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Morpho-physiological and biochemical responses of radish (*Raphanus sativus* L.) under cadmium stress

Heba I. MOHAMED*, Aya I. MOHAMED, Esraa W. GOMAA, Kholoud A. MOSTAFA, Marwa S. MAHMOUD, Nada A. RAHOMA

*Ain Shams University, Faculty of Education, Biological and Geological Science Department, 11341, Cairo, Egypt; hebaibrahim79@gmail.com (H.I.M.) (*corresponding author); biology2021_e9@edu.asu.edu.eg (A.I.M.); biology2021_e6@edu.asu.edu.eg (E.W.G.); biology2021_e15@edu.asu.edu.eg (K.A.M.); biology2021_e39@edu.asu.edu.eg (M.S.M.); biology2021_e77@edu.asu.edu.eg (N.A.R.)*

Abstract

The accumulation of cadmium (Cd) in plants poses a major risk to consumer health, in addition to affecting plant growth, development and quality. The study aimed to examine the effects of cadmium on the plants' ability for photosynthesis and antioxidant enzyme activity. In this study, radishes were planted in Petri dishes and pots containing soil supplemented with different concentrations of cadmium sulphate (25, 50, and 100 mg Cd kg^{-1} soil). The results showed that the percentage of germination, seedlings length, and fresh and dry matter significantly declined its increasing cadmium concentrations. In addition, cadmium hindered plant growth, as evidenced by the fresh and dried weight of radish roots and leaves after a 100 mg Cd kg^{-1} soil treatment. There was also a notable decrease in chlorophyll a and chlorophyll b, total chlorophyll, and total leaf area per plant. The leaves of radish plant exhibited a significant increase in lipid peroxidation and electrolyte leakage contents under Cd stress, while, the relative water content (RWC) decreased. However, leaves and roots of radish plant showed a considerable increase in antioxidant enzymes (catalase; CAT, peroxidase; POD, and superoxide dismutase; SOD). Furthermore, radish showed a significant increase in Cd accumulation in all applications, however, there were no obvious symptoms of Cd toxicity following the 25 and 50 mg Cd kg^{-1} soil applications. In conclusion, the radish plants accumulated cadmium at higher concentrations (100 mg Cd kg^{-1} soil). So, we recommended cultivating the radish plants in soil that has low concentrations of cadmium.

Keywords: antioxidant enzymes; cadmium; chlorophyll content; electrolyte leakage; lipid peroxidation; radish

Introduction

Global industrialization and contemporary farming methods have resulted in the release of heavy metals from man-made activities into the air, water, and soil (Dawood *et al*., 2023; Abu-Shahba *et al.,* 2022). These releases include emissions from automobiles and industrial facilities as well as agrochemicals like fertilizers, insecticides, and herbicides (Adnan *et al.,* 2024). The possible negative effects of heavy metal pollution on human health resulting from the consumption of food products polluted with heavy metals at various stages of

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the food chain have made heavy metal pollution a significant ecological concern (Le and Nguyen, 2024). Heavy metals are accumulated by crops from contaminated soil, which seriously hinders plant growth and harming the safety of agricultural products (Ahmed *et al.,* 2023).

Because of its toxicity and extensive distribution, cadmium (Cd) has become one of the most dangerous environmental contaminants (Wang *et al.,* 2021). Anthropogenic activities such as disposal of urban refuse, smelting, mining, metal manufacturing, and the application of synthetic phosphate fertilizers enhance the concentration of Cd in the environment and are carcinogenic to human health (Zhao *et al.,* 2024). Despite being a nonessential element, Cd is easily absorbed by plants, which results in severe damage to their physiology and biochemistry. This damage includes elevated membrane permeability, respiration, photosynthesis restriction, mineral nutrient uptake, protein integrity and synthesis, and negative effects on protective enzyme activities (Manzoor *et al.,* 2022).

Plant growth and agricultural output are inhibited when cellular metabolic functions are disrupted (Kumar *et al.,* 2024). In response to heavy metal stress, plants have developed a variety of defence mechanisms. Some of these systems allow plants to selectively avoid heavy metal toxicity by controlling the accumulation of heavy metals at the root level through decreased absorption and increased exclusion (Labudda *et al.,* 2022). Moreover, vacuolar compartmentation or binding with several heavy metal chelators, such as amino acids, organic acids like humic acid, glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs), can greatly reduce the toxicity caused by heavy metal ions in plant cells (Liu *et al.,* 2015; Molina and Segura, 2021).

Furthermore, reactive oxygen species (ROS) produced by heavy metals harm plant cells' lipids, proteins, and DNA (Mansoor *et al.,* 2023). Enzymatic and non-enzymatic ROS scavenging machinery reduces ROS buildup and may provide heavy metal tolerance. According to reports, when ROS are produced in excess, the oxidation of cellular macromolecules by ROS may cause oxidative damage to lipids, proteins, and pigments in thylakoid membranes. Additionally, ROS may reduce photosynthesis, which may ultimately result in the death of the plant (Ningombam *et al.,* 2024). Superoxide dismutase, peroxidase, catalase, reduced glutathione, and ascorbic acid are examples of the enzymatic and nonenzymatic antioxidants that plants have created ROS-scavenging systems from (Abbas *et al.,* 2021; Li *et al.,* 2024). Not only do different plant species respond differently to Cd, but genotypes within the same species also differ in how their scavenging systems react to it (Sana *et al.,* 2024).

