

Effect of Cadmium on Physico-biochemical Characteristics and Bioactive Compounds of *Lycopersicon esculentum* Mill. (Var. Arka Abha)

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ABSTRACT

Crop production, yield, quality, and sustainable agriculture are all hindered by cadmium stress. The purpose of this research is to determine how heavy metal contamination in the soil affects the physico-biochemical characteristics of tomato fruit. It was discovered that cadmium harms the quantity and quality of tomato plant yield. In tomato fruits grown in Cd-contaminated soil, characteristics such as fruit weight, total soluble solids, titratable acidity, and lycopene content were measured. Additionally, some secondary metabolites (total phenols and flavonoids) were also assessed. The outcomes were contrasted with tomato fruits grown in non-contaminated soil (control). The findings show that cadmium contamination of the soil has a negative impact on the characteristics of tomato fruits, titratable acidity, total soluble solids, lycopene, and ascorbic acid content. In addition, phenol and flavonoid levels in fruits from plants grown in contaminated soil are higher than in control fruits. To prevent an excessive buildup of heavy metals in the body, it is advised against consuming large quantities of fruits grown in that region.

Keywords: Ascorbic acid, Cadmium, Heavy metals, Titratable acidity, Total soluble solids.

Highlights

- Increasing cadmium concentrations led to a progressive accumulation of cadmium in the soil.
- The findings underscore the importance of monitoring and managing cadmium levels in soil to ensure the health and productivity of tomato plants.
- Cadmium contamination has detrimental effects on *L. esculentum* Mill, leading to reduced growth and potential alterations in fruit quality and bioactive compound composition.

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INTRODUCTION

One of the most significant vegetable crops grown and consumed globally is the tomato (*Lycopersicon esculentum* Mill.), which is valued for both its delicious flavor and its high concentration of beneficial compounds. Tomatoes serve as a nutritional source for chemical elements that may have a positive impact on health in addition to their role in the human diet because of their abundance in vitamins, minerals, lycopene, β -carotene, and anticancer agents (Balan *et al.*, 2023). Especially for carotenoids like lycopene and β -carotene, but also for phenolic compounds, ascorbic acid, tocopherols, and flavonoids, which have high antioxidative capacities, tomato is regarded as an excellent source of bioactive compounds. Because tomatoes and tomato-based products contain high levels of antioxidants, there is growing interest in them because eating tomatoes is associated with a lower risk of developing certain cancers, cardiovascular diseases (CVDs), or neurodegenerative diseases (Rivero *et al.*, 2022). In the Solanaceae family, which has taken on a significant role in fruit biology, tomatoes are a significant horticultural vegetable and fruit crop. Due to their small diploid genome, rapid generation, easy transformation, and abundant genomic resources, tomatoes have recently been recognized as a model plant for functional genomics (Vats *et al.*, 2023). Due to their rising commercial, marketable, and dietary values, tomato crops have gradually become more important (Rajameto *et al.*, 2021).

With 64.9 million tons produced, China is the world's top tomato producer, followed by India with 20.6 million tons,

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Turkey with 13.2 million tons, and the United States with 12.2 million tonnes (FAOSTAT2020). Large amounts of chemical contaminants, especially heavy metals released into the environment, have recently drawn attention from around the world due to rapid population growth and intensive human activities (Liu *et al.*, 2023). Plants, which are sessile organisms, are indiscriminately exposed to environmental factors (Tokic *et al.*, 2023). Since their emergence, plants have grown in a variety of agricultural ecosystems. Therefore, they must have the ability to react to various situations. Heat, salinity, chill, flooding, a lack of water, and heavy metals are just a few examples of abiotic stresses that have a devastating effect on plant growth and yield. Additionally, heavy metal-polluted soil is now widespread in various terrestrial ecosystems (Kazerooni *et al.*, 2022). Heavy

metals (HMs) harm all plant processes and are also extremely toxic to humans, who may experience serious health issues as a result. The accelerated growth of manufacturing and industry seriously pollutes the environment, particularly the soil.

Plant roots take up HMs from the soil, which build up in all plant tissues and significantly slow down several physiological and molecular processes (Khakimova *et al.*, 2023). Cadmium (Cd) is one of the most phytotoxic heavy metals and one of the main environmental pollutants. This unnecessary component is very mobile in the soil-plant system and can interfere with several critical functions, which will cause poor plant growth and a low economic yield. In plants, exposure to Cd delays germination, stunts growth, induces chlorosis, changes the ultrastructure of the chloroplasts, prevents photosynthesis, and reduces carbon assimilation by inhibiting CO₂ fixation enzymes (Pirelová and Ondruková, 2021).

