Our results suggest that Hg treatment in radish plants induces concentration dependent oxidative stress, primarily characterized by the accumulation of H_2O_2 due to CAT activity. The observed significant reduction in CAT may also result from its degradation by induced peroxisomal proteases or from photoinactivation of the particular enzyme.

SOD is a critical component of the enzymic antioxidant defense system that protects plant cells from oxidative damage by catalyzing the dismutation of superoxide radicals (O_2^-) into H_2O_2 and molecular oxygen (Monk *et al*., 1998). Initially, the activity of SOD in plants increases, reaching a peak in response to oxidative stress. Such increased SOD activity is often linked to de-novo synthesis of the enzyme and have been shown to provide increased protection against oxidative damage, particularly in transgenic plants overexpressing SOD (Allen *et al*., 1997). In this study, the activity of antioxidant enzymes, including SOD, exhibited significant variations when plants were exposed to varying Hg concentrations. It is hypothesized that the activation of SOD plays a key role in mediating resistance to HMs induced oxidative stress and in maintaining the overall integrity of the plant's antioxidant defense system under such conditions (Slooten *et al*., 1995). However, our results indicate a marked decrease in SOD activity in Hg treated radish plants, with lower enzyme levels observed at all concentrations compared to control plants, which is in consistent with previous findings by Vijay *et al*., 2024. These findings underscore the notion that the reduction in SOD activity correlates with the intensity of oxidative stress induced by Hg exposure, with a concentration dependent decrease reflecting the extent of the stress.

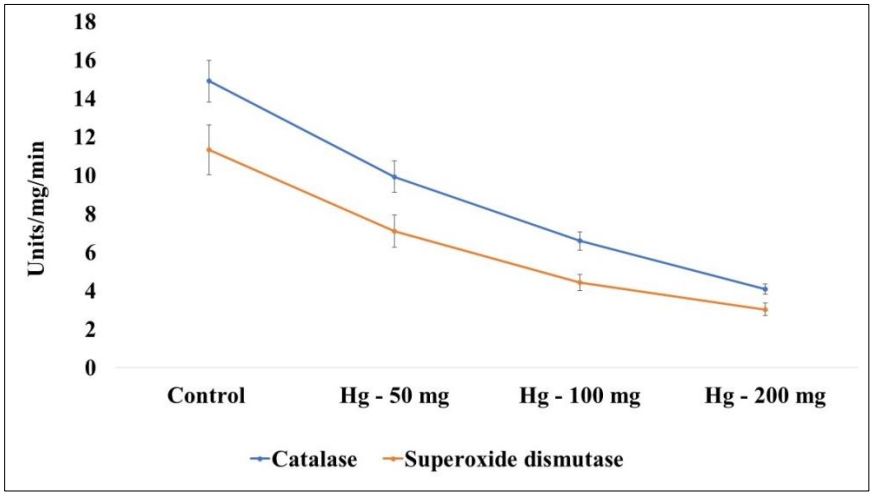

Figure 4: Effect of mercury on Catalase and Superoxide dismutase in different experimental groups of radish plants

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

In conclusion, the present findings confirm that Hg toxicity exerts detrimental effects on radish plants, as evidenced by significant reductions in important growth parameters, including germination percentage, root and shoot lengths, fresh and dry weights, and vigour index, when compared to control plants. Additionally, Hg exposure led to a significant decrease in the carbohydrate and protein contents of the plants, indicating impaired metabolic processes. The observed reduction in growth and metabolic functions due to Hg toxicity may be linked to the disruption of key cellular processes, such as photosynthesis, nutrient uptake, and cell division, which are critical for normal plant development. Furthermore, the activities of antioxidant enzymes, such as catalase and superoxide dismutase, were significantly lowered,

suggesting that Hg induced oxidative stress overwhelmed the plant's defense mechanisms. These results collectively highlight the toxicological impact of Hg on radish plants, disrupting both growth and physiological functions through oxidative damage and metabolic disturbances.

Future research could investigate the use of Hg tolerant cultivars or the application of external treatments, such as plant growth regulators and antioxidants, to improve the resilience of radish plants to Hg toxicity, potentially boosting crop productivity in contaminated soils. Additionally, gaining a deeper understanding of the molecular mechanisms governing Hg uptake and transport in plants could facilitate the development of crops with enhanced resistance to Hg or reduced Hg accumulation, ensuring safer food production in polluted environments.

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