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Different doses of cadmium in soil negatively impact growth, plant mineral homeostasis, and antioxidant defense of mung bean plants

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Abstract: Heavy metal (HM) pollution of soil has become a gigantic issue across the globe. Metals enter the food chain and cause problems in plants, animals, and humans. We performed this work to evaluate the impacts of different cadmium (Cd) levels on growth, physiology, mineral nutrition, and yield attributes of *Vigna radiata*. Changes in growth fund are statistically significant and directly linked with an increase in Cd doses. Various concentrations of cadmium exhibited significant ($p \leq 0.05$) vicissitudes in biochemical parameters such as in the contents of chlorophyll, amino acids, soluble proteins, and total soluble sugars in experimental plants. The shoot and root calcium contents were highly reduced by higher concentrations of Cd in the following trend $Cd_{40} > Cd_{30} > Cd_{20} > Cd_{10}$ than the control. Likewise, shoot and root potassium (K^+) contents were less influenced by Cd_{10} as compared to other levels of Cd. The elevation in these enzymatic contents was maximum under the higher concentration of Cd (Cd_{40}), and with the decreasing Cd level, a decline in concentrations of these estimated antioxidants was recorded ($Cd_{30} > Cd_{20} > Cd_{10} > Cd_0$). A significant ($p \leq 0.05$) reduction in seed yield per plant and thousand seed weight was estimated with the increase in the concentration of Cd as compared to the control. The seed yield and their weights were less influenced in Cd_{10} treated plants followed by Cd_{20} and then Cd_{30}. On the basis of the reported findings, our recommendation is to conduct research with an explicit focus on the mechanistic elucidation of damages caused by Cd. Additionally, target enzymes or metabolites in plants should be explored for use in the development of HM-tolerant crop varieties.

Key words: Mung bean, HMs, Chlorophyll, growth, yield, minerals

1. Introduction

Soil pollution with diverse metals is one of the most protuberant and severe disturbances in a terrestrial ecosystem (Awa and Hadibarata, 2020; Poschenrieder et al., 2009). Heavy metal buildup may occur naturally or as a result of human activity, including industrial processes, the burning of fossil fuels, and the disposal of wastes inland. (Khalid et al., 2021b; Saljnikov et al., 2019; Wang et al. 2019). The stability of the ecosystem and global food security is negatively affected by the mixing of heavy metals in soil (Chen et al., 2022; Zwolak et al., 2019). Certain HMs i.e. copper (Cu), manganese (Mn), and zinc (Zn) are considered as essential metals, which are required to regulate the cellular processes of other metals e.g., lead (Pb), cadmium (Cd), or nickel (Ni) [nonessential and extremely lethal] (Aqeel et al., 2021; Chen et al., 2022). By moving through the food web, these metals cause serious complications in plants, animals, and humans (Amoakwah et al., 2020).

Cadmium (Cd) is the most toxic metal for plants in different concentrations due to its high water solubility (Brião et al., 2020). Due to its characteristic mobility from soil to plant, Cd is of immense importance with respect to uptake and translocation by a plant as well as accumulation in the food web impacting human health (Akca et al., 2022; Ngugi et al., 2021). Naturally, Cd can be found in combinations with other elements. As a small component, Cd is typically extracted from Zn, Pb, and Cu ores (Ćwieląg-Drabek et al., 2020; Rahman and Singh, 2019). Growth and productivity of major crops such as wheat, maize, mungbean, sunflower, and barley are harmfully influenced by Cd (Carvalho et al., 2020; Yang et al., 2020b). Inhibition of germination, decreased seedling growth, decreased biomass production, low photosynthesis, respiratory deficit, disequilibrium in mineral nutrition, and translocation of assimilates are just a few of the cellular and plant processes that are impaired by increased levels of heavy metals (HMs), specifically Cd.s (Haider et al., 2021; Huang et al., 2020; Irshad et al., 2020).

Legumes are cultivated worldwide and are usually used on an annual rotation basis (Zhao et al., 2022). Among the legumes, the most significant legume crop with enormous nutritional value is mungbean (*Vigna radiata* (L.) Wilczek.) (Costa et al., 2020). It is usually grown during spring and

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autumn and is a cheap source of dietary protein and it substantially is added to total protein intake and is the best substitute for expensive animal proteins (Sehrawat et al., 2019; Shanthala et al., 2020). Additionally, mungbeans are used as food, fodder, and green manure. On the other hand, the role of mungbean like other legume crops in improving soil fertility by fixing nitrogen is matchless (Haque et al.; Tarahi et al., 2022).

Crop output and human health are negatively impacted by soil contamination with HMs. Multiple factors including an increase in HM-affected regions, highlight the need to evaluate crop plants with high genetic survival potential. A thorough review of the literature reveals a lack of information regarding soil repair and mungbean survival tactics in metal-affected soils. This study hypothesized that a newly developed variety of mungbean grown in Cd-contaminated soil could have good survival potential. We assessed the potential of mungbean in soil with various Cd contamination amounts. Morphological, physiological, biochemical, and yield metrics were evaluated for these traits, and changes in these traits were interpreted as a measure of tolerance and survival ability.