MATERIAL AND METHODOLOGY

Tomato seeds (var. Arka Abha) were acquired from the Indian Institute of Vegetable Research, Varanasi (IIVR). For the experiment, seeds of the same shape, color and weight were used. The soil used in the experiment was a mixture of red soil 40%+sandy loam 60% with a pH of 7.2. Cadmium was not present in this experimental soil, which comprises primary nutrients of 118 kg available N, 88 kg P and 106 kg k/ha and micronutrients of 21.89 mg available Cu, 219.11 mg Fe, 168 mg Mn and 28.13 mg Zn/kg. The cadmium source employed was cadmium chloride (Cd Cl₂ ½ H₂O).

The trials of pot culture were carried out in the greenhouse of the Department of Botany at Glocal University. Tomato plants were raised in pots with untreated soil (Control) and soil mixed with cadmium at various concentrations (*viz.*, 25, 50, 75 and 100 mg/kg). The inner surfaces of pots were lined with a polythene sheet. About 3 kg of air-dried soil were used in each pot. Six seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of three per pots after a week of germination. Each treatment, including the control, was replicated three times. Three plants from each replicate of the pot were examined for the various physicochemical and bioactive parameters.

Physical Parameters

Germination

We used the following calculation to calculate the percentage of germination:

$$G (\%) = (\text{number of germinated seeds}/\text{total number of seeds}) \times 100$$

Plant Height (cm)

Using a scale, the height of the plant was calculated from the ground up to the tip of the uppermost leaf and stated in centimeters (cm) (Fig. 1).

Root length

Root length was measured using a cm scale.

Number of Fruit per Plant

This was estimated by calculating the mean of the total fruits produced by each plant in each treatment and replication.



Fig. 1: Using a scale, the height of the plant was calculated

Fruit weight

Fruit weight was calculated using an analytical balance with 0.0001 g precision (PR-series, Ohaus, NJ, US).

Chemical parameters

pH

A pH meter LI 120 (ELICO Pvt. Ltd) was used to estimate the pH values of the tomatoes, which consists of a glass electrode attached to a digital meter that aids in measuring the acidity degree of tomato juice from semi-ripe and ripe samples.

Total soluble solids

TSS was calculated by placing 1 to 2 drops of clear juice on the prism of a refractometer (Model Misco®) with a range of 0 to 32° Brix and a resolution of 0.2° Brix. The prism of the refractometer was cleaned with distilled water between samples and dried before use. The refractometer was calibrated using distilled water (0° Brix TSS) as the standard (Tigist *et al.*, 2013).

Titrateable acidity (TA)

Tomato juice was collected from both semi-ripe and ripe tomatoes. The extracted juice is diluted in distilled water (10 ml) and titrated with 0.1 N sodium hydroxide (NaOH) solution and a few drops of phenolphthalein as an indicator against 0.1 N sodium hydroxide (NaOH) solution. The arrival of pink color was regarded the titration's endpoint. The acidity of the samples was estimated using the following equation, which expressed the acidity in percent anhydrous citric acid (Gaikwad *et al.*, 2020).

$$\text{Titrateable acidity (\%)} = M \times 0.1 \text{ N} \times N \times C \times W \times 100$$

Were

M = Titre value

N = volume made up, ml

C = Equivalent weight of citric acid

W = weight of sample, gm.

Total Reducing Sugars

Reducing sugars were calculated utilizing Miller's method (Miller, 1959). 0.1 g of the oven-dried material was then homogenized in 80% pure alcohol. To the previously mentioned homogenate, 3 mL DNSA was added. About 100 mL of 1% NaOH, 50 mg of Na₂

503, and 200 mg of phenol crystals were combined in a 100 mL flask at 4°C to create fresh DNSA. A 1-mL of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ at 40 percent was then added after that. It was then cooled to a temperature of 25°C, and the optical density at 510 nm was determined.