Of the heavy metals, cadmium (Cd) is particularly detrimental to organisms, even at low concentrations, and can cause a number of cancerous disorders in humans (El-Beltagi and Mohamed, 2013; Budi *et al.,* 2024). Plants that are overexposed to cadmium experience a range of responses, including oxidative stress induction, chloroplast damage, decreased transpiration and photosynthetic rates, which ultimately lead to restricted plant growth and restriction of seed germination (Rhimi *et al.,* 2024). Cadmium generally tends to accumulate in roots first, then in other plant parts including stems and leaves (Shaari *et al.,* 2022), proving that when compared to leafy plants, root vegetables often collect higher quantities of cadmium in the edible part (Xu *et al.,* 2022).

An essential component of fundamental plant physiology is photosynthesis. It makes it possible to transform solar energy into a form that can be stored, typically glucose, which is what plants need to develop and flourish. Cd generally has a deleterious effect on photosynthesis in a number of ways, including causing damage to different parts of the equipment involved in photosynthetic processes (Parmar *et al.,* 2013; Song *et al.,* 2019), suppression of the photochemical process (Zhou *et al.,* 2024), disruption of the Calvin cycle's enzymatic activity and disordering of the chloroplasts' natural reactive oxygen species (ROS) balance (Soni *et al.,* 2023).

In Egypt, radish (*Raphanus sativus* L.) is an essential root vegetable (El-Beltagi *et al.,* 2022). Therefore, it is imperative to find a potential radish cultivar with a low accumulation of Cd for safe consumption and to study Cd tolerance mechanisms to increase the production of safe radishes. A previous study investigated the

characteristics of Cd and Pb accumulation under two different concentrations of Cd and Pb in one radish cultivar (Semhi *et al*., 2014). The results of another study indicate that the Cd- and Pb-tolerant cultivar CR inhibits Cd and Pb accumulation and activates physiological responses such as nonprotein thiols (NPT) production and H_2O_2 scavenging. As root vegetables are increasingly produced in heavy metal-contaminated soils (Ku *et al.,* 2021).

The aim of this study was to investigate the growth, photosynthesis, metabolic processes, and antioxidant systems of radish plants with varying levels of Cd tolerance. This research could potentially enhance our comprehension of the cellular processes underlying Cd stress.

Materials and Methods

Plant material

Raphanus sativus cv. 'Longioinnatus' or white radish has been used in the current study. The seeds were obtained from the Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

Seed germination

The seeds washed several times with sterile distilled water, and then the seeds were surface-disinfested with a diluted sodium hypochlorite solution (containing 0.02% free chlorine). Cadmium sulphate (25, 50, and 100 mg/kg soil) and distilled water (control) were the three solutions in which they were soaked for 24 hours. In sterile Petri dishes covered with Whatman No. 1 filter paper, ten seeds were used for each treatment. Five replicas (Petri plates), each containing ten seeds, were kept for each treatment. After seven days, the number of germinated seeds was recorded. The seeds were left to germinate in the dark at 20 ± 1 °C. Ten seedlings were randomly chosen on the seventh day, one from each Petri dish, and their seedling length, fresh and dry weight of seedlings were noted.

Growth conditions and stress conditions

The uniform, healthy radish seeds were surface sterilized for ten minutes using a 0.1% sodium hypochlorite solution, and then they were washed with double distilled water. In December 2023/2024, the seeds soaked in water for 24 hours then the radish seeds were planted into 25 cm-diameter pots filled with 8 kg of well-mixed, air-dried soil. The seeds were sown at 3-4 cm depth in each pot under the environmental condition of day/night temperature of 16/8 h of light/dark, 20/22 °C and 60-70% relative humidity. Four groups participated in the experiment; the first group was the control without any Cd addition. In the other three groups, cadmium applied at three different concentrations (25, 50, and 100 mg Cd/ kg soil) as cadmium sulphate ($3CdSO4.8H_2O$). The pots mixture was combined with the Cd determined for each pot, and the pots were filled to a volume weight of 8 kg on average. The soil's properties were as follows: its texture was sandy loam, with 80% sand, 15% silt, 5% clay, a pH of 7.8, EC of 0.4 dS m^{-1}, and 0.45% organic content. The pots were maintained at 28±1 °C with a 12-hour photoperiod and 80% relative humidity. Four replications of each treatment were used for testing. Plant samples were taken at day 40 from pot culture and their biochemical parameters examined.

Morphological parameters

Three radish plants were taken up and their leaves and roots separated. After being air dried, these samples were heated to 65 °C in a hot air oven. Weighing dried samples was done.

Leaf area

The leaf area-meter type LI-3000 was used to estimate the leaf area.

