Impact of Cadmium on root growth and ascorbate peroxidase activity (APX) and catalase activity (CAT) in wheat (*Triticum aestivum*)

SAFIA SAHOULI* and YAMINA BOUKERCH

Department of Biology, Faculty of Sciences of Nature and Life, University of Djelfa, city 05 Juillet, route Moudjbara, POBox 3117, 17000, Djelfa, Algeria

Abstract

Cadmium (Cd) is recognized as a major environmental pollutant that, upon absorption by plants, disrupts various physiological processes, leading to significant stress. This study investigates the effects of different Cd concentrations on root growth parameters and antioxidant enzyme activities in soft wheat *(Triticum aestivum*). Treatments with 50 and 100 mg/L Cd reduced root biomass by 28.70% and 30.91%, respectively, compared to the control. The Tolerance Index (TI) peaked at 80% under 50 mg/L Cd but declined to 50% at 100 mg/L, indicating moderate tolerance at lower Cd levels. Exposure to higher Cd concentrations (200 and 500 mg/L) resulted in biomass reductions of 95% and 80%, respectively, demonstrating severe toxicity. Antioxidant enzyme analysis revealed that ascorbate peroxidase (APX) activity was stimulated across all Cd treatments, while catalase (CAT) activity exhibited a non-linear response to increasing Cd concentrations. Overall, cadmium exposure negatively affected root development in wheat by impairing physiological mechanisms and inducing oxidative stress.

Keywords: Cadmium, *Triticum aestivum*, toxicity root, tolerance index, ascorbate peroxidase, catalase.

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Introduction

Heavy metals serve as environmental pollutants and contribute to a range of significant environmental and health issues. Their presence in soil and water can be natural or due to industrial activities such as the metal industry, as well as other sources of contamination in the agricultural sector, such as pesticides, herbicides, fungicides, or fertilizers (Rashid et al., 2023). Plants manifest distinct patterns of metal accumulation and dispersion in various plant parts (Haider et al., 2021). Cadmium enters the roots via the apoplast and symplast pathways, and is subsequently delivered to various plant parts by the xylem and phloem, the two main vascular systems. The xylem, which conducts raw sap, carries cadmium to the aerial portions of the plant, such as the stems and leaves. Similarly, the phloem, the tissue that conducts processed sap, redistributes cadmium throughout the plant, including storage organs (Ai et al., 2022).

Heavy metals bound in complex forms are transported either through the apoplast or stored in vacuoles. These cellular compartments play a key role in water storage, enzyme regulation, and

* *Corresponding Author*
E-mail Address: s.sahouli@univ-djelfa.dz
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in the mechanisms of heavy metal tolerance and detoxification, by sequestering heavy metals and preventing their free circulation in the cytoplasm (Sterckeman and Thomine, 2020).

When plants are exposed to cadmium in the soil, they undergo osmotic stress, resulting in a reduction of the relative water content of the leaves, stomatal conductance, and transpiration, which leads to physiological damage (Rizwan et al., 2016; Haider et al., 2021).

Moreover, cadmium affects the redox potential of the cell and causes oxidative damage to cell membranes, leading to programmed cell death and a significant reduction in overall plant biomass (Imran et al., 2020; Hussain et al., 2021).

The plant responds initially by rapidly modifying the ionic flow across its plasma membranes. These changes in ionic flow directly influence various reactions directed against pathogens and heavy metals. One of the early responses involves the production of reactive oxygen species (ROS) following the introduction of calcium ions into cells. These species include superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) (Jost and Jost-Tse, 2016). These molecules cause necrotic damage to plants and function as signaling molecules.

Cadmium, like other heavy metals, leads to the formation of ROS while inhibiting antioxidant enzymes, exacerbating lipid peroxidation reactions (Rizwan et al., 2019). Furthermore, the accumulation of toxic heavy metals in plant tissues increases ROS production, disrupting the redox balance (Feng et al., 2023).

Ascorbate peroxidase (APX, EC 1.11.1.11) is among the enzymatic components involved in ascorbate metabolism, enabling the degradation of H_2O_2 and thus regulating its cellular levels. Ascorbate is produced, consumed, and regenerated by a group of enzymes, allowing it to maintain optimal levels according to cellular needs (Corpas et al., 2024). Increased Cd levels have been found to significantly enhance APX enzyme activity.

