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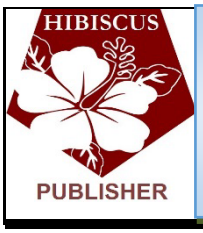


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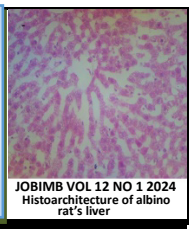


# Effect of Mercury Exposure on the Growth and Physiological Characteristics of Lowland Tomato (*Solanum lycopersicum*)

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**ABSTRACT**

This study evaluates the effects of mercury (Hg) on tomato plants exposed to varying concentrations (0.01, 0.05, 0.10, and 0.25 ppm) for 14 and 30 days. Hg exposure led to significant reductions in plant height and leaf diameter, with more severe effects at higher concentrations. Notably, 0.01 ppm Hg caused increased branching and earlier ripening, whereas higher concentrations diminished flower and fruit counts, with 0.25 ppm Hg resulting in severe reductions and plant deterioration. Chlorophyll content was slightly higher at 0.01 and 0.05 ppm Hg but decreased at 0.10 and 0.25 ppm, indicating disrupted photosynthesis. Proline content, a stress marker, increased significantly in fruit and roots with higher Hg concentrations, peaking at 0.25 ppm. MDA levels, a marker of lipid peroxidation, increased with Hg concentration and duration, especially at higher levels. Visual symptoms of toxicity, such as wilting and chlorosis, were evident at 0.25 ppm Hg, indicating severe plant stress. The study highlights Hg adverse effects on tomato growth, morphology, and reproductive processes, with high concentrations causing severe toxicity and low concentrations having minor effects. Further research is needed to explore biochemical responses and establish Hg toxicity thresholds in tomato plants.

## INTRODUCTION

Mercury (Hg) poses a significant environmental threat, entering soil and water primarily through human activities [1]. For instance, the gold amalgam technology employed in many developing countries for gold mining leads to substantial Hg pollution in soil and water [2]. Additionally, industrial activities such as coal combustion, cement production, and waste incineration are significant sources of Hg emissions globally. Consequently, Hg-containing waste is increasing globally [3]. In Malaysia, significant concentrations of Hg have been documented in various regions due to industrial activities. For instance, sediment samples from West Port and Sungai Pulau in Johor have shown total Hg concentrations ranging from 0.11 to 0.41 mg/kg. Similarly, elevated Hg levels (ranging from 0.03 to 0.08 mg/L) have been detected in rivers such as Sungai Pok Kecil, Sungai Pok Besar, and Teluk Buih in Johor, as well as in seawater around Merambong Island. In addition to water bodies, high concentrations of Hg have been found in food samples along the west coast of the peninsula, particularly in seafood sourced from the Straits of Malacca, where levels have been reported as 1.1–3.2 µg/g. It's important to note that the permitted Hg level in food in Malaysia is 0.5 µg/g. Furthermore, Hg contamination in tropical fruits in Malaysia is linked to the use of agrochemicals and fertilizers [4].

Hg is known to accumulate in vegetables and fruits such as tomatoes, eggplants, and cucumbers, highlighting the potential health risks associated with consuming produce grown in contaminated environments [5]. Recently, attention has turned to vegetable crops due to growing concerns about food safety, as Hg is particularly harmful to human tissues. The body lacks a mechanism to eliminate Hg, emphasizing its non-biodegradable nature and extended half-life, which underscores the persistent threat it poses [6]. In plants, elevated levels of Hg induce phytotoxicity, significantly impacting key physiological

processes such as transpiration, photosynthesis, and carbohydrate metabolism [7]. This disruption triggers secondary nutritional and oxidative stress within plant tissues, ultimately impairing growth and development. Hg accumulation in various plant parts, including leaves, roots, and fruits, has been well-documented, highlighting the potential health risks associated with consuming contaminated vegetables such as tomatoes [8]. Understanding the distribution of Hg within tomato plants, particularly its concentration in fruits, is crucial for assessing overall food safety and the environmental impact of Hg contamination in agricultural settings.

In this study, we aimed to investigate the physiological growth of the lowland tomato cultivar Mardi Tomato 1 (MT1) under the influence of Hg, with a focus on examining chlorophyll content, proline contents, and lipid peroxidation in different plant parts. This research contributes to a better understanding of how Hg exposure affects plant physiology and biochemistry, thereby informing strategies for mitigating its adverse effects on agricultural productivity and food safety.

## MATERIALS AND METHODS

### Plant materials

The hydroponic system, adapted from the Kratky method, was utilized for cultivating tomato plants [9]. These matured plants were subsequently grown in a greenhouse located at the Faculty of Biotechnology and Biomolecular Sciences, Universiti of Putra Malaysia. They were nourished with a nutrient-rich water solution (Hougland's solution), ensuring direct delivery of essential nutrients to the plant roots. The MT1 tomato seeds were sourced from the Malaysian Agricultural Research and Development Institute (MARDI).

### Germination of MT1 tomato seeds

### Preparation of Hoagland Solutions

Hoagland's solution was used as nutrient medium for hydroponic system for seed germination and plant growth [10].

### Preparation of rockwool cubes

Rockwool cubes served as the germination medium in the hydroponic system. Initially, the cubes were placed in a clean plastic container to prevent contaminants from affecting the germination process. They were then thoroughly soaked with distilled water until fully saturated. Following this, the cubes underwent immersion in pH-adjusted water for up to 24 hours, aiming to achieve a pH level between 5.5 and 6.5 crucial for optimal plant growth. This soaking procedure ensured the cubes reached the desired pH. Subsequently, the cubes underwent three rinses with distilled water to eliminate excess salts and residual pH-adjusting substances, essential for ensuring the cleanliness of the growing medium and preventing potential harm to plant roots. Once rinsed, the cubes were ready for use as a germination medium in the hydroponic system, offering an ideal environment for seed germination and early plant growth.

