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Toxicological impacts of mercury on the growth and biochemical profiles of pumpkin (*Curcurbita moschata duchesne*)

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Abstract

Industrialization and urbanization have led to the accumulation of heavy metals in urban soils, water, roadway dust, sediments and living organisms. Heavy metal contamination in agricultural soils is the most concerning issues throughout the world. So, our aim of the study was to evaluate the toxicological impacts of Mercury on the growth and biochemical profiles of Pumpkin (*Curcurbita moschata* duchesne). Experimental plants were divided into 4 groups. Group 1 plants in bag not received mercury treatment and served as control, whereas groups 2, 3, and 4 were subjected to mercury treatment of 50, 100, and 200 mg, respectively. Our findings indicated that mercury treated Pumpkin plants exhibits significant reduction in growth parameters such as germination percentage, root length, shoot length, fresh weight, dry weight and vigour index. Biochemical analysis revealed that mercuric stress decreased the levels of carbohydrates, proteins, chlorophyll and carotenoids contents. Additionally, mercury treated plants showed a marked decrease in the enzymic antioxidants levels such as catalase & super oxide dismutase. Overall, our results highlight that mercury exhibits toxic effects in Pumpkin plants can adversely affect their growth and physiological processes, emphasizing the necessity for strategies to alleviate the heavy metal pollution in agricultural systems.

Keywords: Pumpkin; Mercury; Heavy metals; Pollution; Toxicity; Photosynthesis

1. Introduction

Heavy metals (HMs) are major significant environmental pollutants having toxic consequences on human nutrition, evolutionary, ecological and environmental systems. Metals represent a significant category of toxic substances encountered in daily life during environmental and occupational settings. Nowadays, the consequences of these toxic agents on human health have become an area of significant interest because of widespread exposure, due to increased utilization of wide variety of the metals in industry applications and also in day-to-day activities (Christoforidis and Stamatis, 2009).

In the industrialized world, soil contamination due to HMs is of most significant concern (Hinojosa et al., 2004). Pollution caused by HMs results in adverse effects on various plant quality and yield parameters. Additionally, HMs also altering the composition, size and microbial community activities (Yao et al., 2003). These metals can also create impact in enzymatic activities in soil by altering the microbial community which is responsible for enzyme synthesis (Shun-hong et al., 2009). Moreover, HMs are toxic to soil biota, exert influence on important microbial processes, reducing the activity and number of soil microorganisms. The uptake of HMs by plants and their subsequent accumulation in the food chain pose a significant threat to human health (Sprynskyy et al., 2007).

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HMs are potentially toxic resulting in stunted plant growth, chlorosis, reduced yield and impaired uptake of nutrients. Additionally, these consequences can also disrupt the metabolism of plants and inhibit the ability of leguminous plants to fixate molecular nitrogen (Guala et al., 2010). The main pathway of HMs entrance in the food chain happens through its absorption by plant roots. In plant tissue, HMs accumulation and absorption depends upon various factors such as moisture, nutrient availability, organic matter, pH and temperature. HMs uptake by plants from soils with elevated concentrations might results in a significant health risk due to food chain implications. The consumption of food crops through food chain route which is already contaminated with HMs is a substantial route for human exposure.

Mercury (Hg) uptake by plants depends upon plant species, such that Hg speciation in the soil and properties of soil. Even at low concentrations, Hg exerts toxic effects in plants resulting in photosynthesis inhibition (Assad et al., 2016), growth retardation (Ahammad et al., 2018), protein and DNA damage (Malar et al., 2015), reactive oxygen species (ROS) generation (Kim et al., 2017) and oxidation of lipid membrane (Zhou et al., 2008). Additionally, within plants, Hg exists in different species and transported to different organs through transporters. However, a substantial portion of Hg absorbed by plants is retained in the roots and less translocation to the shoots (Cho and Park, 2000).