2. Material and methods

2.1. Experimental conditions and plant material

The work was conducted to assess the Cd effect on morphology, physiology, photosynthetic pigment, mineral homeostasis, and yield traits of Mungbean [*Vigna radiata* (L.) Wilczek] grown under natural conditions. Seeds of an Indian-origin Mungbean variety namely IPM 410-3 (Shikha) were used in this study. The seeds of the same size and good vigor were sown in individual plastic pots of size 10 × 10 × 10 inches (L × W × H), each having an 8 kg mixture of soil, sand, and plant manure (3:1:1, v/v). Fifteen seeds were sown in every pot. After germination, six plants were retained in each pot and the rest were uprooted and discarded. During the course of experiments, environmental conditions were: mean day temperature 27.6 ± 1 °C, night temperature 13.3 ± 8.6 °C, relative humidity (RH) 60.9 ± 7.5 and the day length from 12 to 14 h at 790–1430 μmol m^{-2} s^{-1} PPFD.

2.2. Treatment evaluation

Twenty-four days old mung bean plants of uniform health, height, and stem diameter were selected for treatment with different doses of Cadmium (Cd) and additional experimentation. Each treatment was performed in five replicates. The source of cadmium was $CdCl_2$. The treatments schedule was as follows (Table).

2.3. Evaluation of morphology, biomass, and yield

Data about various growth and yield traits were evaluated 15 days post-Cd treatment to appraise the impacts due to metal exposure. From each replicate, three plants were randomly selected and harvested. Shoots and roots were separated and weighed for assessing fresh weights. Later on, separated shoots and roots were dried in an oven at 65 °C for three days. Following the drying process, shoot and root dry weights were recorded. These dried plant parts were kept safe and used later for mineral nutrient quantification. The remaining plants in each pot kept on growing in the prevailing growth conditions for observing other traits and yield.

2.4. Chlorophyll estimation by SPAD

Total chlorophyll content was measured via soil and plant analyzer development (SPAD) value from the top third expanded leaf of both treated and untreated plants with the help of a chlorophyll meter (Model SPAD-502, Konica Minolta Sensing, Inc., JAPAN).

2.5. Antioxidants activity, MDA, and H_2O_2 measurements

Fresh leaves (0.25 g) were crushed and homogenized in phosphate buffer (50 mM with 7 pH) to determine catalase (CAT) activity (Chance and Maheley, 1955). After centrifugation at 15,000 × g for 20 min, 0.1 mL supernatant, and 1 mL H_2O_2 were mixed. Absorbance was recorded at 240 nm after every 30 s. For peroxidase (POD) activity estimation, 50 μL supernatant, was mixed with 100 μL H_2O_2 (400 mM) and 100 μL guaiacol (200 mM). Buffer (750 μL) was added and absorbance was noted at 470 nm after each 20 s.

For MDA determination, fresh leaves were homogenized with 3 mL of 1% trichloroacetic acid (TCA). The mixture was centrifuged at 20,000 × g and 0.5 mL

Table. Treatments application schedule used in the experiment.

Treatment		Description	Cadmium (mg/kg)
T_0	Cd_0	Control (no application)	0 mg/kg
T_1	Cd_{10}	Soil application of cadmium in the form of $CdCl_2$	10 mg/kg
T_2	Cd_{20}	Soil application of cadmium in the form of $CdCl_2$	20 mg/kg
T_3	Cd_{30}	Soil application of cadmium in the form of $CdCl_2$	30 mg/kg
T_4	Cd_{40}	Soil application of cadmium in the form of $CdCl_2$	40 mg/kg

supernatant was mixed with 2 mL of thiol barbituric acid (0.5%). Incubation of samples was performed at 95 °C for 50 min. After chilling, the optical densities of samples were noted at 600 nm and 532 nm (Heath and Packer, 1968).

The method of Patterson et al. (1984) was used to estimate H_2O_2. Already crushed leaves in TCA 0.25 were centrifuged for 15 min at 12,000 × g. KI (1 mL) and potassium buffer with a pH of 7 (0.5mL) were mixed with supernatant. The mixture was vortexed and absorbance was noted at 390 nm.

2.6. Soluble proteins, total free Amino Acids, and total soluble sugars estimation

After homogenising 0.5 g of fresh plant material in 10 mL of 50 mM chilled sodium phosphate buffer with 7.0 pH, the sample was centrifuged for 30 min and collected the supernatant. Total soluble protein was determined by following the Bradford (1976) method.

Amino acid and soluble sugars were determined by following the protocols of Hamilton and Van Slyke (1943) and Riazi et al. (1985), respectively.