### Germination of seeds and plant growth

Two MT1 tomato seeds were placed in the hole located on the top of each rockwool cube. The seeds were gently pressed down to the bottom of the hole using forceps and tweezers. Each cube with the seeds was then positioned in a 500 mL plastic container containing 5 mL of distilled water to maintain moisture. The entire container was covered with aluminum foil to simulate darkness and was placed in a growth room at a temperature of 24 ± 2°C for 7 days. Once germination commenced, the aluminum foil was removed.

Subsequently, the plastic containers with germinated seeds were exposed to light-emitting diode (LED) lights for an additional 7 days in the growth room under a Tubular 8 (T8) daylight LED providing 1080 lumens. After 14 days of growth, the young seedlings reached a height of 2 to 3 inches and were carefully transplanted into growing cups. These cups were then placed into a hydroponic system containing 200 mL of Hoagland's solution. The water level in the hydroponic container was regularly monitored and adjusted to ensure the roots remained in contact with the nutrient solution. Additional nutrient solution was added as necessary. The seedlings were maintained in the growth room until they reached 30 days of age. Afterward, they were transferred to a greenhouse and allowed to continue growing until they reached 60 days of age.

### Preparation of hydroponic system

A 20 L container was used to accommodate a sufficient volume of nutrient solution for the plants. The container was constructed from light-blocking material to inhibit algae growth within the nutrient solution. It was filled with the prepared nutrient solution, leaving adequate space at the top to facilitate air exchange, crucial for root respiration in plants. The growing cups were positioned on the lid of the container, ensuring they were correctly aligned above the nutrient solution. These cups were designed to allow the plant roots to extend into the solution, ensuring continuous contact with the nutrient solution to support plant growth.

### Hg treatment

The 60-day-old plants were transferred to a 20 L container filled with Hoagland's solutions containing varying concentrations of Hg: 0.01, 0.05, 0.10, and 0.25 ppm. Hoagland's solutions without Hg served as the control in this study. Each treatment was replicated 7 times. Tomato leaves were sampled at 14- and 30-days post-treatment, while unripe fruits (18 days post-anthesis) and fully ripe fruits (28 days post-anthesis) were also collected. Roots were harvested after 120 days of treatment to study the long-term effects of Hg exposure on root development. All harvested tissues were stored at −80 °C for subsequent analysis.

### Determination of plant growth

The height, leaf number, flower number, leaf morphology, and root growth of tomato plants were recorded and assessed weekly throughout the experiments.

### Determination of chlorophyll content

Fresh leaf samples were weighed, and 0.5 g of leaves were cut into small pieces and placed in a mortar. Subsequently, 5 mL of 80% (v/v) acetone was added, and the leaves were ground for 3 to 5 minutes to disrupt the cell membrane and release chlorophyll into the solution. An additional 10 mL of 80% (v/v) acetone was added to the mixture, and the resulting solution was transferred to a test tube. The volume was adjusted with 80% (v/v) acetone to reach a total volume of 40 mL. Total chlorophyll, chlorophyll a, and chlorophyll b contents were then measured using a spectrophotometer at 645 nm and 663 nm wavelengths, with 80% acetone serving as the blank reference. The total chlorophyll, chlorophyll a, and chlorophyll b contents were calculated using the formula described by [11].

### Determination of total proline content

The proline content in the roots, leaves, and fruits was estimated using the method described by [11]. Roots, leaves (from the still growing fourth leaf from the shoot tip), and fruits were collected from three tomato plants for each treatment.

Samples of 0.5 g of roots, leaves, and fruits were ground until homogenized, and 10 mL of 3% aqueous sulfosalicylic acid was

added. The homogenized samples were filtered through Whatman No. 2 filter paper. The samples were then centrifuged at 13,362 × g for 30 minutes at 4°C, and the clear supernatants were collected. Next, 2 mL of supernatant was added to a test tube using a micropipette. To this, 2 mL of 6 M phosphoric acid, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid (99%) were added. The sample was then placed in a water bath at 100°C for 1 hour. The solution was immediately transferred to ice for 5 minutes to terminate the reaction. Subsequently, 4 mL of toluene was added and shaken vigorously. Two layers of solution were formed, and the pink layer developed. The colorless layer was removed, and the upper pink layer was collected. The absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight (FW) basis.

**Determination of lipid peroxidation**

To assess lipid peroxidation, the thiobarbituric acid (TBA) method was employed. Malondialdehyde (MDA) was used as the standard for this analysis. Initially, 250 mg of fresh plant sample was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and then centrifuged at 10,000 × g for 5 minutes at 4°C. A 1 mL aliquot of the supernatant was mixed with 4 mL of 20% TCA and 0.5% TBA. This mixture was incubated at 95°C for 30 minutes, followed by rapid cooling in crushed ice and subsequent centrifugation at 10,000 × g for 10 minutes at 4°C. The absorbance of the supernatant was measured at 532 nm and 600 nm using a spectrophotometer. The nonspecific absorbance at 600 nm was subtracted from the absorbance at 532 nm to obtain the specific absorbance of MDA-TBA adducts. The MDA content was calculated using the formula provided by [12] and expressed as µmol/g FW of the sample.

## RESULTS AND DISCUSSION

**Physiological effects of Hg on tomato plant**

The results after 14 days revealed that the growth and responses of the tomato plants varied according to the concentrations of Hg used in the treatments. The introduction of Hg adversely affected both the morphology and development of the tomato plants. Furthermore, increasing the Hg concentrations up to 0.25 ppm resulted in severe consequences, ultimately leading to substantial disturbance and plant mortality.

The results presented in **Fig. 1** indicate that Hg supplementation in the hydroponic solution after 14 days caused a decrease in both plant height and leaf diameter, and this effect is statistically significant ($p < 0.05$). The control samples, which did not receive any Hg treatment, had average plant height and leaf diameter of 89 cm and 6 cm, respectively. The reduction in plant height and leaf diameter was more pronounced at higher Hg concentrations. For example, at 0.01 ppm Hg, the average plant height was 97 cm, and the leaf diameter was 6.5 cm, whereas at 0.25 ppm Hg, the average plant height was reduced to 65 cm and the leaf diameter to 2 cm. The results also suggest that Hg treatment affected plant morphology, as plants treated with 0.01 ppm Hg exhibited more branching and earlier ripening compared to other treatment concentrations. These findings suggest that Hg contamination may negatively impact plant growth and morphology, which could have implications for plant productivity and yield.