Determination of chlorophyll content

In order to perform the chlorophyll a and b assay, about 100 mg of fresh leaves were ground in a mortar using 4 mL of 80% acetone. The mixture was centrifuged for 15 minutes at 4 °C at 3000 rpm, and the supernatant's volume was completed to 10 ml using 80% cold acetone. The amount of chlorophylls a, b and carotenoids (mg g^{-1}) in the supernatant was measured using a spectrophotometer (Thermo Scientific Evolution 350 UV-Vis spectrophotometer, MA, USA) to measure the absorbance at 663 and 645 nm (Lichtenthaler and Buschmann, 2001).

The calculation formula is as follows:

$$\text{Chl-a (mg g}^{-1}) = (12.7 \times 663\ \text{nm}) - (2.69 \times 645\ \text{nm}) \times \frac{\text{The extraction volume}}{\text{Sample weight}} \times 100$$

$$\text{Chl-b (mg g}^{-1}) = (22.91 \times 645\ \text{nm}) - (4.68 \times 663\ \text{nm}) \times \frac{\text{The extraction volume}}{\text{Sample weight}} \times 100$$

Total Chl (mg g^{-1}) = Chl-a + Chl-b.

Determination of electrolyte leakage (EL) and leaf relative water content (LRWC)

After the leaves were cleaned with distilled water, circular portions measuring 5 mm in length were cut from fresh leaves and put in receptacles holding 40 mL of distilled water in order to measure EL (Murray *et al.,* 1989). After that, the containers were put on a shaker set to run for 120 hours at 120 rpm. Using an electrical conductivity measuring equipment (Hanna, HI8633, North Smithfield, RI), the solution's initial electrical conductivity (EC1) was determined. After that, the samples were autoclaved for 20 minutes at 121 °C and 15 atmospheres of pressure. The samples' electrical conductivity (EC2) was tested once more as the temperature decreased to room temperature (25 °C). Ultimately, the sample proportion of EL was determined using the following formula:

$$\text{EL \%} = \frac{EC1}{EC2} \times 100$$

The method used to determine the leaf relative water content (LRWC) was Shams *et al.* (2019). Fresh weight (FW) was measured right away after leaf discs with a diameter of 10 mm were cut. To find the turgor weights (TW), leaf discs were then floated in ultrapure water at 25 °C for 24 hours. Leaf discs were then oven-dried for 48 hours at 70 °C and weighed to determine their dry weight (DW). LRWC (%) was calculated by the following formula

$$\text{LRWC (\%)} = \frac{FW - DW}{TW - DW} \times 100$$

Determination of malondialdehyde (MDA)

The amount of malondialdehyde (MDA) in cell membranes was utilized to measure lipid peroxidation. The MDA was ascertained using minor changes in accordance with Hodges et al. (1999). After homogenizing 100 mg of leaves in 2 mL of 0.1% trichloroacetic acid (TCA) solution, the mixture was centrifuged at 12,000 rpm for approximately 25 min at 4 °C. Subsequently, 1 mL of 0.6% thiobarbituric acid (TBA) in 10% TCA was added to 1 mL of supernatant. After being boiled in boiling water for thirty minutes, the mixture was rapidly chilled in an ice bath. Following centrifugation at 10,000 rpm for 10 min, the mixture's absorbance was measured at 532 and 600 nm. To obtain the non-specific absorbance, the absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of malondialdehyde was measured in micromoles per gram of fresh weight (μmol g^{-1} FW) and expressed using a correction factor (μmol^{-1} cm^{-1}) of 0.155.

Preparation of crude enzyme extracts

The tissues of the roots and leaves were ground using this procedure in a cold pastel and mortar with 100 mM potassium phosphate buffer (pH 7.5) that contained 5% (w/v) insoluble polyvinyl pyrrolidone, 1 mM EDTA, and 3 mM DL-dithiothreitol. Following a 30-minute centrifugation at 10,000 ×g for the homogenates, the supernatants were separated and stored at -20 °C until the analysis.

Protein determination

Soluble protein was estimated by using the Coomassie Brillent Blue G-250 reagent according to the method of Bradford (1976) with bovine serum albumin as standard.

Antioxidant enzyme activities

The Catalase (CAT) activity was measured using the Cakmak and Marschner (1992) technique. A suitable amount of enzyme, 20 mM H_2O_2 and 50 mM sodium phosphate buffer (pH 7.0) made up the 3 mL reaction mixture. At 240 nm, the absorbance began to decrease (the molar exclusion coefficient of H_2O_2 was 0.04 mM^{-1} cm^{-1}).

The assay for peroxidase (POD) activity was conducted using Hemeda and Klein (1990) methodology. 10 mL of 1% guaiacol (v/v), 10 mL of 0.3% H_2O_2, and 80 mLof 50 mM phosphate buffer (pH 6.6) were added to a 100 mL reaction mixture. To the reaction mixture, 75 µL of enzyme extract was added, resulting in a final volume of 3 mL. At 470 nm, the guaiacol oxidation-induced rise in absorbance (extinction coefficient: 26.6 mM^{-1} cm^{-1}) was observed.