2.7. Mineral elements in root and shoot

According to Wolf (1982), dried shoots and roots were crushed finely and a 0.1 g plant sample was digested. Digested plant material was filtered and further used for elemental quantification. Calcium and potassium concentrations were measured by a flame photometer (JENWAY PFP 7). Atomic absorption spectrophotometer was used for the measurement of Cd concentration (Perkin Elmer, Model Analyst 3000, Norwalk). Chloride (Cl) contents were estimated using a chloride analyzer (Model 926, Sherwood Scientific Ltd., and Cambridge, UK) (Jackson, 1962).

2.8. Data Analysis

One-way analysis of variance (ANOVA) was performed to check the significant differences at $p \leq 0.05$ in combination with the least significant difference (LSD) using a computer program Co-stat. All the studied parameters were investigated with a minimum of three replicates, and bar plots were prepared with mean ± standard errors. The impact of different doses of cadmium on mungbean plants was visualized in Origin v.2021.

3. Results

3.1. Morphological attributes

Cadmium stress significantly ($p \leq 0.05$) reduced the morphological attributes of Vigna plants. The T4 (Cd_{40} = 40 mg/kg Cd application in soil) severely reduced the shoot fresh weights as compared to T0 (Control) plants whereas T1, T2, and T3 (Cd_{10} = 10 mg/kg $CdCl_2$, Cd_{20} = 10 mg/kg $CdCl_2$, Cd_{30} = 10 mg/kg $CdCl_2$ applied in soil) were less hazardous than the T4 treated plants. In root fresh weights, the maximum reduction was also recorded in Cd_{40} plants than the other Cd levels. The T1 followed by T2 and T3 were less harmful to Vigna plants in decreasing their root fresh weight than T4. Likewise, shoot and root dry weights were significantly ($p \leq 0.05$) decreased by T4 followed by T3 and T2 as compared to the control. The T1 portrayed a minimum reduction in these attributes than the other applied Cd concentrations (Figure 1).

3.2. Biochemical parameters

Various concentrations of cadmium exhibited significant ($p \leq 0.05$) vicissitudes in biochemical parameters such as in the contents of chlorophyll, amino acids, soluble proteins, and total soluble sugars in experimental plants (Figure 2). Just like the morphological attributes Cd_{40} extremely reduced the chlorophyll SPAD values than T0, while other levels of Cd were less hazardous than this level showing the following trend T3 > T2 > T1 in reducing chlorophyll values. Similar results were recorded in the estimation of soluble proteins where a maximum decline was noted in Cd_{40}-treated plants. The total free amino acids and soluble sugars portrayed maximum elevations in these parameters under higher concentrations of cadmium (Cd_{40}) than the other treatments while their minimum values were calculated under control plants.

3.3. Estimation of ionic contents

The soil application of different levels of cadmium also influenced the concentrations of important ionic contents, a significant ($p \leq 0.05$) decline in these attributes was estimated in Vigna plants. The shoot and root calcium contents were highly reduced by higher concentrations of Cd in the following trend T4 > T3 > T2 > T1 than the control. Likewise, shoot and root potassium contents were less influenced by Cd_{10} as compared to other levels of Cd. In the case of shoot and root phosphorous, the T4 treated plants exhibited the lowest values of this ionic content than T0, while the other concentrations of Cd were less hazardous than the T4 showed following order T3 > T2 > T1 in declining the phosphorus contents (Figure 3). The cadmium and chloride contents were also significantly ($p \leq 0.05$) enhanced by the increase in the level of cadmium stress. Opposite to the other ionic contents higher concentrations of Cd_{40} increased the values of cadmium and chloride in plant tissues than control. The following order T3 > T2 > T1 > T0 was recorded in the elevation of these metallic contents by the application of Cd (Figure 4).

3.4. Enzymatic antioxidants, ROS, and their end products

To overcome the cadmium-stressed conditions the antioxidants were significantly ($p \leq 0.05$) enhanced by the increase in Cd concentration. The concentrations of superoxide dismutase, peroxidase, and catalase were lowest in Cd not treated plants. The elevation in these enzymatic contents was maximum under higher concentration of Cd (Cd_{40}), with the decrease in level