**Fig. 2** summarizes the flower and fruit counts observed after 14 days of Hg exposure. A decrease in both flower and fruit numbers was noted in the groups treated with 0.05, 0.10, and 0.25 ppm Hg compared to the control group. Specifically, Hg contamination led to a reduction in flower and fruit production. Notably, an increase in both flowering and fruiting was observed at 0.01 ppm Hg, with flower and fruit counts rising to 30 and 10, respectively, compared to 25 flowers and 8 fruits in the control group. Conversely, higher Hg concentrations resulted in a significant reduction in fruit production. At 0.25 ppm Hg, only 1 fruit was produced, while 0.05 and 0.10 ppm Hg treatments yielded 4 and 2 fruits, respectively. Additionally, early fruit ripening was observed at 0.01 ppm Hg, suggesting that low concentrations of Hg can impact the reproductive processes of tomato plants.

**Fig. 3** and **Fig. 4** illustrate the observable signs of toxicity in tomato plants under different Hg concentrations. Severe visual symptoms, including wilting, chlorosis, reduced fruit size, and leaf drop, were evident at 0.25 ppm Hg, as shown in **Fig. 3** and **Fig. 4**. The results indicate that tomato plants exposed to this high concentration of Hg experienced significant deterioration and eventual death, as observed over a 60-day exposure period. This suggests that Hg exposure at 0.25 ppm is highly toxic to tomato plants.

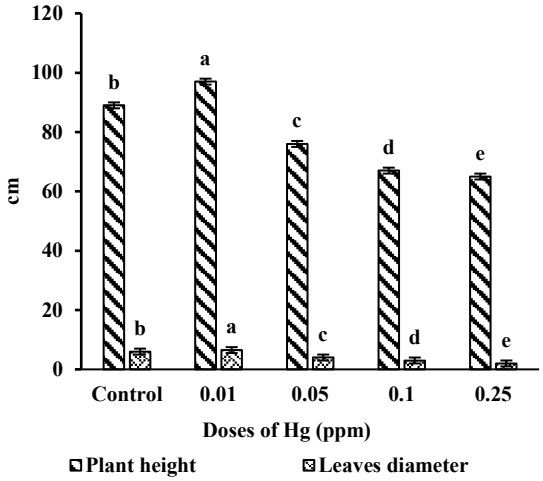


**Fig. 1.** Effect of different doses of Hg on plant height and leaf diameter of tomato after 14 days of exposure. Error bars represent the standard errors (SE) of the treatment means. Significant differences between Hg concentrations, as determined by Tukey's multiple range test ($p < 0.05$), are indicated by different letters.

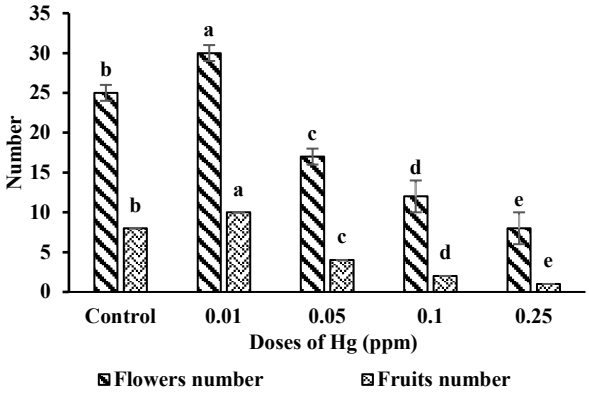


**Fig. 2.** Effect of different doses of Hg on flower and fruit numbers of tomato after 14 days of exposure. Error bars represent the standard errors (SE) of the treatment means. Significant differences between Hg concentrations, as determined by Tukey's multiple range test ($p < 0.05$), are indicated by different letters.

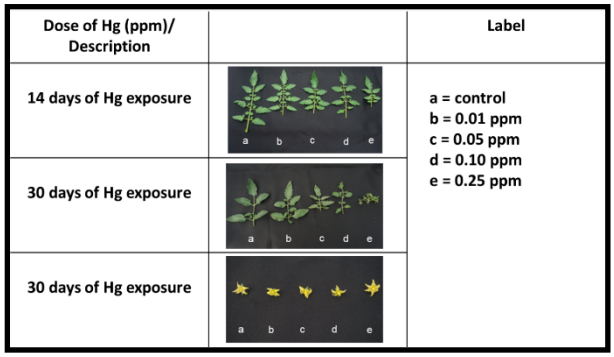


**Fig. 3.** Effects of different doses of Hg on leaf morphology and flower petal development of tomato.

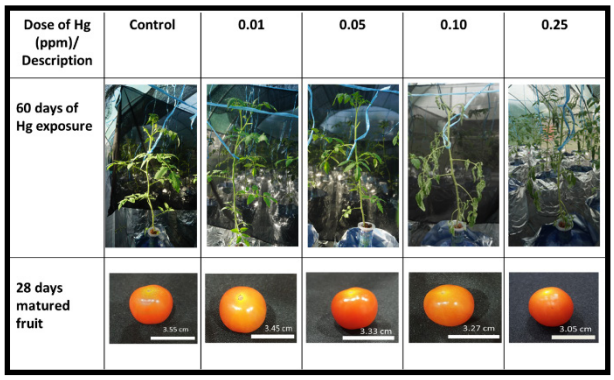


**Fig. 4.** Effect of different doses of Hg on plant growth and fruit size of tomato.

In contrast, no signs of toxicity were observed at 0.01 ppm Hg, indicating that Hg concentrations below this level are likely harmless. This finding suggests a potential threshold for Hg toxicity in tomato plants. Interestingly, exposure to the highest Hg concentration (0.25 ppm) appeared to have an effect on the number of petals in tomato flowers, as depicted in **Fig. 3**. While control flowers had 5 petals, those exposed to 0.25 ppm Hg had an increased petal count of 6, whereas other Hg-treated samples showed no change. Overall, the results indicate that high concentrations of Hg can cause severe harm to tomato plants, while lower concentrations may not produce visible toxic effects. Further research is needed to explore the impact of low Hg concentrations on petal number in tomato flowers.