Determination of chlorophyll content

For chlorophyll estimation, the Harborne approach (Harborne, 1984) was used. 200 mg fresh leaf tissue was homogenized in 80% acetone and centrifuged for 15 minutes at 4°C at 12,000 rpm. After adequate dilutions, the quantities of chlorophyll in the supernatant were quantified spectrophotometrically using the formula below.

$$\text{Total chlorophyll content (mg L}^{-1}\text{)} = 17.3 A_{646} + 7.18 A_{663}$$

$$\text{Chlorophyll a (mg L}^{-1}\text{)} = 12.21 A_{663} - 2.81 A_{646}$$

$$\text{Chlorophyll b (mg L}^{-1}\text{)} = 20.13 A_{646} - 5.03 A_{663}$$

Bioactive parameters

Lycopene content

The lycopene pigment was extracted by dissolving in 10 mL of acetone after a known weight of samples was crushed thoroughly with a pestle and mortar. The mixture was shaken for 30 minutes at 140 rpm before being centrifuged for 15 minutes at 12000 rpm. Acetone was used to make up the final volume of the supernatant solution (100 mL). Finally, the amount of lycopene in the sample was evaluated by measuring the absorbance at 503 nm (Tilahun *et al.*, 2017).

$$\text{Lycopene content (mg/100 g)} = 31.206 \times A \times W$$

Were,

A = absorbance at 503 nm

W = weight of sample extracted (g)

Ascorbic acid

The 2, 6-dichlorophenol indophenol titration method was used to determine the ascorbic acid content of the samples. 50 cc of oxalic acid was added to a known amount of material. The mixture was properly blended before being filtered. A standardized dye was used to titrate 10 mL of the extract. An endpoint is defined as a pale pink tint that lasts for 5 to 10 seconds (Tilahun *et al.*, 2017).

$$\text{Ascorbic acid content (mg/100 g)} = M \times D \times N \times W \times 100$$

Were

M = Titre value

N = volume made up (mL)

D = Dye factor (0.1/titre value)

W = weight of sample (gms)

Estimation of total flavonoids

We have added 250 liters of methanolic extract with 1.25 liters of distilled water and 75 liters of a 5% NaNO_2 solution. 150 L of a 10% $\text{AlCl}_3 \cdot \text{H}_2\text{O}$ solution was added after 5 minutes and filtered for 6 minutes. The A510 was calculated after adding 500 L of 1 M NaOH and 275 L of distilled H_2O to the mixture. The standard was quercetin (QU) (Jia *et al.* 1999) the results were represented in milligrams of quercetin equivalents per 100 grams of fresh weight.

Estimation of total Phenolics

The method described by Singleton *et al.* was used to determine phenolic chemicals (Singleton *et al.*, 1999). One ml of methanolic extract and 1-mL of Folin Ciocalteu reagent were combined. After 3 minutes, 1-mL of 20% saturated sodium carbonate solution was added to the mixture, which was then adjusted to 10 mL with distilled H_2O . The reaction mixture was shaken intermittently in the dark for 1-hour. A spectrophotometer was used to measure the absorbance at A725 (UNICAM UV300). Based on the standard curve for Gallic acid (GAL), the concentration of phenolic compounds was determined, and the results were represented as mg Gallic acid equivalent (GAE) 100 g¹ FW.

Proline Content

Bates *et al.* described a technique for determining the Proline content (Bates *et al.* 1973). A 0.5 g leaf sample was homogenized with 5 mL sulfo salicylic acid (3 percent, m/v) and centrifuged at 12,000 g for 20 minutes at 4°C. The supernatant, glacial acetic acid, and acid ninhydrin were then added in equal amounts (2 mL), followed by 1-hour of boiling at 100°C and 20 minutes in ice. A 4 mL toluene was quickly vortexed into the mixture. The Proline concentration was measured at 520 nm after the top toluene layer was removed.

RESULT

The effect of cadmium on the physical parameters of *Lycopersicon esculentum* Mill (var. ArkaAbha) are presented in (Table 1). When cadmium-treated plants (25, 50, 75, and 100 mg/kg) were compared to untreated plants, all growth parameters steadily declined. The percentage of germination rapidly reduced as the concentration was increased. It was 94, 86, 79, and 66 percent in 25, 50, 75, and 100 mg/kg treated samples, respectively. Seedlings in the control samples survived in the field 93% of the time. The percentage of seedlings that survived in all of the Cd-treated samples rapidly decreased (Table 1). In 25 mg/kg, it was 88 percent. 84, 72, and 61 percent in 50, 75, and 100 mg/kg treated samples, respectively. The height of Arka Abha

