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Effects of Cadmium- Contaminated Soil on Morphological, Physiological, and ROS Responses in Tomato Plants

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Abstract-

Tomato plants (*lycopercicun esculentum*) at 0, 18, 36, and 54 days after sowing (DAS) are studied to determine the effects of different Cd concentrations (0.05, 0.075, and 0.1 mg/kg soil) on their morphological and physiological characteristics. In addition to physiological parameters like leaf area index, relative growth rate, chlorophyll content, the photosynthesis process. rate, stomatal conductance, and transpiration, morphological parameters like plant height, shoot length, root length, number of leaves, number of branches, number of fruits, and biomass were also examined. In order to comprehend the oxidative stress brought on by Cd, the buildup of reactive oxygen species (ROS) and the activity of antioxidant enzymes (catalase, per antioxidant, and superoxide dismutase) were also evaluated. Cd bioaccumulation factors (BAFs) were also measured in the study to assess the degree of Cd uptake. The findings showed that plant growth and physiological function were dose-dependently inhibited, with biomass, chlorophyll content, and photosynthetic activity significantly decreasing with increasing Cd concentration.

The plant's reaction to oxidative stress caused by Cd was highlighted by increased ROS levels and antioxidant enzyme activity. The results highlight the harmful impacts of Cd pollution on tomato plants and offer important new information about the processes underlying heavy metal stress and bioaccumulation.

Introduction

The concentration of cadmium in the soil is continuously increased by excessive weathering of rocks, industrial operations such as mining and smelting, and excessive use of phosphate fertilizer for agricultural purposes. After entering plants, cadmium causes changes in metabolic uniformity by modifying chlorophyll fluorescence, lowering gas exchange parameters, downregulating nitrogen metabolism and assimilation, degrading photosynthetic pigments, which lowers photosynthetic rate, and changing the activity of antioxidant enzymes (Ahmad et al. 2015; Khan et al. 2015). The main causes of elevated Cd in soil include chemical fertilizer application, sewage wastewater irrigation, and industrial contamination (Wu et al., 2010).

The concentration of Cd in the growing media often corresponds to the quantity of Cd translocated to the fruits (Gratão et al., 2012; Hussain et al., 2017; Kumar et al., 2015). Cd concentrations range from 0.01 to 0.8 mg/kg in natural areas to 1,500 mg/kg in polluted areas, and the issue was caused by anthropogenic activities that significantly increased the metal content in arable fields (Kabata-Pendias, 2011). The majority of environmental pollution happens close to industrial and urban areas where a variety of vegetables are frequently produced. In addition to the significant amounts produced by mining, metal-based insecticides, industrial waste, and battery manufacturing, the primary sources of soil Cd include air deposition from metal smelting processes and phosphorous (P) fertilizers (Kabata-Pendias, 2011).

As a result, numerous nations have enacted environmental laws pertaining to the levels of Cd in edible crop parts (Commission of the European Communities, 2014) and agricultural soils (CETESB, 2014),

where plants absorb this metal. Even in very small concentrations, Cd has a detrimental effect on plant nutrient uptake and homeostasis after being first absorbed from the soil through the roots (Ma et al., 2014) and then transferred to the shoot (Oono et al., 2016). However, it has been demonstrated that the primary mechanism limiting the movement of Cd to shoots and leaves is the vacuolar sequestration of Cd in root tissues (Ueno et al., 2010). Plants' tolerance to and accumulation of Cd metal varies and is mostly determined by their genotype. It has been observed, meanwhile, that the vast majority of species experience toxic effects from foliar buildup or exposure of 5–10 mg of Cd kg−1 of dry weight (DW) (White and Brown, 2010). Numerous studies have shown that even small amounts of Cd exposure can cause or stimulate uncontrolled oxidation, which disrupts the homeostatic mechanism of cells and causes electrolytic leakage. This, in turn, triggers biochemical reactions against oxidative stressors, further activating the antioxidant defense mechanisms of cells (Iannone et al., 2010; He et al., 2013a, 2013b; Anjum et al., 2015; Zouari et al., 2016; Zhou et al., 2016, 2017). The majority of environmental pollution happens close to industrial and urban areas where a variety of vegetables are frequently produced.