Pumpkin (*curcurbita moschata duchesne*) is known to possess numerous biological activities which includes antioxidant (Dang, 2004), anti-inflammatory (Wahid et al., 2021), anticancer (Cheong et al., 1997; Soltani and Darbemamieh, 2021), antimicrobial (Hussain et al., 2021), antidiabetic (Kwon et al., 2007), hepatoprotective (Radic et al., 2021), antiulcer (Gad et al., 2019) effects. Moreover, pumpkin is most important in HMs research due to its capacity to absorb and subjected to accumulate these metals from contaminated soils, making it an effective model for studying bioaccumulation. Additionally, studies on pumpkin's responses to HMs provide insights that can improve agricultural practices, thereby enhancing yield of crops and quality while addressing pollution of soil. Moreover, pumpkins can serve as ecological indicators, helping in evaluation of soil's health and guide environmental management strategies, which ultimately contributing to public health and sustainable agriculture.

However, Hg contaminated food consumption represents a significant pathway for human exposure to Hg and Hg's presence in edible parts of the plants can exerts significant threat to human health. Therefore, this study was aimed to explore the toxicological impacts of Mercury on the growth and biochemical profiles of Pumpkin (*curcurbita moschata duchesne*).

2. Materials and methods

Experimental protocol deduced in order to achieve the objectives were carried out using standard procedures. Pumpkin seeds obtained from an agricultural shop, Puducherry. Mercuric Chloride was used to induce mercury toxicity.

2.1. Seed Sterilization

Uniform sized seeds were selected. All the seeds were surface sterilized with 0.1% mercuric chloride for about 2-3 minutes to avoid infection of fungus. These seeds were taken out immediately and washed several times with distilled water.

2.2. Polyethylene bag experiment

Polyethylene bag culture experiments were conducted to investigate the effects of HMs toxicity in pumpkin plants. The growth medium in the polyethylene bags consist of artificially polluted soil with Hg concentrations of 50, 100 and 200 mg. With wooden stick, 2cm deep holes were made and then sowed seven sterilized seeds in each bag. Afterwards each seed was covered with a small amount of soil for proper supplement of germination factors. Soil moisture content was adjusted regularly according to its water holding capacity with tap water.

2.3. Experimental design

After the initial phase, the Pumpkin plants were divided into four different groups. Group 1 bag with soil served as control, that did not receive any 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 relative humidity, natural photoperiod and average temperature.

2.4. 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 (in cm)**

The root length from the ground level to the tip of the root is measured using standard centimeter scale.

- **Shoot Length (in cm)**

The shoot length from the ground level to the tip of the shoot is measured using standard centimeter scale.

- **Fresh Weight (in gm)**

The fresh weight of the whole plant is determined using an electronic balance.

- **Dry Weight (in gm)**

The dry weight of the whole plant is determined using an electronic balance.

- **Vigour Index**

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 - Baki and Anderson, 1973.

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

2.5. Biochemical Estimations

2.5.1. Estimation of Carbohydrates

The carbohydrate content was estimated by the method of Hedge and Hofreiter (1962). The absorbance of the solution was measured at a wavelength of 640 nm.

2.5.2. Estimation of proteins

The protein content was estimated by Lowry's method (1951). The color develops, which is measured at a wavelength of 660 nm.

2.5.3. Photosynthetic pigments analysis

Chlorophyll content was estimated by using the method of Arnon, (1949), the absorption readings was read at 645 nm and 663 nm. Carotenoid contents was estimated by using the method of Lichtenthaler, (1987) and was measured at 473 nm.

2.6. Enzyme assays and analysis

2.6.1. Estimation of Catalase

The activity of catalase (CAT: EC 1.11.1.6) was assayed by the method of Sinha (1972). The absorbance of the resulting color solution was measured at 620 nm.

2.6.2. Estimation of Superoxide dismutase

Superoxide dismutase (SOD: EC 1.15.1.1) was assayed by the method of Kakkar et al., 1984. The colour solution formed at the end of the reaction was measured at 560 nm.

2.7. Statistical analysis

Results were expressed as means ± standard deviation of 6 plants per group. Data were analyzed by oneway 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).