Table 1: Physical parameters of *L. esculentum* Mill (Arka Abha)

Cadmium concentration (mg/kg)	% of germination	Survival percentage	Plant height (cm)	Root length (cm)	Fruit number (per plant)	Fruit weight (g)
Control	97 ± 0.02	93	71.23 ± 0.003	10.8 ± 0.004	24 ± 0.001	66.14 ± 0.05
25	94 ± 0.05	88	70.96 ± 00.04	10.1 ± 0.004	22 ± 0.002	61.12 ± 0.10
50	86 ± 0.03	84	68.54 ± 0.001	9.6 ± 0.001	19 ± 0.002	53.32 ± 0.12
75	79 ± 0.01	72	56.1 ± 0.002	8.3 ± 0.002	13 ± 0.001	46.74 ± 0.12
100	66 ± 0.02	61	47.8 ± 0.003	6.1 ± 0.005	9 ± 0.003	40.10 ± 0.09

± Standard deviation.

Table 2: Chemical parameters of *L. esculentum* Mill (Arka Abha)

Cadmium concentration (mg/kg)	Total soluble solids (°brix)	Titrateable Acidity (%)	Reducing sugars (%)	PH	Chlorophyll (mg/g FW)	Carotenoid (mg/g FW)
Control	4.25	0.66	2.17	4.24	0.54 ± 0.018	0.28 ± 0.011
25	4.29	0.74	2.19	4.16	0.49 ± 0.012	0.26 ± 0.014
50	4.37	0.84	2.26	3.98	0.38 ± 0.015	0.26 ± 0.013
75	4.49	0.91	2.41	3.87	0.30 ± 0.017	0.24 ± 0.012
100	4.61	0.98	2.57	3.71	0.21 ± 0.019	0.21 ± 0.016

± Standard deviation.

Table 3: Bioactive parameters of *L. esculentum* Mill (Arka Abha)

Cadmium concentration (mg/kg)	Lycopene (mg/100g)	Vitamin c (mg/100g)	Flavonoids (mg 100g-1 FW)	Phenolic content (mg GAE/100g)	Proline (mg/g)
Control	2.88 ± 0.03	25.51 ± 0.03	297 ± 0.04	22.16 ± 0.02	2.231 ± 0.02
25	2.89 ± 0.04	25.63 ± 0.06	378 ± 0.06	23.54 ± 0.06	6.298 ± 0.03
50	2.91 ± 0.01	25.79 ± 0.04	564 ± 0.01	24.37 ± 0.05	11.998 ± 0.05
75	2.94 ± 0.01	25.98 ± 0.02	781 ± 0.02	25.11 ± 0.05	17.437 ± 0.04
100	2.97 ± 0.02	26.17 ± 0.03	897 ± 0.04	25.82 ± 0.04	24.864 ± 0.03

± Standard deviation.

plants treated with 25 M Cd did not differ significantly from the height of control plants. Higher doses (50 mg/kg, 75mg/kg, and 100 mg/kg), however, lowered plant height. The root length of tomato plants exposed to 100 mg/kg Cd was likewise drastically reduced. All of the cadmium concentrations studied reduced the number of fruits per plant from 24 in control plants to nearly 9 in cadmium-treated plants. The weight of the fruit decreased as the concentration of cd increased.

The PH of the fruit decreased at all Cd values (25, 50, 75, and 100mg/kg), but in a similar fashion (Table 2). TSS content (°brix) increased at only 50 mg/kg, while Titrable acidity increased similarly (Table 2). Chlorophyll and carotene levels in control samples were 0.54 and 0.28, respectively. The amount of chlorophyll and carotenoid content decreased considerably in a concentration-dependent manner (Table 2).

Cadmium concentrations had an increasing effect on ascorbic acid levels, although only at low Cd doses (25 and 50 mg/kg) were the effects substantial. Under the influence of cd toxicity, the levels of flavonoids in *L. esculentum* plants were up-regulated in comparison to control. Color features and lycopene concentrations in the fruit mesocarp were unaffected by CdCl₂ concentrations. Cd poisoning resulted in higher amounts of total reducing sugars than the control group (Table 3). The presence of cd resulted in a significant increase in proline as compared to control plants (Table 3). Following treatment with 25, 50, 75, and 100 mg/kg, the Proline content increased by 6.298, 11.998, 17.437, and 24.864 fold, respectively. When tomato plants were exposed to 25, 50, 75, and 100 mg/kg, total phenolic content increased by 23.54, 24.37, 25.11, and 25.82 times, respectively, compared to control.