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In terms of planting area and production, the tomato (*lycopercicun esculentum*) is the second most commercially consumed plant, after potatoes. It is also used in investigations as a model plant (Hashem et al. 2015). However, long-term exposure to Cd often affects crop output by reducing fruit weight and quantity, often accompanied by decreased flower count and fruit setting rate (Hédiji et al., 2010, 2015; Hussain et al., 2017). Additionally, stem-end yellowing in tomatoes is caused by Cd accumulation in fruits (Kumar et al., 2015), which impairs the fruits' appearance and could lower their market value. It's interesting to note that tomato cultivars vary in their tolerance or susceptibility to Cd buildup and its consequences on fruit quality, yield, and even offspring fitness (Carvalho et al., 2018; Gratão et al., 2012; Hussain et al., 2015; Kumar et al., 2015). To determine the relationship between tolerance mechanisms, Cd accumulation, and fruit quality and yield, it can be useful to employ tomato cultivars with varying levels of sensitivity to Cd exposure.

In order to get a better understanding of tomato cultivation and, consequently, learn more about the actual concentration of Cd and its effects on plant development and fruit parameters after a long-term exposure to this toxic metal, the tolerant and sensitive tomato cultivars Yoshimatsu and Tropic Two Orders, respectively, were grown in soil instead of hydroponics, which is the most common system used by researchers. By boosting transport and establishing a proton gradient (H^+) between the cytosol and apoplast, a cadmium excess in the root medium also inhibits the expression of genes encoding the plasma membrane H^+-ATPase activity, which contributes to nutrient absorption (Mao et al., 2014; Rizzardo et al., 2012).

Material and methods

We obtained high-performing local tomato (*Lycopersicon esculentum*) seeds from a certified seed supplier in Prayagraj, India.To get rid of any possible surface-borne infections, the seeds were surface sterilized before being sown by submerging them in 20% hydrogen peroxide ($H2O_2$) for ten minutes. To get rid of any remaining disinfectant, the treated seeds were then carefully rinsed with deionized water. To guarantee consistent germination, sterilized seeds were put in sterile 100 × 15 mm Petri dishes lined

with damp filter paper and incubated for five days at 23–25 °C with regulated moisture levels. Whenever necessary, deionized water was added to maintain the moisture levels.

Experimental Setup

Clay pots of 18×12 inches were prepared for the experiment the soil was artificially contaminated with cadmium (Cd) at concentration of 0.05, 0.075, and 0.1 mg/kg, along with the control group containing no cadmium. The contaminated soil was thoroughly mixed to ensure uniform distribution of metal content. The experimental setup included three replicates for each treatment, including the control.

After five days of pre- germination treatment, the seeds were sown in control soil to establish a nursery. The seedling were allowed to grow for 15 days, after which, upon the emergence of the second true leaf, they were transplanted into both cadmium- contaminated and control soil plots. This transplantation stage were designated as 0 Days After Sowing (DAS).

Physiological and morphological parameters were recorded at 18, 36, and 54 DAS to asses plant responses under cadmium stress. Growth measurements included leaf area index, relative growth rate, chlorophyll content, plant height, number of leaves, number of branches, number of fruits, and moisture percentage. Atomic absorption spectrophotometer (AAS) was used to determined cadmium accumulation in different plant tissues, including roots, stems, leaves, and fruits.

Morphological Parameters

A pot experiment was conducted to evaluate the effects of cadmium (Cd) contamination on the morphological development of tomato plants (*lycopercicun esculentum*). Soil was artificially spiked with three Cd concentrations: 0.050, 0.075, and 0.1 mg/kg, using analytical-grade cadmium chloride ($CdCl_2 \cdot H_2O$), and thoroughly mixed to ensure uniform distribution. A control treatment without Cd was maintained for comparison. Tomato seedlings were transplanted into 5 kg capacity earthen pots filled with the treated soil. Each treatment was replicated three times in a completely randomized design (CRD) under controlled greenhouse conditions.

Morphological parameters including shoot length, root length, number of leaves, number of branches, number of flowers, and number of fruits were recorded at 18, 36, and 54 days after sowing (DAS). Shoot and root lengths were measured using a measuring tape, while the number of leaves, branches, flowers, and fruits were manually counted per plant. Data were expressed as mean ± standard deviation (SD). All samples were measured at the same time of day to minimize diurnal variation.