The nitro blue tetrazolium chloride (NBT) complex's light absorption was decreased while the enzyme was active in order to evaluate the activity of superoxide dismutase (SOD) (Beauchamp and Fridovich 1971). Using this approach, 3 mL of reaction mixture contains 13 mM L-methionine, 50 mM phosphate-potassium buffer (pH = 7.8), 4 µM riboflavin, 75 µM NBT, 0.1 µM EDTA, and 70 µL of the recovered enzyme extract. After being exposed to approximately 300 mol m^{-2} s^{-1} fluorescent lamps for 15 minutes, the samples were taken out of the light chamber and their absorbance was measured at 560 nm using a Jenway-7315 spectrophotometer. 50% of the uptake read for the control was the uptake rate.

Determination of Cd content

Samples were first surface sterilized with 1 M HCl to assess the amount of absorbed cadmium. Excess surface-bound Cd was then resolved with 1 mM Na_2EDTA, and the samples were dried in an oven at 70 °C for three to four days. The $HNO_3/HClO_4$ digestion procedure was used to grind the dried samples into a fine powder using a mortar and pestle. Using an atomic absorption spectrometer (Unicam Sp 1900 model), the cadmium concentration of the digested samples was determined after they had been dissolved in deionized distilled water (El-Beltagi *et al.,* 2010b). The amount of Cd present in the roots and leaves, expressed as µg g-1 dry weight.

Statistical analysis

Complete randomized block design was used in the experiment. For the four replications of each treatment, three plants per pot were maintained. The experimental data was analyzed using one-way analysis of variance (ANOVA), with a probability level of 95% or $p \leq 0.05$ for the LSD and Duncan's multiple range tests.

Results

Effect of cadmium on germination and seedling index

As indicated in Table 1, the radish treatments with varying levels of cadmium significantly lowered the growth of seedlings. The percentage of germination, length of the seedlings, and their fresh and dry matter all significantly declined as the concentration of cadmium increased. The percentage of germination, seedling length, fresh and dry matter of seedlings, and ratio of fresh to dry matter were all decreased by 33.1%, 49.2%, 65.8%, and 61.5%, respectively, in 100 mg Cd kg^{-1} soil as compared to the control.

Table 1. Effect of cadmium on germination and seedling growth of *R. sativa*

Treatments	Germination (%)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
Control	97.2±2.0[a]	6.1±0.3[a]	3.8±0.2[a]	0.26±0.01[a]
25 mg Cd kg^{-1} soil	92.5±1.9[b]	5.2±0.2[b]	2.6±0.1[b]	0.21±0.01[b]
50 mg Cd kg^{-1} soil	80.0±1.5[c]	4.9±0.1[c]	2.0±0.0[c]	0.17±0.01[c]
100 mg Cd kg^{-1} soil	65.0±1.0[d]	3.1±0.1[d]	1.3±0.1[d]	0.10±0.01[d]

Vertical column represent means of three independent determinations ± standard deviation (SD). Duncan's test indicates that there is a significant difference between treatments at the ≤ 0.05 level for the means with different letters.

Effect of cadmium on morphological parameters

The growth of the radish plants was significantly suppressed by each of the Cd treatments. With rising Cd concentrations, there was a considerable decrease in the amount of fresh and dry matter deposited in the radish leaves and roots. The fresh and dry weight of radish roots and leaves were lowered by 62.5% and 71.4%, respectively, and 53.8% and 55.0%, respectively, in 100 mg Cd kg^{-1} soil as compared to the control (Table 2 and Figure 1). Plant photosynthetic efficiency and dry matter production are closely related. The area of photosynthetic tissue, which is primarily leaves, and the amount of chlorophyll in leaves determine the photosynthetic efficiency. Every cadmium treatment significantly lowered the amount of leaf area on each plant. After receiving 25 mg Cd kg^{-1} of soil, radish plants reduced their leaf area per plant by approximately 12.7%, but after receiving 100 mg Cd kg^{-1} of soil, their leaf area declined by approximately 60.5%.

Table 2. Effect of cadmium on fresh, dry weight of leaves and roots and leaf area per plant of *R. sativa*

Treatments	Roots		Leaves		Leaf area per plant (cm)
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	
Control	0.80±0.02[a]	0.42±0.01[a]	3.25±0.2[a]	2.0±0.09[a]	280.0±1.15[a]
25 mg Cd kg^{-1} soil	0.70±0.01[b]	0.35±0.01[b]	2.95±0.1[b]	1.7±0.08[b]	244.5±1.2[b]
50 mg Cd kg^{-1} soil	0.55±0.01[c]	0.20±0.01[c]	2.31±0.2[c]	1.3±0.04[c]	200.5±1.0[c]
100 mg Cd kg^{-1} soil	0.30±0.01[d]	0.12±0.01[d]	1.50±0.1[d]	0.9±0.02[d]	110.5±0.8[d]

Vertical column represent means of three independent determinations ± standard deviation (SD). Means with the different letters are significantly different at ≤ 0.05 level among treatments according to Duncan's test.