The present findings indicate that increasing concentrations of Hg significantly impact plant growth parameters, in line with previous research. These impacts manifest in various forms, including stunted growth, reduced biomass production, changes in leaf morphology, and alterations in physiological processes such as photosynthesis and nutrient uptake [13]). Hg exposure has been shown to significantly hinder the growth of various plants, including tomato [14], cucumber [15]), fern [16], Indian mustard [17, chickpea [18], alfalfa [19], wheat [20], and maize [21], as demonstrated by multiple studies. The negative impact on plant growth is linked to structural disturbances such as changes in cell shape, decreased intercellular spaces, and vascular abnormalities [22]. At 0.25 ppm Hg, there was a drastic reduction in plant height, leaf diameter, flower number, and fruit number compared to the control (**Fig. 1** and **Fig. 2**).

These results are consistent with [23], who observed reduced tomato plant growth parameters in response to Hg exposure at 20 mg/kg in soil. Similarly, Israr et al. [24] reported comparable findings for *Sesbania drummondii*, attributing the reduction in growth parameters to the strong affinity of roots for Hg, which leads to Hg accumulation in the roots. The study also noted that immature leaves were particularly affected, turning yellow and exhibiting a spider-like appearance.

High Hg concentrations caused a noticeable reduction in leaf area, likely due to disruptions in iron absorption by the roots. This aligns with Wang et al. [25], who demonstrated that iron deficiency in Chinese cabbage negatively impacted plant growth, causing yellowing of leaves and affecting nitrogen metabolism, photosynthesis, reactive oxygen metabolism, root medium pH, and $Fe^{3+}$ reductase activity. Marrugo-Negrete et al. [26] concluded that Hg disrupts plant physiological processes, impeding growth and development. Interestingly, at a low concentration of 0.01 ppm Hg, a hormetic effect was observed, promoting growth and improving physiological processes in tomato plants. This finding is consistent with prior research by Ren et al. [27], who observed a hormetic effect in rice seedlings exposed to a Hg concentration of 0.05 mg/L.

The findings of this study highlight the potential consequences of Hg contamination on plant growth and development, emphasizing concerns in agricultural settings where Hg contamination could severely affect crop production and food security. Furthermore, the study suggests that Hg exposure impairs root iron absorption, leading to reduced leaf area. This discovery is significant for understanding the mechanisms of Hg toxicity in plants and developing strategies to mitigate its effects on crop productivity.

**Effect of Hg on chlorophyll content in tomato leaves**

The levels of chlorophyll a, chlorophyll b, and total chlorophyll at various Hg concentrations are illustrated in **Fig. 5**. In the control group, chlorophyll a content was 1.9 ± 0.006 mg/g FW, chlorophyll b content was 0.66 ± 0.006 mg/g FW, and total chlorophyll content was 2.6 ± 0.006 mg/g FW. At 0.01 ppm Hg, the chlorophyll a content remained at 1.9 ± 0.006 mg/g FW, while chlorophyll b content decreased to 0.60 ± 0.002 mg/g FW, resulting in a slightly lower total chlorophyll content of 2.5 ± 0.007 mg/g FW compared to the control. Conversely, at 0.05 ppm Hg, chlorophyll a content increased to 2.0 ± 0.006 mg/g FW, and chlorophyll b content rose to 0.72 ± 0.002 mg/g FW, leading to a higher total chlorophyll content of 2.8 ± 0.007 mg/g FW. At 0.1 ppm Hg, chlorophyll a content decreased to 1.7 ± 0.006 mg/g FW, and chlorophyll b content dropped to 0.54 ± 0.002 mg/g FW, resulting in a total chlorophyll content of 2.3 ± 0.007 mg/g FW, which was lower than the control. Similarly, at the highest concentration of 0.25 ppm Hg, chlorophyll a content further decreased to 1.7 ± 0.006 mg/g FW, chlorophyll b content fell to 0.51 ± 0.002 mg/g FW, and total chlorophyll content was 2.2 ± 0.007 mg/g FW, also lower than the control.

These findings suggest that the impact of Hg on chlorophyll content varies with Hg concentration. Lower concentrations (0.01 and 0.05 ppm) showed an increase in chlorophyll content compared to the control, while higher concentrations (0.1 and 0.25 ppm) resulted in decreased chlorophyll content. However, statistical analysis indicated no significant difference between the control and 0.01 ppm Hg.

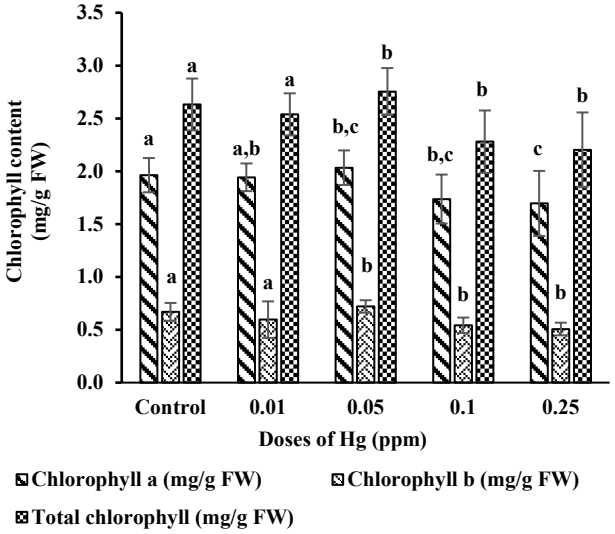


**Fig. 5.** Effect of different doses of Hg on chlorophyll content in tomato leaf after 14 days of exposure. Standard errors (SE) of the treatment means are represented by the bars. Significant differences between Hg concentrations, as determined by Tukey's multiple range test ($p < 0.05$), are indicated by different letters.