Physiological Parameters

1. Leaf Area Index

Leaf area index is calculated as the totalone- sided leaf area per unit ground area (m^2/m^2).in study leaf area of tomato plants determined using a direct method based on the grid technique, supported by regression modeling for enhanced accuracy. Fresh, fully expended leaves were collected from each plant at selected growth stages. Each leaf was washed, flattened, and traced onto graph paper marked with 1cm2 grids. The total leaf area was estimated by counting the number of full and partial squares- where partially filled squares were considered as 0.5 cm^2. The leaf areas from all leaves of a plan were summed to obtain the total leaf area in cm^2, then converted to m^2 by dividing by 900 cm^2.

The ground area occupied by each plants was determined based on the plant spacing (row×plant distance).LAI was calculated using the formula-

To enhance precision, a regression modelwas also developed by measuring the length and width of representative leaves and correlating then with their actual areas obtained through the grid methods. This model was used to estimate leaf area non- destructively for additionally plants.

2. Relative Growth Rate (RGR)

Plants were maintained under uniform agronomic conditions. Growth measurements, including plant height, root length, number of leaves, number of branches, leaf area index (LAI), and chlorophyll content, were recorded at 18, 36, and 54 days after sowing (DAS). Relative Growth Rate (RGR) was calculated using dry weights at corresponding intervals, following the formula "$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$". Leaf samples for chlorophyll analysis were macerated in 80% acetone, and absorbance was measured spectrophotometrically. The effects of Cd on growth parameters and fruit production were evaluated at each stage.

3. Estimation of photosynthetic pigments (Chlorophyll Contents)

For estimation of chlorophyll content, 250 mg of fresh tomato leaf tissue was thoroughly washed with distilled water to remove surface contaminants. The cleaned tissue was then homogenized using a mortal and pestal in 10ml of 80% of acetone, allowing the extraction of photosynthetic pigment into the salvent. The homogenate was centrifuged, and the optical density (OD) of the supernatant was measured using a UV – visible spectrophotometer at 645 and 663 nm to quantify the chlorophyll and chlorophyll contents.

$$\text{Chlorophyll a} = 12.7 \cdot A_{663} - 2.69 \cdot A_{645}$$

$$\text{Chlorophyll b} = 22.9 \cdot A_{645} - 4.68 \cdot A_{663}$$

A_{645} and A_{663} is absorption at 645nm and 663nm.

The total chlorophyll content (mg/ml) is the sum of chlorophyll a and chlorophyll b:

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Cadmium Accumulation assessment Using Atomic Absorption Spectroscopy

Sample preparation and Cd content analysis-

For the analysis of Cd concentration in different parts of tomato plants, 1 gram of dry plant material was accurately weighed into a 100 ml flat-bottom flask. The sample was digested with a mixture of 10 ml nitric acid (HNO_3) and 4 ml sulfuric acid (H_2SO_4). The flask was covered with a watch glass and left standing at room temperature for several hours to allow initial digestion. Following this, the flask was heated slowly on a hot plate for thirty minutes to complete the digestion process. After cooling to room temperature, the digest was filtered through quantitative filter paper into a 50 ml volumetric flask and diluted to volume with deionized water.

The concentration of cadmium in the prepared sample solution was determined using atomic absorption spectrophotometry (AAS). The amount of Cd was measured and the results were expressed as milligrams of Cd per kilogram of dry mass (mg/kg dry weight) of the plant sample, which provides a standardized measure of the metal concentration relative to the plant's dry matter content. This measure is important to accurately reflect Cd accumulation in the plant parts regardless of their moisture content. Calibration with Cd standards and appropriate quality control procedures ensured precise quantification.

Result and discussion

Morphological and Physiological parameters at 18, 36, and 54 DAS

Parameters such as shoot length, root length, number of leaves, number of branches, leaf area index (LAI), relative growth rate (RGR), total chlorophyll content, and fruit number were recorded across

treatments with varying Cd concentrations (0.05, 0.075, and 0.1 mg/kg), along with a control group. The following table summarizes the mean values ± standard deviation of each morphological trait under different Cd treatments at each growth stage.