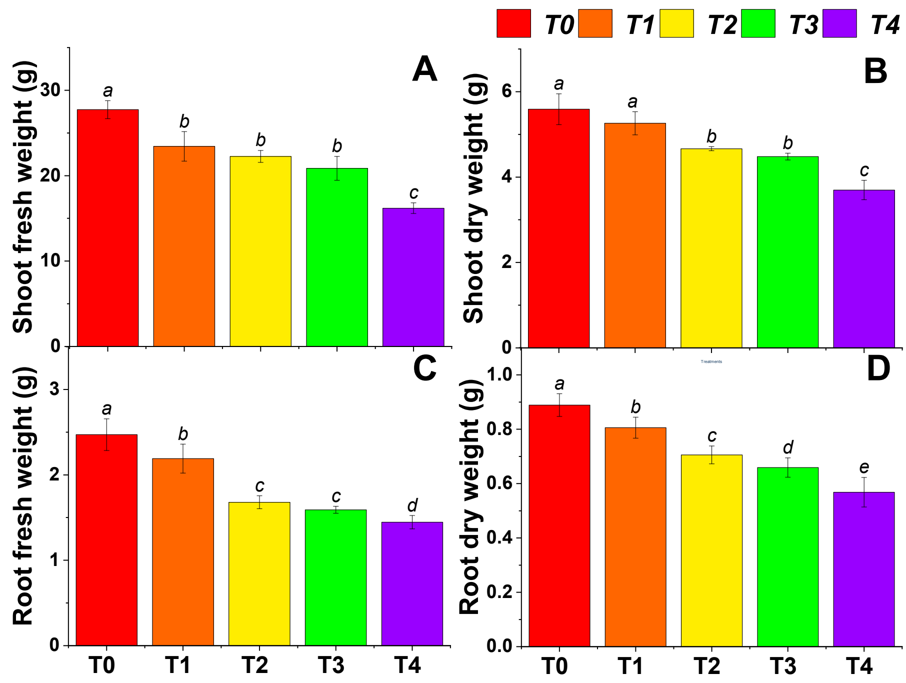

Figure 1. Shoot root fresh and dry weights of Vigna plants influenced by variable levels of cadmium. The values are mean standard deviation (n = 3). Various alphabets over the bars demonstrate significant differences whereas similar letters exhibited nonsignificant differences ($p \leq 0.05$). T0: Control; T1: 10 mg/kg $CdCl_2$, T2: 20 mg/kg $CdCl_2$; T3: 30 mg/kg $CdCl_2$; T4: 40 mg/kg $CdCl_2$.

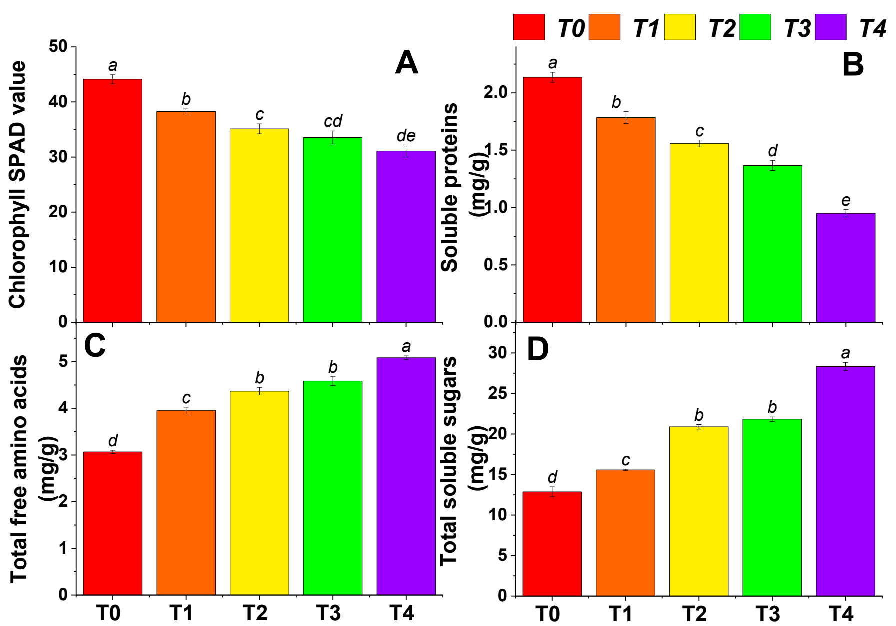

Figure 2. Chlorophyll and secondary metabolites in Vigna plants influenced by variable levels of cadmium. The values are mean standard deviation (n = 3). Various alphabets over the bars demonstrate significant differences whereas similar letters exhibited nonsignificant differences ($p \leq 0.05$). T0: Control; T1: 10 mg/kg $CdCl_2$, T2: 20 mg/kg $CdCl_2$; T3: 30 mg/kg $CdCl_2$; T4: 40 mg/kg $CdCl_2$.