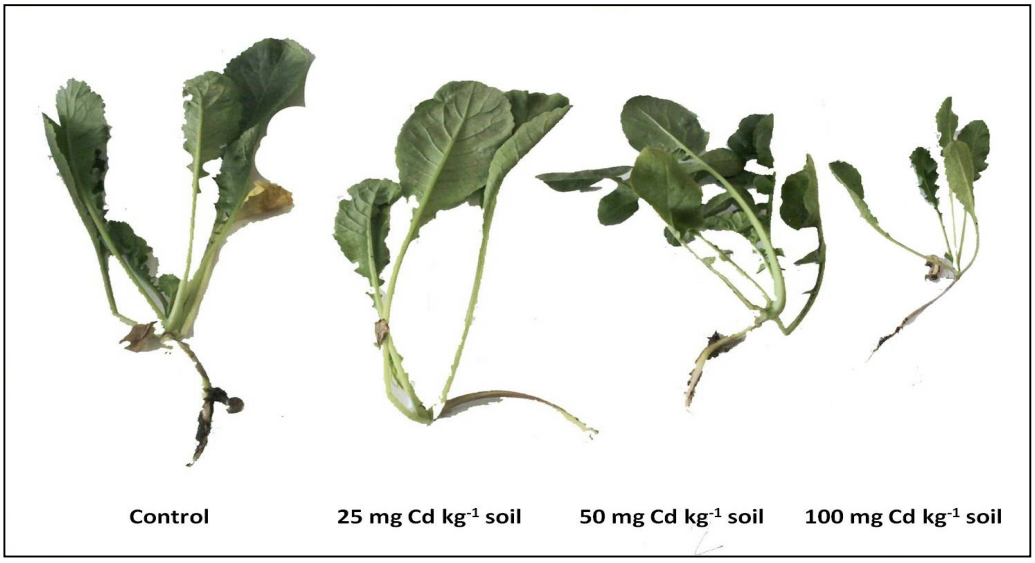

Figure 1. Morphological changes in radish plants under different concentrations of cadmium stress

Effect of cadmium on photosynthetic pigments

Radish's chlorophyll concentration decreased in line with cadmium-induced reductions in leaf area. The Cd-treated radish leaves showed a significant decrease in total chlorophyll, chlorophyll a, and chlorophyll b content, but a rise in the ratio of chlorophyll a/b (Figure 2). Following the application of 100 mg Cd kg^{-1} soil, the amount of chlorophyll a, chlorophyll b, and total chlorophyll content in leaves of radish plant dropped by 52.2%, 62.3%, and 34.4%, respectively, in comparison to controls. Although Chl a content also reduced as a result of Cd treatments, the rise in carotenoids and the Chla/Chlb ratio—which was determined to be about 45.5% and 26.7%, respectively—may have been caused by either a decrease in the synthesis of Chl b or a faster breakdown of Chl a relative to Chl b.

Effect of cadmium on electrolyte leakage, relative water content and lipid peroxidation

The increment in membrane leakage is indicative of a loss of membrane integrity brought on by cadmium treatments (Table 3) and lipid peroxidation (MDA). On the other hand, the radish leaves showed a much lower loss of membrane integrity when exposed to 100 mg Cd kg^{-1} soil. The radish leaves observed an increment in lipid peroxidation and electrolyte leakage by about 140% and 97.7%, respectively, in comparison to control plants. Furthermore, as Cd concentrations increased, RWC significantly reduced. When comparing plants treated with 100 mg Cd kg^{-1} soil to control plants, the most noticeable reductions in RWC were observed in these plants (34.7%).