Catalase (CAT, EC 1.11.1.6), one of the most important antioxidant enzymes, protects cells from the harmful effects of hydrogen peroxide, a reactive oxygen species produced as a byproduct of regular metabolism (Nandi et al., 2019).

This study aims to explore the effects of cadmium at different concentrations on wheat roots by examining root mass, tolerance index, and changes in APX and CAT enzymatic activities in *Triticum aestivum*.

Materials and Methods

Seed Germination and Root Analysis

Wheat seeds of uniform size and color were surface-sterilized in a 2% sodium hypochlorite solution for 2 minutes, followed by rinsing two to three times with sterile water. The seeds were evenly placed in sterile Petri dishes (9 cm in diameter) lined with a double layer of sterilized filter paper circles (Whatman No. 1) and moistened with 5 mL of cadmium sulfate ($CdSO_4$) solution at different concentrations (0, 50, 100, 200, and 500 mg/L). The filter paper was re-moistened with the same solution every 24 hours. Petri dishes were then placed in a growth chamber for 7 days at a temperature of 25 °C.

Determination of Growth Parameters and Tolerance Index

The total fresh root weight of the samples was measured using an electronic balance.

Root toxicity was calculated using the following formula:

Toxicity (%) = [(Fresh weight of control roots – Fresh weight of treated roots) / Fresh weight of control roots] × 100

Measurement of Tolerance Index (TI)

Root length was measured by estimating the maximum length of the primary root, from the base to the tip, using a standard ruler.

The tolerance index (TI) was calculated according to Malecka et al. (2012) using the following equation:

TI (%) = (Mean root length in metal solution / Mean root length of control) × 100

Table 1
Effect of cadmium toxicity on wheat seedling growth.

Concentration (mg/l)	Tolerence index(TI) (%)	Toxicity root (%)
50	80	33.33
100	50	40.55
200	30	82.22
500	17	95

Enzymatic Extraction

Roots from each treatment were collected and cut into small pieces to facilitate grinding. Approximately 500 mg of roots were ground in a mortar with 2 mL of extraction buffer containing 50 mM phosphate buffer (pH 7.0), 1% polyvinylpolypyrrolidone (PVP), and 0.20% Tween 20. The homogenate was centrifuged at 4°C for 30 minutes at 14,000 rpm. The resulting supernatant was collected and kept on ice as the enzymatic extract.

APX Activity

APX activity was determined by monitoring the oxidation rate of ascorbate. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM sodium ascorbate, 0.1 mM H_2O_2, and the enzyme extract, following the method of Nakano and Asada (1987). Absorbance was measured at 290 nm at regular intervals over 4 minutes. APX activity was calculated using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$. Protein concentration was determined by the method of Bradford et al. (1976), using bovine serum albumin as a standard to estimate the specific activity of the enzyme.

Catalase (CAT) Activity

CAT activity was assessed according to Aebi (1974). The final reaction volume was 3 mL, containing 200 µL of crude enzyme extract, 100 µL of 0.3% hydrogen peroxide (H_2O_2), and 2700 µL of 50 mM phosphate buffer (pH 7.2). The spectrophotometer was calibrated without the enzymatic extract. The reaction was initiated by the addition of hydrogen peroxide, and the decrease in absorbance at 240 nm was recorded over 4 minutes. CAT activity was calculated using an extinction coefficient of 43.1 $M^{-1}cm^{-1}$ for H_2O_2 and expressed as µM/min/mg of protein.

Statistical Analyses

Data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, United States). APX data are presented as mean ± standard deviation. To assess differences among the means, a one-way ANOVA was used, followed by Tukey's post hoc test. A significance level of $p < 0.05$ was considered for all statistical tests.

Results

The root system of plants grown at 50 and 100 mg/L of Cd showed a decrease in length compared to the control (Fig.I A–B). A blackening of the roots was observed with an increase in Cd concentration to 100 mg/L (Fig. I C); similarly, a concentration of 200 mg/L reduced both the primary and lateral root lengths (Fig. I D). The highest concentration, 500 mg/L, significantly affected the root system by reducing the number of branches and altering the lengths of the primary and secondary roots (Fig. I. E).