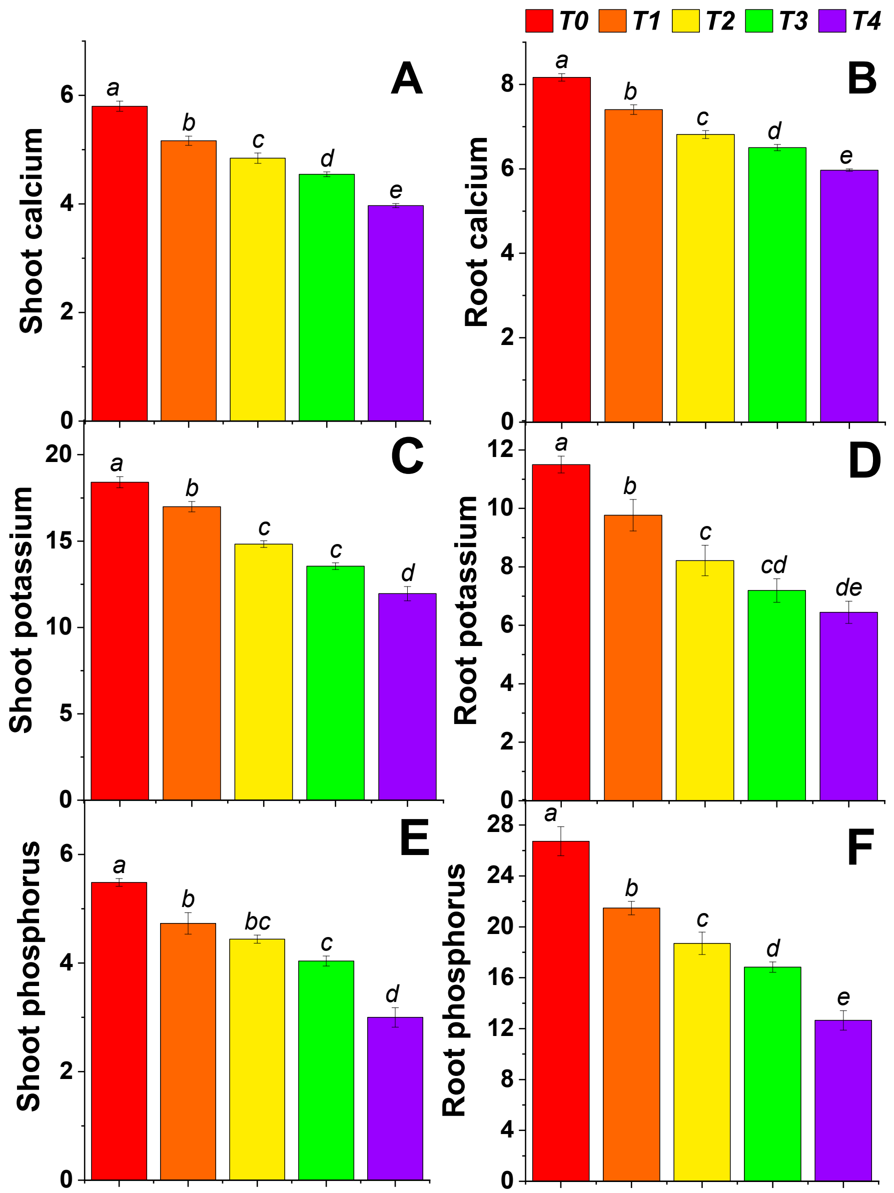

Figure 3. Shoot and root mineral contents of Vigna plants influenced by variable levels of cadmium. The values are mean standard deviation (n = 3). Various alphabets over the bars demonstrate significant differences whereas similar letters exhibited nonsignificant differences ($p \leq 0.05$). T0: Control; T1: 10 mg/kg $CdCl_2$, T2: 20 mg/kg $CdCl_2$; T3: 30 mg/kg $CdCl_2$; T4: 40 mg/kg $CdCl_2$.

of Cd the decline in concentrations of these estimated antioxidants were recorded ($Cd_{30} > Cd_{20} > Cd_{10} > Cd_0$). In the case of Malondialdehyde and hydrogen peroxide, the Cd untreated plants portrayed the lowest values than the other treatments. The T1 proved less harmful for the Vigna plants and showed lower concentrations of these hazardous chemicals than the other levels of Cd. While T4 was the most perilous concentration of Cd exhibited higher values of these harmful chemicals than T0 (Figure 5).

3.5. Yield attributes

The soil application of different Cd concentrations ultimately reduced the yield attributes of the experimental plant. A significant ($p \leq 0.05$) reduction in seed yield per plant and thousand seed weight was estimated with the increase in Cd concentration as compared to control (give some values of decrease/increase). The seed yield and