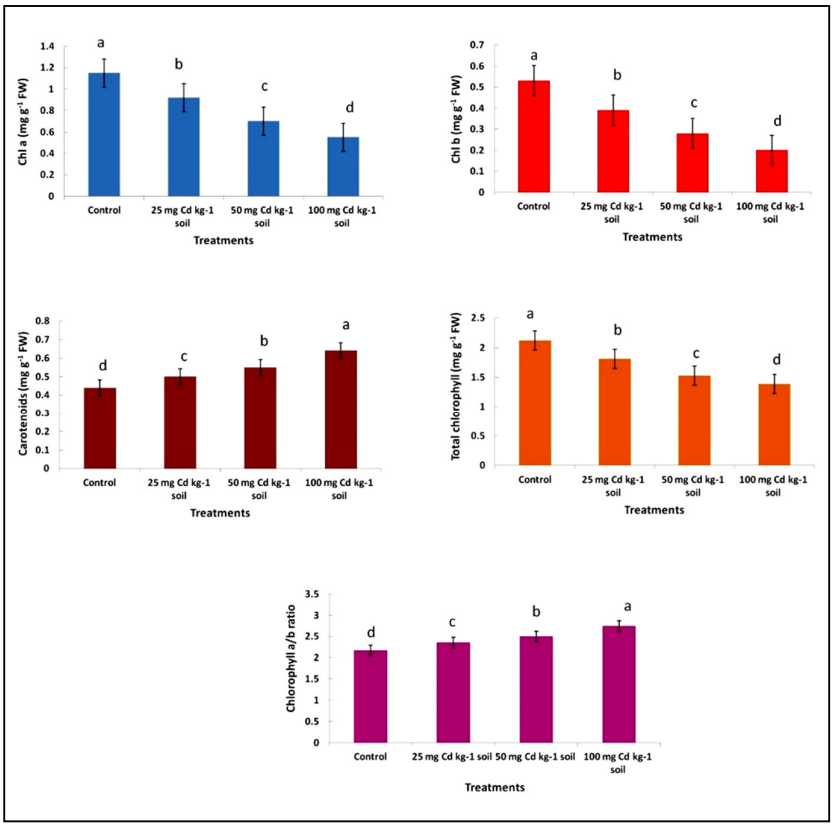

Figure 2. Effect of cadmium on photosynthetic pigments of leaves of *R. sativa*

Vertical bar represent means of three independent determinations ± standard deviation (SD). Duncan's test indicates that there is a significant difference between treatments at the ≤ 0.05 level for the means with different letters.

Table 3. Effect of cadmium on electrolyte leakage, relative water content and lipid peroxidation in leaves of *R. sativa*

Treatments	Electrolyte leakage %	Relative water content %	MDA (μmol g^{-1} FW)
Control	30.5±1.2^{d}	80.2±2.6^{a}	3.0±0.2^{d}
25 mg Cd kg^{-1} soil	35.9±1.5^{c}	71.2±2.2^{b}	3.5±0.6^{c}
50 mg Cd kg^{-1} soil	45.4±2.0^{b}	60.3±1.8^{c}	4.9±0.8^{b}
100 mg Cd kg^{-1} soil	60.3±2.2^{a}	52.4±1.5^{d}	7.2±1.0^{a}

Vertical column represent means of three independent determinations ± standard deviation (SD). Duncan's test indicates that there is a significant difference between treatments at the ≤ 0.05 level for the means with different letters.

Effect of cadmium on antioxidant enzyme

Therefore, Table 4 enhanced activity of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) lends indirect support to the idea that Cd treatment causes reactive oxygen species (ROS) to be produced. When the content of Cd in the soil increased, the specific activity of the enzymes CAT, POD, and SOD also increased. This enhanced activity reached the maximum at 100 mg Cd kg^{-1} in the roots and leaves of the plants. The most noticeable increases in POD (166%, 487%), SOD (66%, 56%), and CAT (394%, 310%) enzymes were found in the roots and leaves, respectively, of plants treated with 100 mg Cd kg^{-1} soil.

Table 4. Effect of cadmium on antioxidant enzyme content in leaves and roots of *R. sativa*

Treatments	CAT Units mg^{-1} protein min^{-1}		POD Units mg^{-1} protein min^{-1}		SOD Units mg^{-1} protein min^{-1}	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	4.52±0.5^{d}	3.55±0.4^{d}	4.25±0.1^{d}	9.41±0.1^{d}	12.11±1.0^{d}	14.51±0.9^{d}
25 mg Cd kg^{-1} soil	6.28±0.8^{c}	8.67±0.4^{c}	6.33±0.1^{c}	30.22±0.2^{c}	14.32±1.4^{c}	16.33±1.1^{c}
50 mg Cd kg^{-1} soil	15.24±0.9^{b}	13.21±0.5^{b}	7.85±0.2^{b}	41.31±0.5^{b}	17.50±1.6^{b}	19.47±1.4^{b}
100 mg Cd kg^{-1} soil	22.33±1.0^{a}	14.55±0.6^{a}	11.30±0.1^{a}	55.20±0.9^{a}	20.11±2.0^{a}	22.65±1.2^{a}

Vertical column represent means of three independent determinations ± standard deviation (SD). Duncan's test indicates that there is a significant difference between treatments at the ≤ 0.05 level for the means with different letters.

Effect of cadmium-on-cadmium content

With an increase in the applied Cd concentration, the cadmium content of the edible portions of radish (roots) increased. Radishes' roots and leaves had amounts of 4.1 μg g^{-1} and 1.2 μg g^{-1} of cadmium in the control treatment, respectively. The Cd concentration in the radish roots and leaves increased to 50.3 and 27.6 μg g^{-1} dry weights, respectively, after 100 mg Cd kg^{-1} soil was applied (Figure 3). Nevertheless, at 25 and 50 mg Cd kg soil, no phytotoxic symptoms resulting from the application of Cd were noticed; but, at 100 mg Cd kg^{-1} soil, leaves began to exhibit chlorosis, a phytotoxic indication. This indicates that the radish plants have accumulated a significant amount of cadmium. It seems that radish may store large amounts of cadmium without the plant displaying any symptoms, and this accumulation could pose a major risk to human health rather than cadmium's phytotoxic effects being the reason it may be hazardous.