3. Results

3.1. Effect of mercury in Pumpkin plant on germination percentage, root length and shoot length

Table 1 shows the effect of Hg in pumpkin plants on germination percentage (%), root length and shoot length of different experimental groups. Observations were recorded at 30th day after sowing. Upon increasing the Hg concentration of about 50 mg (T1), 100 mg (T2) and 200 mg (T3), which resulted in significant decrease in gemination percentage, root length and shoot length as compared to control pumpkin plants.

Table 1 Effect of mercury in Pumpkin 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 (C)	95	7.89±0.52	10.90±0.93
Test (T1)	65	4.04±0.39	6.13±0.45
Test (T2)	45	2.06±0.20	4.62±0.24
Test (T3)	30	1.73±0.12	3.75±0.21

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).

3.2. Effect of Mercury in Pumpkin plant on Fresh weight, Dry weight and Vigour index

Table 2 shows the effect of Hg on fresh weight, dry weight and vigour index on various groups of pumpkin plants. These observations are recorded at 30th day after sowing. A significant reduction in fresh weight, dry weight and vigour index were noted under Hg toxicity in pumpkin plants as compared to control plants. Higher Hg concentration leads to more decreased pronounced effects which was evidenced by observation in 200 mg of Hg treated plants which showed greater significant reductions as compared to untreated control, 50 mg of Hg and 100 mg of Hg treated plants.

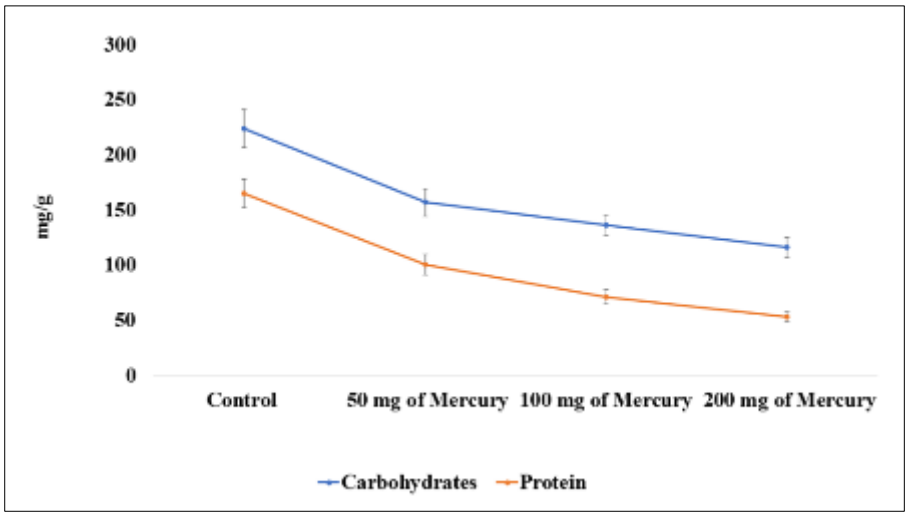
Table 2 Effect of mercury on fresh weight, dry weight and vigour index on different experimental groups of pumpkin plants

Groups	Fresh weight (g)	Dry weight (g)	Vigour index
Control (C)	8.94±0.49	2.39±0.12	1785.05±112.64
Test (T1)	6.27±0.26	1.78±0.05	661.05±36.94
Test (T2)	4.03±0.23	1.64±0.04	300.60±26.05
Test (T3)	3.22±0.13	1.27±0.02	164.4±9.04

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).

3.3. Effect of Mercury on carbohydrate and protein contents of Pumpkin plants

Figure 1 shows the effect of Hg on the total carbohydrate and protein levels in three groups of tested experimental pumpkin plants. These observations were recorded at 30th day after sowing the seeds. The results revealed that significant decrease in carbohydrates and protein levels in Hg tested groups when compared to untreated control group.