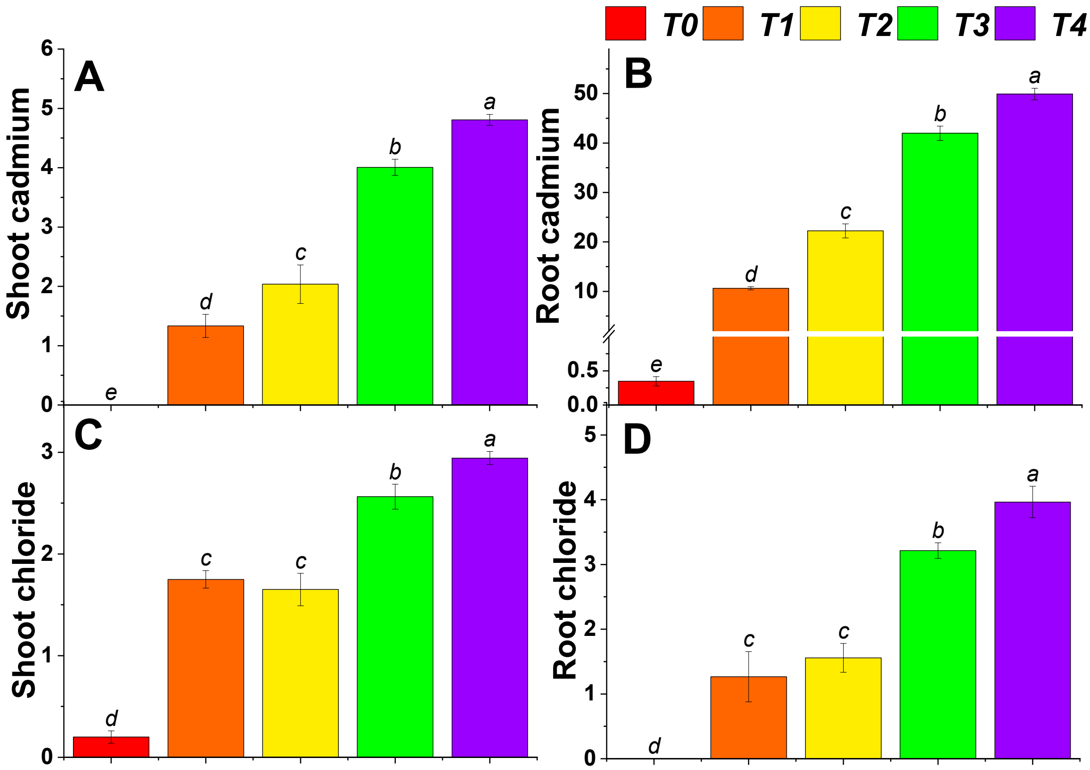

Figure 4. Shoot and root cadmium and chloride contents in Vigna plants influenced by variable levels of cadmium. The values are mean standard deviation (n = 3). Various alphabets over the bars demonstrate significant differences whereas similar letters exhibited nonsignificant differences ($p \leq 0.05$). T0: Control; T1: 10 mg/kg $CdCl_2$, T2: 20 mg/kg $CdCl_2$; T3: 30 mg/kg $CdCl_2$; T4: 40 mg/kg $CdCl_2$

their weights were less influenced in Cd_{10}-treated plants followed by T2 and then T3. T4 dose was highly lethal for the plants and showed an extreme decline in these estimated yield attributes (Figure 6).

4. Discussion

The observations recorded in our work have attested to the soil problems due to HM pollution. With rising Cd doses, *V. radiata* plants showed deterioration in growth and yield and became more susceptible to the dangers of Cd in the root zone. (Irshad et al., 2020; Irshad et al., 2022). The recorded decline in biomass of *V. radiata* is positively correlating with a decrease SPAD value. Changes in metabolism and morphological characteristics are unmistakable signs of plant health and life cycle destruction. (Eid et al., 2021; Lian et al., 2020). Our opinion about decreased growth is attested by its gradual link with metal toxicity (Figure 1). This decrease was further and mainly aggravated by the decrease in SPAD value that is indicative of photosynthetic incapability (Anwar et al., 2021; Sharma et al., 2021). We believe that the functional disruption in the organelles engaged in this process is related to the sensitivity of photo-assimilation and photosynthetic pigments to HMs. Nutrient disequilibrium also contributed to the poor growth responses and pigment decay that occurred concurrently with the reduction in yield. This conclusion was supported by numerous studies that showed a cumulative decline in the listed traits following exposure to stressful situations (Anwar et al., 2021; Khalid et al., 2019; Rahman et al., 2022). Khalid et al. (2017), similarly, presented that absorption and accumulation of HM in plant bodies lowered the biomass and yield (Khalid et al., 2019).

Shortage or disparity in essential minerals in plants under stress conditions leads to chlorosis (Mengel et al., 2001). Production and maintenance of compatible solutes and antioxidant enzymes for scavenging ROS are the primary physiological responses of plants to HM stress (Akhter et al., 2021; Khalid et al., 2021a). Typically, during HM stress, plant cells' starch and soluble proteins are consumed, which raises the amounts of soluble sugars and free amino acids (Hanif et al., 2021; Ozturk et al., 2021). In contrast to the Control, we noticed noticeably higher amounts of soluble sugars in various treatment