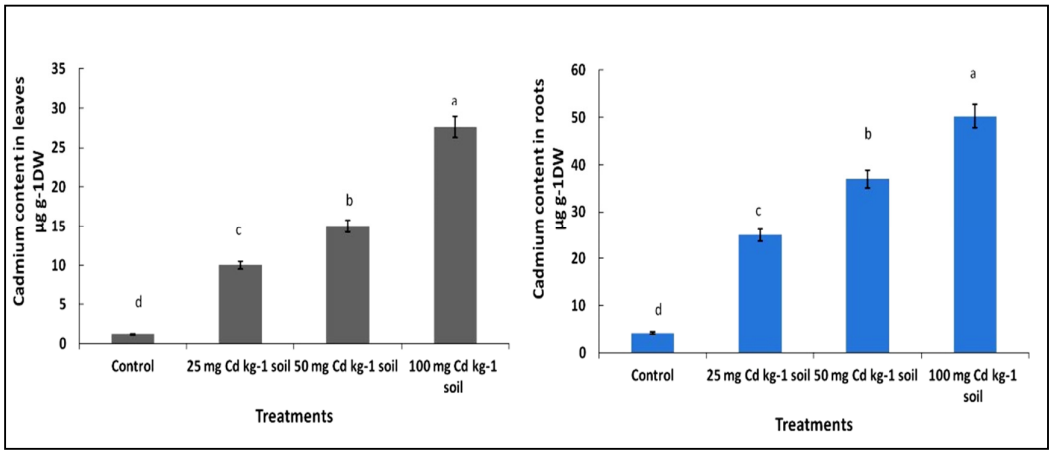

Figure 3. Effect of cadmium-on-cadmium content in leaves and roots of *R. sativa*
Vertical bar represent means of three independent determinations ± standard deviation (SD). Duncan's test indicates that there is a significant difference between treatments at the ≤ 0.05 level for the means with different letters.

Discussion

Because heavy metals build up excessively in edible plant parts, agricultural soil contamination poses a threat to human health and food output (Ahmed *et al.,* 2023; Dawood *et al.,* 2024). Growth of seedlings is a crucial aspect of Cd tolerance. Indeed, Cd can shorten the lengths of the roots and shoots and lower the biomass production of seedlings (Ali *et al.,* 2015; Tao *et al.,* 2015). Plants affected by cadmium undergo a number of morphological, physiological, and biochemical alterations, including stunted growth and water imbalance (Figure 4) (Zulfiqar *et al.,* 2022). The suppression of mitosis, decreased synthesis of cell-wall components, and modifications in the metabolism of polysaccharides may all contribute to the reduction of the root and shoot systems under Cd stress (Wang *et al.,* 2023). Kaur and Jhanji, (2016) furthermore noted a decrease in the quantity and area of leaves on a plant after receiving Cd treatment, attributing this decline to the meristems' repressed cell division activity. The observed decrease in radish plant growth may be the result of altered metabolism and osmotic adjustments (Tuver *et al.,* 2022). The presence of excessive heavy metals in plant cells impairs many plant functions and metabolic processes in a number of ways. These include the disruption of key cellular molecules' functional groups and protein structures, as well as the functionality of essential metals in pigments and enzymes, which represses essential processes like photosynthesis, respiration, water balance, and enzymatic activities (Shahid *et al.,* 2017; Fouda and Sofy, 2022; Goncharuk and Zagoskina, 2023).

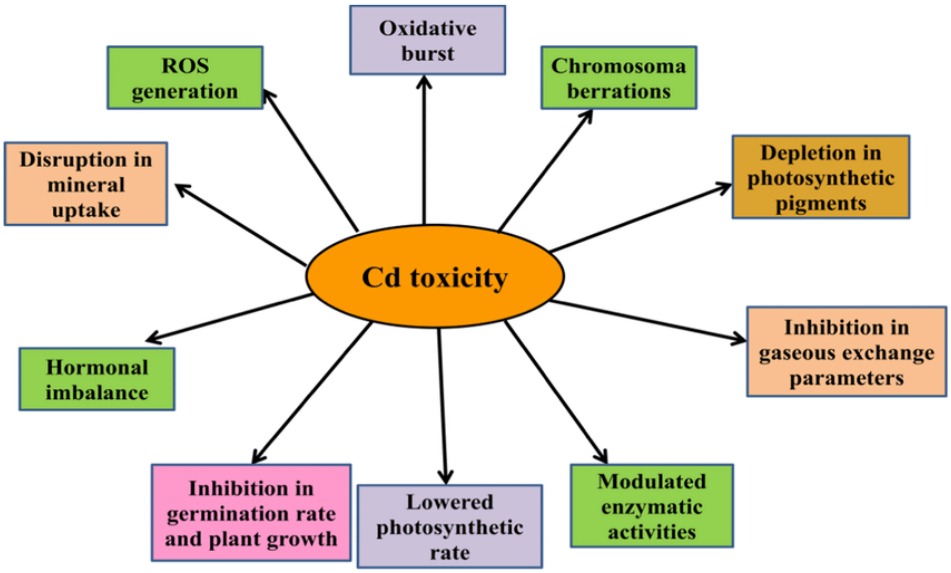

Figure 4. Morphological, physiological, and biochemical alterations in plants under cadmium stress