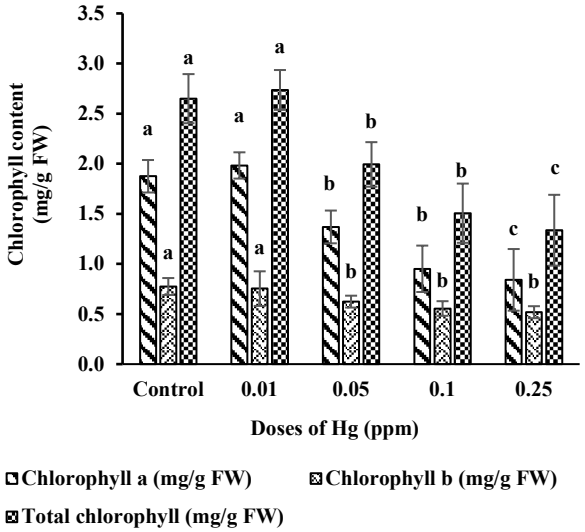


**Fig. 6.** Effect of different doses of Hg on chlorophyll content in tomato leaf after 30 days of exposure. Standard errors (SE) of the treatment means are represented by the bars. Significant differences between Hg concentrations, as determined by Tukey's multiple range test ($p < 0.05$), are indicated by different letters.

**Fig. 6** shows the levels of chlorophyll a, chlorophyll b, and total chlorophyll at various Hg concentrations. In the control group, chlorophyll a content was 1.9 ± 0.006 mg/g FW, chlorophyll b content was 0.77 ± 0.002 mg/g FW, and total chlorophyll content was 2.6 ± 0.007 mg/g FW. At the lowest Hg concentration (0.01 ppm), chlorophyll a content decreased to 1.7 ± 0.006 mg/g FW, and chlorophyll b content dropped to 0.69 ± 0.002 mg/g FW, resulting in a total chlorophyll content of 2.4 ± 0.007 mg/g FW, slightly lower than the control. At 0.05 ppm Hg, chlorophyll a content further decreased to 1.4 ± 0.006 mg/g FW, while chlorophyll b content fell to 0.62 ± 0.002 mg/g FW, leading to a total chlorophyll content of 2.0 ± 0.007 mg/g FW.

At 0.1 ppm Hg, chlorophyll a content dropped to 0.95 ± 0.006 mg/g FW, and chlorophyll b content decreased to 0.55 ± 0.002 mg/g FW, resulting in a total chlorophyll content of 1.5 ± 0.007 mg/g FW. At the highest Hg concentration (0.25 ppm), chlorophyll a content was 0.84 ± 0.006 mg/g FW, a reduction of approximately 55.2% compared to the control. Chlorophyll b content was 0.49 ± 0.002 mg/g FW, about 36.1% lower than the control, leading to a total chlorophyll content of 1.3 ± 0.007 mg/g FW, approximately 49.6% lower than the control. These results indicate that Hg exposure significantly reduces the levels of chlorophyll a, chlorophyll b, and total chlorophyll. This pattern is consistent with previous studies, which show that Hg disrupts photosynthetic processes by inhibiting chlorophyll synthesis and impairing the photosynthetic machinery.

Numerous studies have explored the impact of heavy metals on chlorophyll synthesis in plants, focusing on how these metals either directly inhibit specific enzymatic processes or induce deficiencies in essential nutrients. For example, research on dark-grown wheat leaves has shown that Hg interferes with protochlorophyllide photoreduction, a critical step in chlorophyll synthesis [28]. The effects of increasing Hg concentrations on chlorophyll levels were assessed over 14 days (**Fig. 5**) and 30 days (**Fig. 6**). The findings revealed a decrease in chlorophyll a and total chlorophyll levels as Hg concentrations increased.

This reduction reflects the phytotoxic effects of Hg, with chlorophyll concentrations in tomato plants decreasing from 0.01 to 0.25 ppm compared to the control. These results suggest that Hg impairs nutrient assimilation, depriving plants of essential elements needed for physiological functions, which exacerbates stress responses and hampers growth [29; 30]. Hg also affects magnesium (Mg) uptake, a crucial component of the chlorophyll molecule. Inadequate Mg supply impairs chlorophyll synthesis, leading to decreased chlorophyll content [31]. Hg exposure was found to decrease chlorophyll levels and cause thylakoid degradation [32], as well as suppress the activity of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH):protochlorophyllide oxidoreductase (POR), an enzyme critical for chlorophyll biosynthesis [33; 34; 35].

In this study, chlorosis was observed, where mature leaf tissue turned yellow due to chlorophyll deficiency, indicating that Hg also disrupts nutrient uptake and causes nutrient deficiencies. For instance, Hg exposure affected uptake and accumulation of essential elements such as phosphorus (P) and manganese (Mn) [36]. Additionally, increased levels of thiol and malondialdehyde (MDA) were detected, signaling oxidative stress [37; 38]. Furthermore, Hg ions replaced metal ions in photosynthetic pigments, leading to a reduced photosynthetic rate [39]. These disruptions have significant implications for the overall health and growth of the plants.

Hg toxicity also extends to nucleic acids, disrupting critical cellular processes such as spindle development and chromosomal integrity, eventually leading to cell death [40]). Given these extensive effects, further research is needed to investigate the long-term exposure of plants to varying Hg concentrations. These findings align with previous research indicating that heavy metals can diminish the availability of crucial elements like Fe and Mg, essential for chlorophyll synthesis [41].

**Effect of Hg on proline content in tomato**

The proline content measured in leaves under various Hg treatments is presented at two time points, day 14 and day 30, as shown in **Fig. 7**. On day 14, all treatments exhibited similar values for the measured leaves, with the control treatment registering a value of 1.41 ± 0.004 µmol/g FW, comparable to the values of the other treatments. Generally, values for each treatment increased from day 14 to day 30. For example, treatment with 0.01 ppm Hg showed a decrease in proline levels between day 14 and day 30, while the treatment with 0.25 ppm Hg exhibited a significant increase.