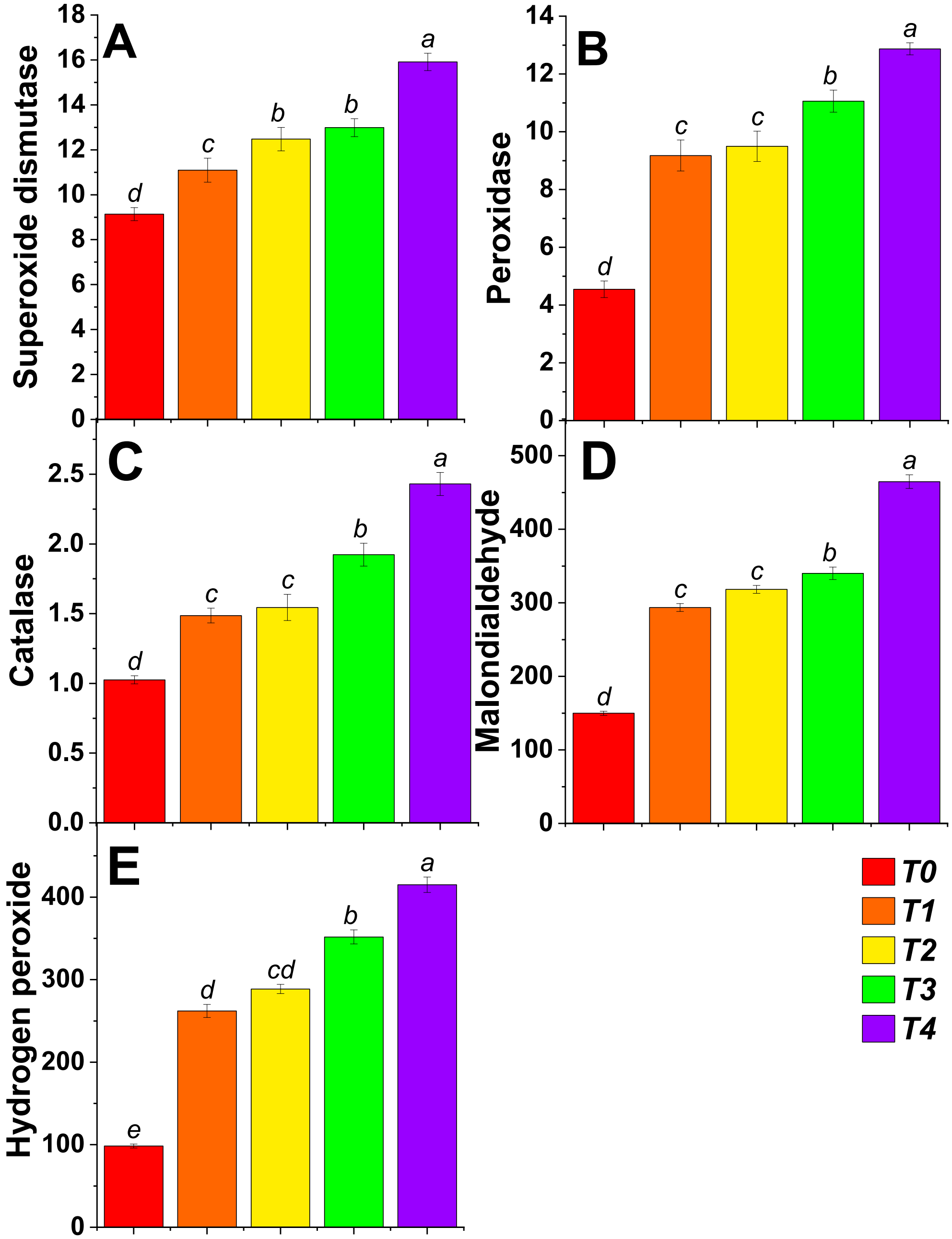

Figure 5. Antioxidant profile of Vigna plants influenced by variable levels of cadmium. The values are mean standard deviation (n = 3). Various alphabets over the bars demonstrate significant differences whereas similar letters exhibited nonsignificant differences ($p \leq 0.05$). T0: Control; T1: 10 mg/kg $CdCl_2$, T2: 20 mg/kg $CdCl_2$; T3: 30 mg/kg $CdCl_2$; T4: 40 mg/kg $CdCl_2$.

groups (Figure 2). Meanwhile, it was found that total soluble protein and total soluble sugars have an Inverse relationship (Figure 5). The deterioration of enzymes involved in the conversion of starch into sugar is the primary cause of this rise in soluble sugars (Amirjani, 2012 (Hasan et al., 2022)). The maintenance of osmotic functions is done by various cellular osmolytes (Alhaithloul et al., 2020). Any decrease in their concentrations curtails plant growth and development (Jogawat, 2019). The same has been observed in our study and Cd more negatively influenced total soluble proteins. As shown by the state of other traits, such osmolytic care actually condensed