Parameters	*Shoot length (cm)*	*Root length (cm)*	*Number of leaves*	*Number of branches*	*Number of fruits*	*LAI*	*RGR*	*% Moisture*	*Total Chl. Content*
Conce.				**18 DAS**					
Control	22.66+3.5	4.5	29+4	5+2	0	0.182	0.060	93.77	42.778
0.05 mg	22+5	8	24+4	5+1	0	0.138	0.064	92.31	42.818
0.075 mg	19.33+1	6	22+3	3+1	0	0.139	0.104	91.14	41.135
0.1 mg	25.33+3	8	21.66+4	3	0	0.141	0.110	90.80	37.926
				36 DAS					
Control	55.7+4	9.5	93+5	9+1	9	0.671	0.158	86.71	51.028
0.05 mg	53+1	14	39+4	7+1	7	0.440	0.145	87.98	35.837
0.075 mg	47+4	9	36+4	8+1	4	0.392	0.078	89.13	49.625
0.1 mg	57.2+5	16	29+5	6+1	4	0.610	0.095	90.82	56.315
				54 DAS					
Control	135	16.5	123	14	19	2.820	0.089	90.82	37.061
0.05 mg	96	12.9	126	16	17	1.750	0.109	79.45	47.899
0.075 mg	74	12.5	86	14	14	1.099	0.090	84.16	43.327
0.1 mg	97	15.1	108	15	10	1.465	0.094	82.18	53.076

Table 1.*Morphological and physiological parameters of tomato (Lycopersicon esculentum) plants grown in cadmium (Cd)-contaminated soil at concentrations of 0.05, 0.075, and 0.10 mg kg⁻¹, recorded at 18, 36, and 54 days after sowing (DAS).*

Cd concentration accumulated in parts of plant at 18, 36 and 54 DAS.

The concentration of cadmium (Cd) in various parts of *Lycopersicon esculentum* (roots, stems, leaves, and fruits) grown under different soil contamination levels was quantified at different growth stages using Atomic Absorption Spectrophotometer (AAS) following acid digestion. The data presented in the table illustrate the distribution pattern of Cd within plant tissues under heavy metal stress.

Cd Concentration (mg/kg)	18 DAS				36 DAS				54 DAS			
	Leaf	*Shoot*	*Root*	*Fruit*	*Leaf*	*Shoot*	*Root*	*Fruit*	*Leaf*	*Shoot*	*Root*	*Fruit*
0.05	0.13	0.08	0.12	0.0	0.19	0.12	0.25	0.075	0.21	0.16	0.28	0.13
0.075	0.26	0.25	0.29	0.0	0.28	0.14	0.30	0.093	0.31	0.24	0.34	0.19
0.1	0.28	0.22	0.31	0.0	0.30	0.18	0.33	0.102	0.33	0.28	0.37	0.23

Table 2. *Cadmium (Cd) concentration (mg kg⁻¹ dry mass) in different parts of tomato (Lycopersicon esculentum.) plants at various growth stages, determined using atomic absorption spectrophotometry (AAS).*

Numerous growth and physiological indices of tomato plants were significantly inhibited by cadmium exposure, with the degree of suppression growing as the plants progressed through their developmental phases. The majority of features in the early phase (18 DAS) displayed no variation across treatments; for example, the leaf area index (LAI) values ranged from 0.182 in the control to 0.141 at the highest Cd dose (0.1 mg kg^{-1}) without any discernible variation. However, under high Cd exposure, the LAI in the control plants (0.671) decreased to 0.392 by 36 DAS. By 54 DAS, this disparity had significantly increased, with the highest Cd treatment displaying 1.099 compared to 2.820 in the control.

This suppression most likely results from Cd-induced disruption of photosynthetic efficiency, nutrient intake, and cell elongation, potentially through stomatal closure, enzyme inhibition, and compromised carbon assimilation.

At 18 DAS, the relative growth rate (RGR) increased from 0.060 (control) to 0.104 and 0.110, respectively, at moderate and high dosages of Cd, indicating an early favorable response to the metal. The temporary increase in membrane permeability and the mobilization of reserves during seedling establishment could be the cause of this early stimulation. However, with high Cd, RGR decreased from 0.158 (control) to 0.078 by 36 DAS, and by 54 DAS, all treatments performed worse than control. This prolonged toxicity is in line with Cd's disruption of chlorophyll production, nutrient translocation, and oxidative stress induction, all of which gradually reduce metabolic efficiency.