Significant differences between treatments were observed by day 30. The control treatment maintained a value of 1.4 ± 0.05 µmol/g FW, similar to the day 14 value. In contrast, the 0.01 ppm treatment showed a decrease in proline content, with a lower value of 1.2 ± 0.05 µmol/g FW. Treatment with 0.05 ppm Hg also showed a decrease, with a value of 3.9 ± 0.05 µmol/g FW. Conversely, treatment with 0.1 ppm Hg showed a slight increase, with a value of 5.44 ± 0.05 µmol/g FW. The 0.25 ppm Hg treatment exhibited the highest value of 12 µmol/g FW, indicating a significant increase in proline levels, surpassing all other Hg treatments. The data revealed that the 0.25 ppm Hg treatment had the most significant effect on day 30, resulting in a substantial increase compared to all other treatments. The 0.1 ppm Hg treatment also displayed a slight increase, whereas the control treatment and the 0.05 ppm Hg treatment showed minor decreases.

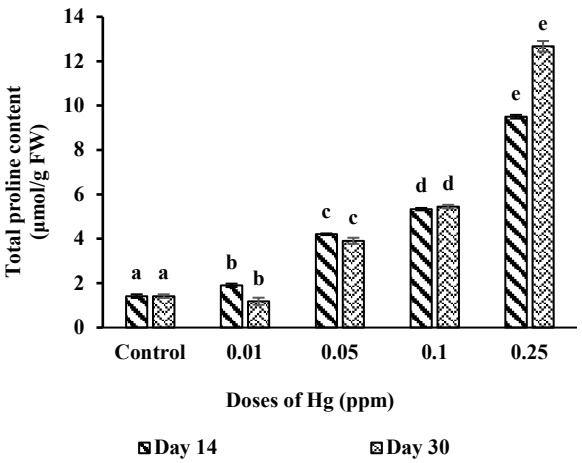


**Fig. 7.** Effect of different doses of Hg on proline content of tomato leaves after 14 and 30 days of exposure. Standard errors (SE) of the treatment means are represented by the bars. Significant differences between Hg concentrations, as determined by Tukey's multiple range test ($p < 0.05$), are indicated by different letters.

The proline content of unripe and ripe fruit is presented in **Fig. 8**, comparing the Hg levels in the fruit at different Hg concentrations for two stages of ripeness (18 days after anthesis and 28 days after anthesis). The results showed that in the control treatment, the proline content in the fruit was 1.1 ± 0.09 µmol/g FW for ripe fruit and 0.94 ± 0.05 µmol/g FW for unripe fruit.

There was a correlation between increased proline content and the amount of Hg in the fruit. For example, at the lowest Hg concentration tested (0.01 ppm), the proline content was 0.77 ± 0.09 µmol/g FW for ripe fruit and 0.64 ± 0.05 µmol/g FW for unripe fruit. Conversely, at the highest Hg concentration tested (0.25 ppm), the proline content rose to 10 ± 0.09 µmol/g FW for ripe fruit and 8.61 ± 0.05 µmol/g FW for unripe fruit.

Moreover, the data highlighted that the proline content in mature fruit consistently surpassed that in unripe fruit for all Hg concentrations tested. For instance, at the highest Hg concentration (0.25 ppm), the proline content was 10 ± 0.09 µmol/g FW in ripe fruit, whereas it was 8.61 ± 0.05 µmol/g FW in unripe fruit. Additionally, percentage changes indicated that the presence of Hg significantly elevated the proline content in both ripe and unripe fruit. For example, at a Hg concentration of 0.25 ppm, the proline content increased by 809.9% in ripe fruit and by 816.7% in unripe fruit compared to the control, underscoring the substantial impact of Hg concentrations on proline content in the tested fruits, with higher concentrations leading to elevated proline content.

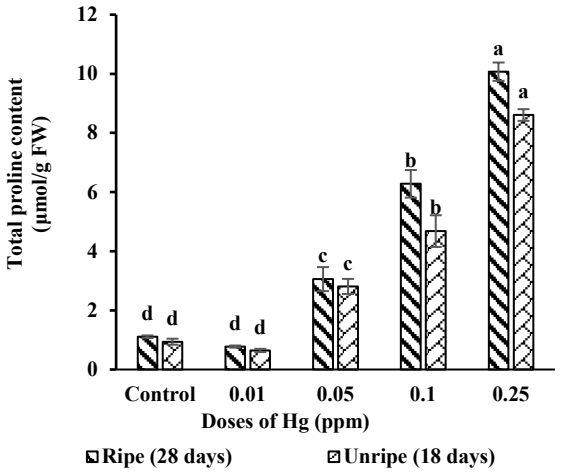


**Fig. 8.** Effect of different doses of Hg on proline content in unripe (18 days after anthesis) and ripe (28 days after anthesis) fruits of tomato. exposed to varying Hg concentrations. Standard errors (SE) of the treatment means are represented by the bars. Significant differences between Hg concentrations, based on Tukey's multiple range tests ($p < 0.05$), are indicated by different letters.

**Fig. 9** shows that the root proline concentration in the control group was 1.2 ± 0.004 µmol/g FW. In contrast, the highest Hg concentration tested (0.25 ppm) resulted in a proline concentration of 14 ± 0.004 µmol/g FW, indicating a substantial increase in proline levels in the roots due to Hg exposure. Additionally, proline content in the roots increased from 1.8 ± 0.004 µmol/g FW at 0.01 ppm Hg to 3.6 ± 0.004 µmol/g FW at 0.05 ppm. The increase continued more dramatically to 9.9 ± 0.004 µmol/g FW at 0.1 ppm and reached 14 ± 0.004 µmol/g FW at 0.25 ppm. Overall, the data clearly demonstrate that higher Hg concentrations significantly elevate proline content in the roots of tomato plants.