The calculation of the percentage reduction in root biomass is a simple yet effective method for assessing the effects of treatment on plant growth. In the case of cadmium exposure, this method enables quantification of root growth inhibition caused by this heavy metal. Roots of wheat plants exposed to cadmium at 50 mg/L showed a reduction in root biomass by 33.33% compared to the controls, while concentrations of 100 mg/L and 200 mg/L resulted in reductions of 40.55% and 82.22%, respectively (Table 1). A significant reduction of 95% in root biomass was recorded in roots treated with 500 mg/L of Cd (Table 1).

The Tolerance Index (TI) is a ratio used to evaluate a plant's ability to withstand environmental stress, particularly heavy metal exposure such as cadmium. This index compares the growth of roots treated with Cd to that of untreated (control) roots.

Roots treated with 50 mg/L Cd exhibited a TI value of 80%. In contrast, roots treated with 100 mg/L

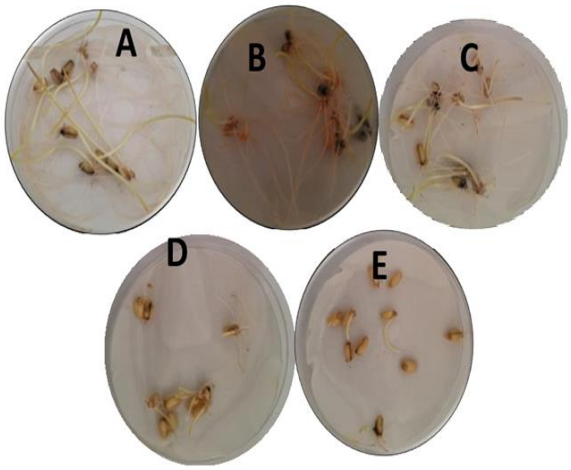

Fig. I. Effect of different concentrations of cadmium (Cd) on the root growth of 7-day-old wheat seedlings. (A) Control; (B) 50 mg/L Cd; (C) 100 mg/L Cd; (D) 200 mg/L Cd; (E) 500 mg/L Cd.

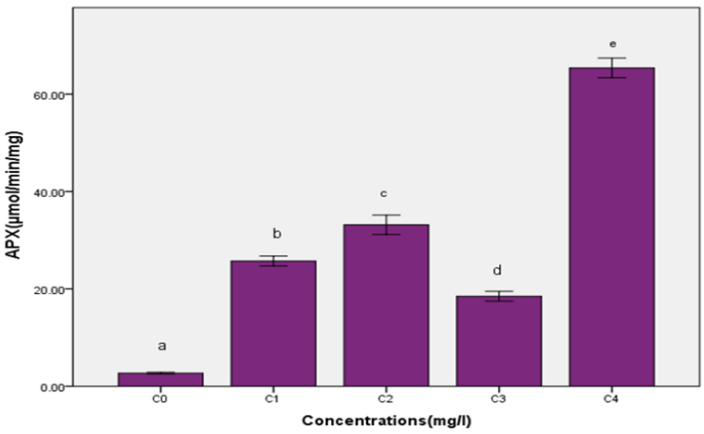

Fig. II. Effect of different concentrations of $CdSO_4$ on APX activity in wheat roots. (C0: Control; C1: 50 mg/L; C2: 100 mg/L; C3: 200 mg/L; C4: 500 mg/L).

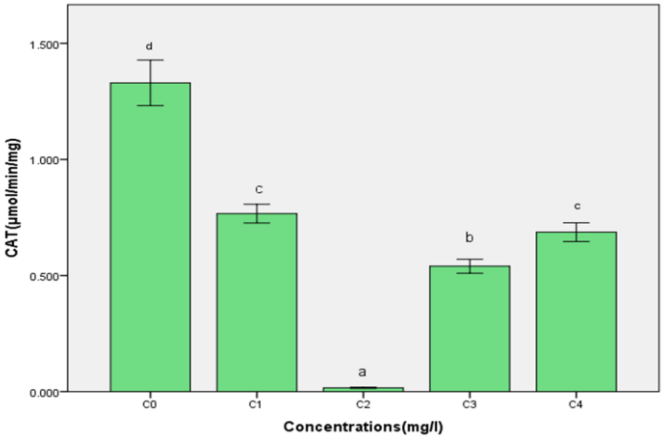

Fig. III. Effect of different concentrations of $CdSO_4$ on CAT activity in wheat roots (C0: Control; C1: 50 mg/L; C2: 100 mg/L; C3: 200 mg/L; C4: 500 mg/L).