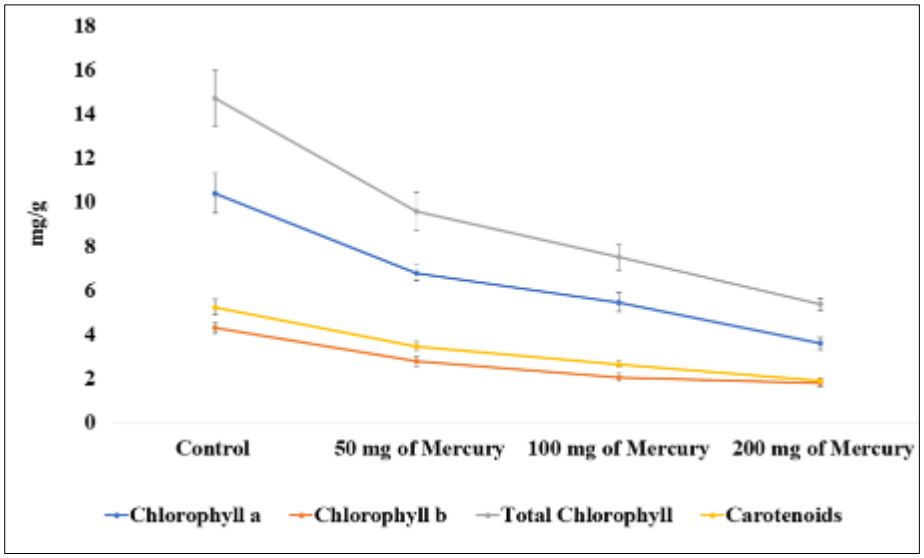


Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at p<0.05. Duncan's multiple range test (DMRT).

Figure 1 Effect of mercury stress on carbohydrate and protein contents on different experimental groups of pumpkin plants

3.4. Effect of Mercury on Chlorophyll a, chlorophyll b, Total chlorophyll and Carotenoid contents of Pumpkin plants

Figure 2 illustrates the impact of Hg on plant photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in pumpkin plants. The results showed that under Hg stress pumpkin plants exhibits significant decrease in these photosynthetic pigments as compared to untreated control plants.

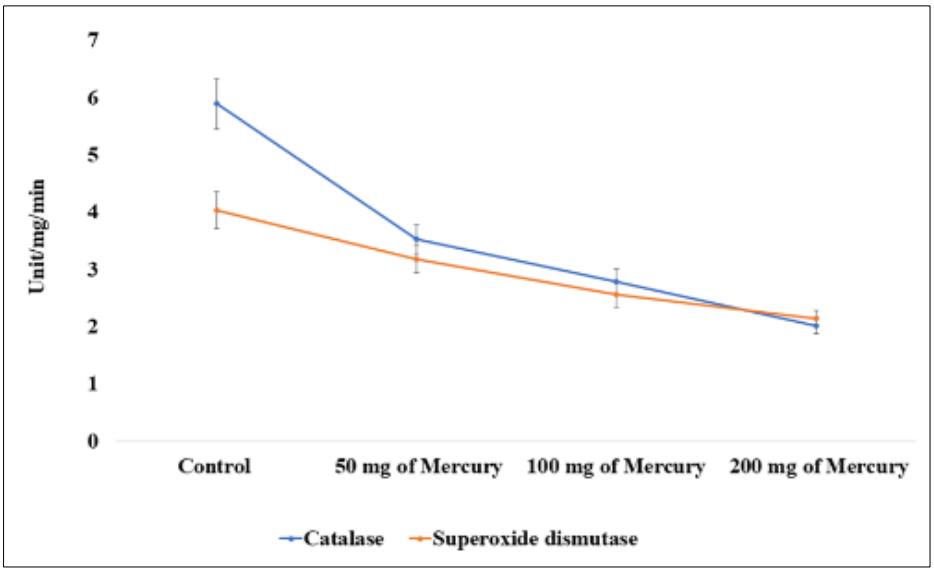


Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at p<0.05. Duncan's multiple range test (DMRT).

Figure 2 Effect of mercury on Chlorophyll a, Chlorophyll b, Total chlorophyll and Carotenoids contents in different experimental groups

3.5. Effect of Mercury on Enzymic antioxidants of Pumpkin plants

Figure 3 shows the effect of Hg toxicity on enzymic antioxidants such as Catalase and Superoxide dismutase on various groups of pumpkin plants of different experimental groups. The results showed that these enzymic antioxidants were significantly reduced in the Hg tested pumpkin plants when compared to untested control group.