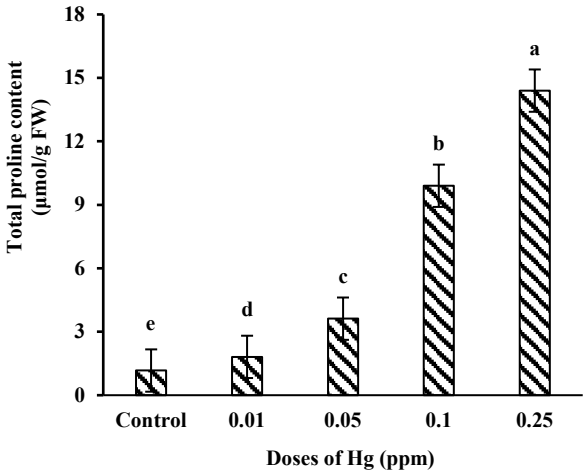


**Fig. 9.** Effect of different doses of Hg on proline content in the roots of tomato. after 120 days of exposure. Standard errors (SE) of the treatment means are represented by the bars. Significant differences between Hg concentrations, as determined by Tukey's multiple range tests ($p < 0.05$), are indicated by different letters.

The accumulation of proline in various plant species is recognized as an adaptive response to stress conditions, enhancing the plant's ability to cope with adverse environmental factors [42]. This phenomenon has been well-documented in recent years. While some researchers have questioned the direct correlation between proline accumulation and stress adaptation, it is generally accepted that elevated proline levels in response to stress benefit plant cells. Proline synthesis is believed to be a response to various injuries, including heat, cold, salinity, and exposure to toxic heavy metals, as supported by several studies. When plants are exposed to toxic heavy metals, proline biosynthesis is accelerated, often accompanied by increased activity of superoxide dismutase (SOD) and membrane damage [43].

Various studies have reported increased proline accumulation in plants exposed to heavy metals [44; 45; 46; 47; 48]. Our findings align with these research, showing that elevated Hg concentrations significantly increase proline accumulation in tomato plants. Elevated proline levels activate stress-response pathways, triggering the expression of stress-related genes and proteins involved in stress tolerance mechanisms [49]. The rise in proline levels with increasing Hg concentration highlights its role in maintaining the functional integrity of critical enzymes and proteins, thereby ensuring the continuous operation of essential metabolic processes.

Proline also plays a crucial role in neutralizing reactive oxygen species (ROS) and enhancing the activity of various antioxidant enzymes [50]. For example, rice plants treated with proline showed increased tolerance to Hg by reducing ROS levels [51]. Similarly, *Triticum aestivum* plants with higher proline content exhibited improved tolerance to cadmium (Cd) exposure [49]. Therefore, our findings from 0.05 ppm to 0.25 ppm Hg align with previous research, highlighting proline's beneficial effects on alleviating oxidative stress and enhancing stress tolerance in various plant species.

**Effect of Hg on lipid peroxidation in tomato**

The data presented in **Fig. 10** indicate that malondialdehyde (MDA) levels, a marker for lipid peroxidation, generally rise with increasing Hg exposure levels and durations. For instance, the control group exhibited an MDA content of 506 µmol/g FW after 14 days of exposure. In comparison, the MDA levels for the 0.01, 0.05, 0.1, and 0.25 ppm exposure groups were 208 ± 342, 688 ± 342, 763 ± 342, and 863 ± 342 µmol/g FW, respectively. After 30 days of exposure, the control group had an MDA level of 599.08 µmol/g FW, while the levels for the 0.01, 0.05, 0.1, and 0.25 ppm groups were 256 ± 342, 711 ± 342, 869 ± 342, and 1433 ± 342 µmol/g FW, respectively. The results further show that the increase in MDA, indicative of increased lipid peroxidation, was more pronounced in the 0.05, 0.1, and 0.25 ppm treatments compared to the 0.01 ppm treatment for both the 14-day and 30-day exposure periods. This suggests that higher Hg exposure leads to elevated MDA levels in tomato leaves, and longer exposure durations contribute to increased lipid peroxidation.

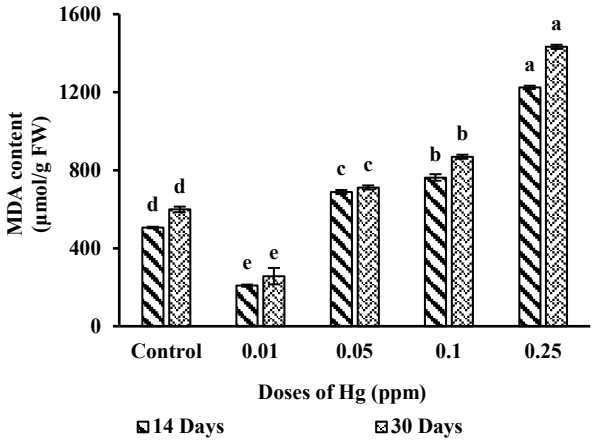


**Fig. 10.** Effect of varying Hg doses on lipid peroxidation (measured as malondialdehyde, MDA) in the leaf of tomato exposed to different Hg concentrations for 14 and 30 days. Standard errors (SE) of the treatment means are represented by the bars. Significant differences between Hg concentrations, as determined by Tukey's multiple range tests ($p < 0.05$), are indicated by different letters.

The data presented in **Fig. 11** show that lipid peroxidation increased in both unripe and ripe fruits with higher Hg exposure levels and extended durations. For ripe fruits exposed to Hg for 28 days, the control group had an MDA value of 541 ± 136 µmol/g FW. In comparison, the MDA values for fruits exposed to 0.01, 0.05, 0.1, and 0.25 ppm Hg were 451 ± 136, 644 ± 136, 723 ± 136, and 851 ± 136 µmol/g FW, respectively. In unripe fruits exposed to Hg for 18 days, the control group showed an MDA concentration of 555 ± 136 µmol/g FW. The MDA values for unripe fruits exposed to 0.01, 0.05, 0.1, and 0.25 ppm Hg were 398 ± 136, 689.33 ± 136, 802 ± 136, and 894 ± 136 µmol/g FW, respectively. These results illustrate that higher Hg concentrations resulted in a more significant increase in MDA levels in both unripe and ripe fruits, particularly at 0.05, 0.1, and 0.25 ppm Hg, compared to the 0.01 ppm Hg exposure.