The total chlorophyll, chlorophyll a, and chlorophyll b content of the radish leaves treated with cadmium significantly decreased. Zhao *et al.* (2021) additionally noted that cadmium decreased the amount of chlorophyll. The reduction in chlorophyll content linked to Cd stress may be ascribed to the inhibition of the enzymes protochlorophyllide reductase and 5-aminolaevulinic acid dehydratase, which are involved in the biosynthesis of chlorophyll, or to the induction of its degradation through the production of free radicals from polyunsaturated fatty acids as a result of increased lipoxygenase activity (Kaur and Jhanji, 2016). Furthermore, a reduction in the amount of chlorophyll caused by cadmium was linked to a problem with the leaves' ability to absorb magnesium and iron (Liu *et al*., 2020). A decrease in the amount of light-harvesting chlorophyll proteins (LHCPs) in the photosynthetic apparatus has been associated with an increase in the ratio of Chl a:b (Kaur and Jhanji, 2016).

Enzymatic and non-enzymatic antioxidants make up a plant's natural defence system, which shields it from oxidative damage brought on by cadmium stress (Gutiérrez-Martínez *et al.,* 2020; Sheteiwy *et al*., 2023). Since SOD is the most effective enzyme in stress resistance and is engaged in the dismutation of O_2^- into H_2O_2 and molecular oxygen in plants under stress (Al-Mokadem *et al.,* 2022), it plays the most significant function in the antioxidant defence mechanism (Jawad *Hassan et al.,* 2020). One plausible explanation could be linked to elevated generation of O_2^- radicals, which triggers the activation of preexisting enzyme reserves (Jawad Hassan *et al.,* 2020). Furthermore, a significant build-up of H_2O_2 is detrimental to cell metabolism. By dissociating H_2O_2, the CAT and POD enzymes enable plants to convert it into water and oxygen, which is essential for their ability to withstand adverse environments (Li *et al.,* 2014; Hewedy *et al.,* 2023). Under 25 µM Cd stress, increased generation of H_2O_2, a by-product of superoxide dismutation by SOD, could lead to significant increases in POD and CAT activity. However, the severe oxidative damage under higher Cd concentrations (50 and 100 µM) in sorghums reduced these responses. The degree of Cd stress has been linked to alterations in antioxidant enzyme activity, according to earlier research on different plant species (Anjum *et al.,* 2015).

The study found that when plants were treated with Cd, the level of Cd in their leaves and roots increased considerably. High concentrations of heavy metal deposition in roots were discovered to have a negative impact on heavy metal tolerance in earlier research (Feng *et al.,* 2018; Ullah *et al.,* 2020). Similarly, it has been found in earlier research that plants growing in Cd-contaminated soils are capable of absorbing and

accumulating a significant quantity of Cd in plant parts without exhibiting any symptoms, beyond the allowable limit (Loi *et al*., 2018).

More cadmium can be accumulated by roots than by leaves. The process of translocating cadmium from roots to shoots involves loading the xylem sap into the transpiration stream and moving it to the above-ground portions of the plant (Loi *et al*., 2018). The root can become immobile due to cadmium buildup through binding to the cell walls of the xylem vessels and roots, complexing with organic acids, or combining with polysaccharides (Seregin and Ivanove, 1998). The endodermis often prevents cadmium transport in the root tissues, which could account for the low allocation ratio of cadmium between the roots and shoots (Loi *et al.,* 2018).

Conclusions

Cadmium resulted in a reduction in fresh and dry mass of roots and leaves as well as leaf area. The levels of each of these growth traits fell as the Cd level increased in a dose-dependent manner. Additionally, RWC, one of the photosynthetic pigments, decreased at all cadmium concentrations, whereas lipid peroxidation and electron leakage increased as cadmium concentrations rose. Nonetheless, it is likely that the elevated activity of antioxidant enzymes safeguarded the metabolic apparatus, stabilized the membranes to avert water loss, and encouraged the intake of nutrients to enhance development efficiency. It appears that Cd is harmful due to its phytotoxic effects and high concentration in edible radish sections, even in the absence of any symptoms that pose a serious risk to human health. Our results could lay the foundation for breeding radish cultivars with low Cd uptake. Moreover, it is important to find radish cultivars with low accumulation of a broader range of heavy metals. The future trend is to study the alleviation of cadmium stress by using ecofriendly compounds.

Authors' Contributions

Conceptualization: AIM, EsWG, KAM, MSM, NA R, HIM; Data curation: AIM, EsWG, KAM, MSM, NA R, HIM; Formal analysis: AIM, EsWG, KAM, MSM, NA R, HIM; Funding acquisition: AIM, EsWG, KAM, MSM, NA R, HIM; Investigation: AIM, EsWG, KAM, MSM, NA R, HIM; Methodology: AIM, EsWG, KAM, MSM, NA R, HIM; Project administration: HIM; Resources: AIM, EsWG, KAM, MSM, NA R, HIM; Software: HIM; Supervision: HIM; Validation; Visualization: AIM, EsWG, KAM, MSM, NA R, HIM; Writing - original draft: AIM, EsWG, KAM, MSM, NA R, HIM; Writing - review and editing: HIM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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