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 3 Effect of mercury on Catalase and Superoxide dismutase in different experimental groups of pumpkin plants

4. Discussion

HMs are always widely acknowledged as important soil component. Plants require different HMs for their growth and development whereas elevated levels of these HMs can be harmful to plants life. When the concentrations of HMs exceed their normal levels, plants are adversely affected leads to alterations in soil quality and fertility, biomagnification, groundwater contamination ultimately cause significant damage to soil ecosystems (Borah et al., 2020). Additionally, elevated concentration of HMs also leads to oxidative stress that can directly affect the plants in terms of cytoplasmic enzymes inhibition and cell assembly destruction. Current anthropogenic pollution sources which include industry and agriculture, have a significant influence on the accumulation of HMs in the soil (Bechambi et al., 2015). Understanding the consequences of HMs contamination is necessary for creating effective remediation strategies and certifying stable agricultural practices.

4.1. Germination percentage, Root length and Shoot length

HMs are one of the significant abiotic stressors that affect plant growth and development. Healthy germination of seeds is pivotal in ensuring desired successful crop production and desired density of plants. Seed germination success and its emergence depends on the interaction between conditions of environment in the seed quality and the seed bed (Almansouri et al., 2001). Understanding the consequences of HMs are very important in the germination environment on a plant species is crucial in investigating germination and seedlings development. Results from our study revealed that significant reduction in germination percentage, root length and shoot length of Hg tested plants when compared to untreated control pumpkin plants. These growth parameters declined significantly just because of increased concentration of Hg. Moreover, these increased concentrations of Hg may negatively affect both crop quality and yield, and also pose a serious threat through the food chain to human health. Additionally, root length and shoot length in the control group was statistically higher than the Hg tested groups. These diminished growth parameters may be attributed to the abiotic stress caused by Hg (Akinci and Akinci, 2011).

4.2. Fresh weight, dry weight and vigour index

Fresh weight measurement is necessary for investigating the total biomass and plant health at the time of sampling. Importantly, fresh weight can be affected by factors which includes evaluating timing, conditions of environment and hydration status. Dry weight offers more sturdy indication of plant's biomass, permitting for comparisons that account for differences in water contents. It is mainly useful in studies investigating overall plant's physiological conditions, growth efficiency and accumulation of nutrients. Vigour index is one of the most important growth parameter in assessing seed quality and agricultural practices. Results from our study shows significant reductions in fresh weight, dry weight and vigour index in Hg treated plants as compared to control groups underlines the detrimental consequences of Hg toxicity on plant growth and development. Reduced fresh weight and dry weight indicates decreased accumulation of biomass, impaired process of metabolism, diminished uptake of nutrients, altered

physiological stability may be attributed to Hg toxic effects on cellular functions. Moreover, decreased vigour index highlights the consequences of Hg exposure reflecting weakened seedling development and resilience. Previous findings (Banadka and Nagella, 2022; Siddique and Dubey, 2016) also underlines our current experimental results which showed that reduced fresh weight, dry weight and vigour index were observed under Hg toxicity.

4.3. Carbohydrates and protein contents

Carbohydrates playing a vital role in the process of photosynthesis and breakdown during respiration by plants. The contents of carbohydrates were decreased in all Hg treated pumpkin plants when compared to untreated plants as evidenced by results shown in figure 1. This in accordance with previous results shown by Rascio and Navari-Izzo, 2011 depicted that various metals having the ability of reducing the biomolecule contents in agricultural crops as their concentration increases. The observed low sugar levels are attributed to reduced synthesis or diversion of the metabolites to other synthesis processes aiming at alleviating the detrimental consequences of Hg. Also, Hg can interfere with enzyme activities that play a significant role in the metabolism of carbohydrates.