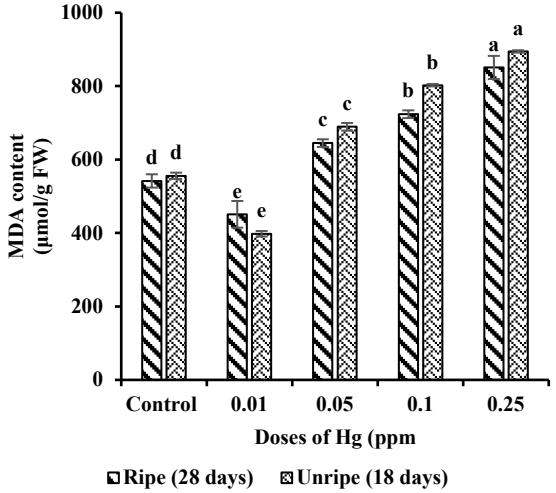


**Fig. 11.** Effect of various Hg doses on lipid peroxidation (measured as malondialdehyde content, MDA) in the unripe (18 days after anthesis) and ripe (28 days after anthesis) fruits of tomato. Fruits were exposed to different Hg concentrations for 28 days in ripe fruit and 18 days in unripe fruit. Standard errors (SE) of the treatment means are shown by the bars. Significant differences between Hg concentrations, based on Tukey's multiple range tests ($p < 0.05$), are indicated by different letters.

**Fig. 12** shows the MDA levels in tomato roots exposed to various Hg concentrations compared to the control group. The data reveal that higher Hg concentrations are linked to increased MDA levels. Specifically, the control group had an MDA level of 69 µmol/g FW. In comparison, MDA levels for the 0.01, 0.05, 0.1, and 0.25 ppm Hg exposure groups were 105 ± 9.7, 172 ± 9.7, 205 ± 9.7, and 238 ± 9.7 µmol/g FW, respectively. These results indicate a clear correlation: as Hg concentration rises, MDA levels also increase.

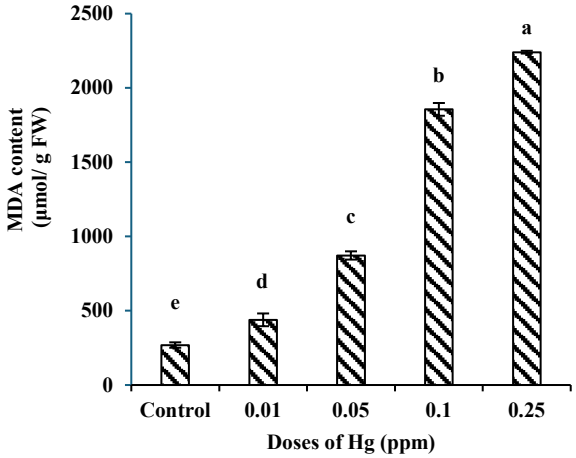


**Fig. 12.** Effect of various Hg doses on lipid peroxidation, measured as malondialdehyde (MDA) content, in the roots of tomato after 120 days of exposure. Standard errors (SE) of the treatment means are depicted by the bars. Significant differences between Hg concentrations, as determined by Tukey's multiple range tests ($p < 0.05$), are indicated by different letters.

Malondialdehyde (MDA) is a reliable biomarker for detecting lipid peroxidation, a type of oxidative damage that can occur within cells. As the most accurate indicator of lipid peroxidation status and cell membrane damage due to ROS, MDA levels reflect the extent of oxidative stress [52]. This study reveals that Hg exposure increases MDA levels, indicating that Hg stimulates ROS production, leading to elevated lipid peroxidation products and oxidative stress in tomato plants.

Under Hg stress, the accumulation of ROS is a critical factor that damages plants. The increased MDA levels observed in tomato organs exposed to Hg are consistent with findings from studies on Hg-treated plants, including wheat [53; 54], water hyacinth [55], radish [31], and okra [56]. Elevated Hg levels can disrupt physiological functions, causing nutrient imbalances and negatively affecting the production and performance of essential biological molecules like enzymes, vitamins, and hormones, leading to adverse effects [56; 57]. Under Hg stress, the accumulation of ROS is a critical factor that damages plants. The increased MDA levels observed in tomato organs exposed to Hg are consistent with findings from studies on Hg-treated plants, including wheat [53; 54], water hyacinth [55], radish [31], and okra [56]. Elevated Hg levels can disrupt physiological functions, causing nutrient imbalances and negatively affecting the production and performance of essential biological molecules like enzymes, vitamins, and hormones, leading to adverse effects [56; 57].

While most studies report increased MDA levels in the roots and leaves of plants exposed to Hg, this trend is less commonly observed in fruits. Studies on plants such as tomato seedlings [58], mustard [59], alfalfa [60], and okra [56] typically do not show elevated MDA levels in fruits. However, our study found increased MDA levels in tomato fruits exposed to 0.01, 0.05, 0.10, and 0.25 ppm Hg, indicating oxidative damage to their membranes under high Hg concentrations. Elevated MDA levels in fruits suggest that oxidative stress also affects these reproductive structures. Roots are often reported to experience higher oxidative stress compared to leaves and fruits, likely due to Hg accumulation and the presence of antioxidant components that scavenge active oxygen species [31]. For instance, [61] observed increased MDA content in the roots of rapeseed treated with 10 ppm Hg, which triggered a strong antioxidative response. However, our study shows that MDA levels in roots are lower than in leaves and fruits, possibly due to the presence of robust antioxidant defense systems in the roots. These antioxidants, including peroxidases and superoxide dismutases, are effective at neutralizing ROS, which may lead to lower MDA levels in the roots.

## CONCLUSION

This study emphasizes the harmful effects of Hg on tomato plants, demonstrating that high concentrations result in severe toxicity, negatively impacting growth, morphology, and reproductive processes. In contrast, lower concentrations cause only minor effects. These findings underscore the importance of determining the precise thresholds at which Hg becomes harmful to tomato plants, which is crucial for developing effective strategies to mitigate its adverse effects in agricultural systems. Future research should focus on a detailed investigation of the biochemical and molecular responses of tomato plants to varying concentrations of Hg. This involves utilizing transcriptome analysis to investigate gene expression changes linked to Hg exposure, potentially uncovering critical regulatory pathways and stress-response mechanisms.

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