DISCUSSION

A wide range of chemical pollutants are being introduced into agricultural soils worldwide by anthropogenic activities.

According to studies, heavy metals like lead, cadmium, nickel, and V enter the soil as the main cause of agricultural soil pollution (Altaf *et al.*, 2021). One of the heavy metals that occurs naturally and is particularly hazardous to both plants and people is cadmium (Cd). Recent years have seen substantial and pervasive pollution of cropland due to the rise in Cd concentration in soils. The buildup of Cd in plants has harmful implications on their regular growth (Li *et al.*, 2023). We examined the physical, chemical, and bioactive features of *Lycopersicon esculentum* Mill to look into the effects of cadmium stress on plant growth and development.

The current study exposed tomato plants (*L. esculentum* Mill.) var. Arka Abha to cadmium. All cadmium-treated samples show a decrease in seed germination percentage with increasing concentration (Table 1). A reduction in water transport and absorption caused by Cd is one potential explanation for decreased seed germination. Research suggests that Cd enters the cytosol through calcium channels found in the plasma membrane, changing the interactions between cells and water (Kaur *et al.*, 2023). (Kranner and Colville, 2011) claims that metals have an impact on seed germination through two different mechanisms: (1) toxicity and (2) altering water intake during the imbibition process. Several studies on peas (Siddiqui, 2009) and sorghum (Kuriakose and Prasad, 2008) have connected the suppression of seed germination to a decrease in the germination medium's osmotic potential, particularly in the presence of HMs like Cd and Cu, which makes it harder for grain to absorb water.

According to the current study, proline accumulated in tomato roots and leaves when exposed to cadmium (Table 3). Proline acts as a protective agent, promoting increased growth, claim (Chandrakar *et al.*, 2018). Proline directly scavenges OH radicals (Per *et al.*, 2017; Chandrakar *et al.*, 2018) and facilitates the removal of ROS (Alyemeni *et al.*, 2018; Alves *et al.*, 2018). Additionally, it serves as a metal chelator under pressure (Aslam

et al., 2017). When exposed to HMs, plants with high levels of proline and phenol in their tissues act as an osmolyte, a radical-free scavenger, and a cellular redox protective agent, according to research by (Brilli et al., 2019). In response to Cd toxicity, wheat plants showed an increase in proline content (Kaya et al., 2020). Proline may lessen the negative effects of ROS by acting as a singlet oxygen scavenger, a radical hydroxyl scavenger, a lipid peroxidizing inhibitor, and more (Gill and Tuteja, 2010). Proline is essential for protein chemistry, membrane strength, cellular redox potential buffering, and free radical scavenging in plant cells. Additionally, in HM, high proline levels are brought on by de novo synthesis or the inhibition of degradation pathways (Sharma et al., 2020). During significant metal load, proline levels frequently rise. Proline rose in Brassica under cadmium stress and lead stress in wheat. Due to proline's function in osmoregulation and as a free radical scavenger, these increases typically translate into increased heavy metal tolerances (Alves et al., 2023).

CONCLUSION

The study on cadmium (Cd) effects on *L. esculentum* Mill (var. Arka Abha) revealed significant negative impacts on plant health with increasing Cd concentrations. Key findings include:

- Growth Inhibition: Dose-dependent decreases in germination rate, survival percentage, plant height, root length, number of fruits per plant, and fruit weight.
- Chemical Alterations: Decreased fruit pH, increased total soluble solids (TSS) at 50 mg/kg Cd, and increased titratable acidity.
- Chlorophyll and Carotenoid Reduction: Significant reductions in chlorophyll and carotenoid content.
- Bioactive Compound Fluctuations: Increased ascorbic acid levels at low Cd doses, up-regulated flavonoid content, and no significant effect on lycopene concentration.
- Proline and Phenolic Content Increase: Considerable increases in proline and phenolic content, indicating stress response activation.

Cadmium contamination negatively affects *L. esculentum* Mill, reducing growth and altering fruit quality and bioactive compounds. Monitoring and managing soil Cd levels is crucial. Further research should explore Cd uptake and detoxification mechanisms and mitigation strategies for crops.

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CONFLICT OF INTEREST

None

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