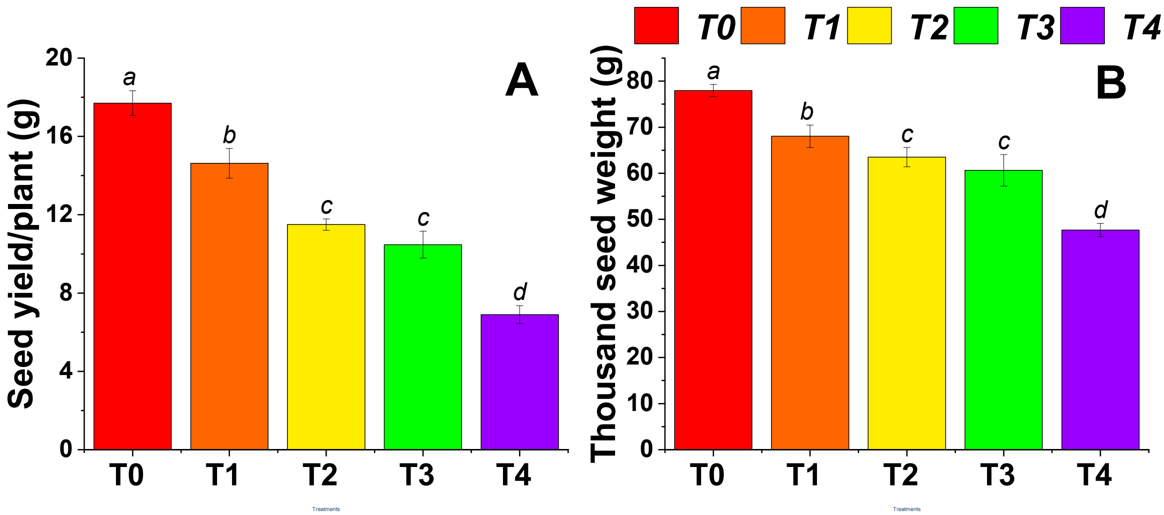

Figure 6. Yield attributes of Vigna plants influenced by variable levels of cadmium. The values are mean standard deviation (n = 3). Various alphabets over the bars demonstrate significant differences whereas similar letters exhibited nonsignificant differences ($p \leq 0.05$). T0: Control; T1: 10 mg/kg $CdCl_2$, T2: 20 mg/kg $CdCl_2$; T3: 30 mg/kg $CdCl_2$; T4: 40 mg/kg $CdCl_2$.

the damages caused by ROS and helped to ameliorate traumas at the plant level. We observed that MDA levels were sufficiently high in the presence of Cd, indicating a degree of danger to the integrity of the membrane. This is also advocated by the increasing H_2O_2 levels with increasing Cd levels. Although changes were apparent for MDA and H_2O_2 levels the regulation of both traits was conspicuously done with antioxidant defense. The activities of antioxidant enzymes i.e. POD, SOD, and CAT were affected by different levels of Cd in the root zone (Figure 5). The ability to tolerate stress is favourably correlated with the antioxidant enzymes' activities. POD typically detoxifies oxygen molecules that are active. The responses of POD and other antioxidant enzymes in other plants to various stresses provide evidence in support of this claim (Che et al., 2020; Huang et al., 2019; Liu et al., 2019). SOD activity levels were greater in plants exposed to various Cd levels, suggesting that they are better able to withstand oxidative stress. According to studies (Akhter et al., 2022a; Akhter et al., 2022b; Homayoonzadeh et al., 2020), antioxidant responses against different levels of stress are considered as a major achievement in the survival strategy of plants. In the event of failing, surviving becomes challenging. This may immediately result in a decrease in other characteristics, such as the rate of photo assimilation. Therefore, it is clear that HMs pose a greater danger to plants than other soil pollutants, even at different doses. We also noted a disproportionate concentration of essential minerals in the roots and shoots (Figure 3). These are crucial ions for plant metabolic activities (Aqeel et al., 2023). Reduced concentrations of ions such as P, Ca^{2+}, Fe^{2+}, and Mg^{2+} in the shoot system impede chlorophyll synthesis and cause chlorosis under HM stress. Such diminution in photosynthetic pigments is a major reason for low biomass production (Shahbaz et al., 2019) (Kotapati et al., 2017). Additionally, the migration and absorption of Cd into plant tissue can disrupt the levels of K, Ca, and P. (Rubio et al., 1994).

These abating mineral concentrations were proportional to repression in the growth of *Vigna* plants. Cd interfered with ionic homeostasis led to the inhibition of transpiration which also impaired xylem transport (Yang et al., 2020a). Supportive to our stance, a reduction in essential ions was recorded in this study. Additionally, restriction in the uptake of macro and micro nutrients due to HMs also impedes cellular transport of those macro and micro elements by altering the structure of plasma membranes (Shamsi et al., 2007). We likewise detected a substantial concentration of Cl in all the treatments against the control. This can be due to the applied $CdCl_2$ in the soil. The Cd concentration was higher in roots than in shoots (Figure, 4). The same has been reported earlier and our finding is in accordance with these reports (Wahid and Ghani, 2008).

5. Conclusion

The outcomes of the study unravelled reduced growth, physiology, and yield of *V. radiata* plants caused by the uptake of cadmium. It was found that the delicate equilibrium between nutrient ions, morphology, and metabolic activities for the synthesis of essential biopolymers had been compromised. On the other hand, differential accumulation of Cd in tissues along with absorption is a better indicator of performance as all perturbations were directly linked to this. Very simply, Cd displayed a toxic effect but this effect was more dependent upon the dose. Damage was directly proportional to the HM dose. On the basis of the reported findings, our recommendation is to conduct research with an explicit focus on the mechanistic elucidation of damages caused by Cd. Additionally, target enzymes or metabolites in plants should be explored for use in the development of HM-tolerant crop varieties.

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