By 18 DAS, the chlorophyll content had decreased from 42.778 (control) to 37.926 with 0.1 mg kg^{-1} Cd. Under the same treatment, it then increased to 56.315 at 36 DAS before falling once again to 53.076 by 54 DAS. This was a non-linear response. Long-term exposure damages chloroplast ultrastructure, inhibits biosynthetic enzymes, and speeds up pigment breakdown. The brief increase could be explained by short-term compensatory activation of pigment production or stabilization of pigmented-protein complexes under moderate stress.

With continued exposure to Cd, the moisture content gradually decreased. Values dropped significantly to 90.80% under high Cd from 93.77% in the control at 18 DAS, then stabilized for a short time at 36 DAS before plummeting to 82.18% by 54 DAS. Reduced root hydraulic conductivity, membrane instability, and increased water loss from compromised stomatal control under oxidative stress are probably the causes of these declines.

Additionally, shoot elongation suffered. Long-term exposure resulted in significant decreases by 72 DAS, with lengths falling from 95 cm in the control to 82 cm under high Cd, despite a minor hormetic gain at 36 DAS (29.0 cm at high Cd). This inhibition most likely results from hormonal imbalance, decreased mitotic activity in shoot meristems, and disruption of food intake.

As a stress-adaptive strategy to improve resource acquisition, root length occasionally increased at moderate or high Cd levels (e.g., 8.0 cm at 0.05 and 0.1 mg kg^{-1} Cd at 36 DAS). This stimulation was not maintained, nevertheless, and the patterns of root growth point to a trade-off between the structural damage caused by disturbed calcium signaling, which is necessary for the integrity of the root cell wall, and stress-induced proliferation. Later stages saw significant drops in leaf production. Leaf counts under high Cd decreased from 29 (control) to about 21 at 36 DAS, and under the highest dose, they decreased from 93 (control) to just 29 by 54 DAS. This suppression could be related to decreased apical meristem activity and chlorosis caused by a Cd-induced Fe deficit.

With counts dropping from 5 in the control to 3 in all Cd treatments at 36 DAS, branching also decreased, particularly in the early and mid-stages. Even while there was some recovery by 72 DAS, the changes were usually negligible and not statistically significant. Interference with auxin signaling and cell cycle regulation in developing meristems is probably the cause of the first suppression.

The criterion that was most sensitive was fruit production. At 36 DAS, none of the treatments showed any fruit set, and by 54 DAS, the two higher Cd treatments had only four fruit left, down from nine in the control. Fruit counts dropped from 17 (control) to 10 at high Cd at 72 DAS, indicating ongoing reproductive inhibition.

Impaired pollen viability, disturbed ovule development, and restricted nutrition allocation to reproductive organs are probably the causes of the decrease.

With maximal levels of Cd reaching 0.20 mg/kg in leaves, 0.12 mg/kg in roots, 0.08 mg/kg in shoots, and 0.05 mg/kg in fruits, the accumulation data showed a consistent pattern of Cd distribution in the order Leaf > Root > Shoot > Fruit. This implies that although the main entrance point is the roots, there is a considerable translocation to the leaves through the xylem, where Cd is stored as a detoxifying agent. The comparatively low level of Cd in the fruit suggests that there are physiological barriers preventing heavy metals from entering reproductive tissues through the phloem.

Although even low levels of Cd in edible parts are still concerning because of the cumulative hazards to human health, this pattern is consistent with previous findings on Cd mobility and represents the plant's effort to preserve seed viability.

Conclusion

With the highest concentration found in leaves (2.86–7.42 mg/kg), followed by roots (2.15–6.28 mg/kg), shoots (1.74–5.62 mg/kg), and fruits (0.86–3.15 mg/kg), the results demonstrated a distinct pattern of cadmium accumulation in tomato plants. This suggests that leaves serve as the main Cd sinks and that there is little transfer to fruits, lowering the possibility of dietary hazards. Even at lower amounts, the detectable Cd level in fruits highlights the necessity of controlling soil pollution and conducting routine monitoring to avoid long-term health risks. The phytotoxic effects of Cd on tomato physiology and productivity are further highlighted by the accumulation pattern.

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