In Plants, Proteins are important constituents that are easily susceptible to gets damaged in conditions of environmental stress (Wu et al., 2010). Hence, any alterations in these protein contents can also be regarded as a vital oxidative stress indicator in plants. Results from our present study shows that reduced protein levels in Hg treated plants as compared to untested pumpkin plants. Increased concentration of Hg leads to more pronounced reduction in protein levels of pumpkin plants. This observed reduction in protein contents at increased concentrations of Hg in pumpkin plants may be attributed to enhanced degradation of proteins as a result of increased activity of protease enzyme (Palma et al., 2002) which is known to increase under toxic conditions.

4.4. Chlorophyll a, chlorophyll b, Total chlorophyll and Carotenoid contents

Results from our present study shows that levels of chlorophyll a, b and total chlorophyll were significantly reduced in Hg treated plants as compared to control plants. The reduction in the levels of these chlorophyll contents underscores the detrimental effects due to Hg toxicity which can hinder essential molecules formation, protein denaturation, displace metal ions like magnesium from chlorophyll and disrupt cell membranes (Chen et al., 2010). Hg exposure can disrupt chlorophyll synthesis, damage membranes of chloroplast results in decreased pigment levels. These impairments lead to diminished growth and low energy synthesis affecting overall plant's health.

Carotenoids are tetraterpenoid compounds functions as accessory pigments in the process of photosynthesis that is widely found in plants, contribute to the colouring aspect of fruits and flowers and also protecting plants against oxidative stress (Crupi P et al., 2023). Moreover, with orange pulp, Pumpkins are exclusively valuable, due to its higher content of carotenoids. Results revealed that carotenoid contents also were adversely decreased in Hg treated plants when compared to untreated plants. This observed reduction in carotenoid contents indicates that Hg can also impairs plant's capability to manage oxidative stress.

4.5. Catalase and Super oxide dismutase

In plants, both enzymic and non-enzymic defense system were activated to overcome the toxicity induced by HMs (Farid et al., 2018). Antioxidants are vital biomarkers of oxidative stress in organisms expose to pollutants in the environment. HMs interfere with developmental and cellular metabolic pathways through various enzyme and protein inactivation in plants binding to their active sites and functional groups such as amino, carboxyl, sulfhydryl and carbonyl groups (Asati et al., 2016).

Plant physiology alterations can be monitored in a situation where the organism experiences the condition of stress due to persistent environmental pollutants, results in ROS generation such as H_2O_2, OH, O_2 in a biosystem (Aljahdali and Alhassan, 2020). Due to increase in the ROS generation, cells can respond through a defense network of enzymic antioxidants with the ability of scavenging these ROS synthesized (Lin et al., 2016). So only antioxidants are used as biomarkers for oxidative stress that will reflect the relationship between increased ROS generation and pollutants in the environment.

Results from our study shows that lowered the levels of antioxidants such as superoxide dismutase (SOD) and catalase (CAT) in Hg treated plants as compared to untreated pumpkin plants. This significant reduction may be attributed to increased production of ROS with increase in the concentrations of HMs which can pose damage to plants. The antioxidative feedback due to Hg bioaccumulation in pumpkin plants at the cellular level causes the production of ROS potentially which results in cellular damage or oxidative stress. This aliens with previous reports illustrate Hg treatment leads to impairment in the activities of antioxidants (Sharaf et al., 2019).

Abbreviations

- Heavy metals -HMs;
- Mercury -Hg;
- Catalase – CAT;
- Superoxide dismutase – SOD;
- milligram – mg;
- percentage - %;
- Gram – gm,
- Centimeter – cm;
- Reactive oxygen species – ROS.

5. Conclusion

Based on the experimental results, it is concluded that Hg stress adversely affect Pumpkin plants resulted in decreased growth parameters such as germination percentage, root length, shoot length, fresh weight, dry weight and vigour index under Hg stress when compared to control plants. Additionally, Carbohydrates and Protein contents are reduced while photosynthetic pigments such as Chlorophyll and Carotenoid contents are also significantly gets decreased in pumpkin plants due to toxicity of Hg. Furthermore, antioxidant enzymes such as catalase and super oxide dismutase levels shows decreased levels in higher Hg concentrations in pumpkin plants. These results highlighting the toxicological impact of Hg on biochemical and physiological health of Pumpkin plants.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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