Cd displayed a 50% tolerance. Very high concentrations of 200 mg/L and 500 mg/L resulted in TI values of 30% and 17%, respectively (Table 1).

Understanding the role of APX in wheat's response to cadmium stress is crucial for developing strategies to enhance plant tolerance to heavy metals. The effect of cadmium on APX activity varied depending on the metal concentration. Cadmium exposure significantly stimulated APX activity. A notable increase compared to the control was observed in roots exposed to 50 mg/L and 100 mg/L, with recorded values of approximately 25.720 ± 0.057 µM/min/mg and 33.170 ± 0.1 µM/min/mg, respectively.

However, exposure to 200 mg/L Cd led to a decrease in APX activity (18.497 ± 0.06 µM/min/mg), although this value remained higher than that of the control (2.7 ± 0.01 µM/min/mg). Interestingly, the highest concentration (500 mg/L) resulted in a maximum increase in APX activity, reaching 65.390 ± 0.09 µM/min/mg (Fig. II).

All cadmium concentrations tested significantly reduced CAT activity compared to the control (1.33 ± 0.04 µM/min/mg). However, the reduction was not linear with increasing Cd concentration. The strongest inhibition was observed at 100 mg/L Cd, where CAT activity drastically dropped to 0.0163 µM/min/mg, indicating almost complete suppression of enzymatic function. Interestingly, CAT activity at 50 mg/L and 500 mg/L was nearly identical, with values of 0.686 ± 0.04 and 0.766 ± 0.02 µM/min/mg, respectively. At the intermediate concentration of 200 mg/L, CAT activity decreased to 0.540 ± 0.02 µM/min/mg (Fig. III).

Discussion

Increasing Cd concentrations resulted in a decrease in the length of primary and secondary roots. The reduction in root elongation may be caused by inhibition of root growth, cell division, and synthesis of cell wall polysaccharides. Likewise, lignification can inhibit root growth if it occurs in the elongation zone (Parrotta et al., 2015; Loix et al., 2017).

The root absorbs Cd, but **it** localizes in all plant tissues and induces several alterations in plant morphological traits and physiochemical processes. In fact, Cd toxicity, in most cases, determines a decrease in root elongation, alterations in root architecture, and a reduction in root system formation (He et al., 2017). Biomass of the entire plant or its organs is an essential measurable parameter for evaluating the effects of various constraints on plants (Daud et al., 2013). This study clearly showed that the higher the Cd concentration, the more significant the effects on root biomass. When wheat seeds are exposed to cadmium, their roots undergo significant morphological alterations: they become thinner and shorter than the control roots. The development and survival of the plant are directly impacted by this decrease in root mass. High concentrations of cadmium also prevent secondary roots from growing and elongating. These results are consistent with the studies of Bouhraoua et al. (2025), which clearly showed that Cd can lead to a reduction in biomass production.

According to Idrees et al. (2015), a progressive decrease in the length of roots and shoots of Cd-contaminated plants was observed along with a modification of root morphogenesis. Rahoui et al. (2008) showed that heavy metals affect seed germination by disrupting the chain of events in germination metabolism. In wheat plants, Cd can reduce length and lead to ROS accumulation.

Results of TI measurement suggest that wheat roots faced problems in withstanding high concentrations of Cd. However, TI remained close to 100, suggesting that wheat roots were able to tolerate this level of Cd with minimal impact on their growth or function and can moderately withstand low concentrations of Cd.
It is likely that toxic effects of the heavy metal caused a significant inhibition of primary root growth and root length in *Zea mays* (Šípošová et al., 2023). Such inhibition of cell division results in a reduction of meristematic cells due to changes in numerous physiological processes of developing seedlings, thereby reducing growth and biomass (Chieb and Gachomo**.** 2023).
Alteration of cellular membrane function due to

cadmium stress is well expressed in terms of increased permeability, which can be easily measured by electrolyte leakage. Cd affects seed germination by poor water absorption during the imbibition process, also leading to difficulties in water absorption by the grains (Kaur et al., 2023). Excess Cd causes alteration of metabolic enzymes, photosynthetic system inhibition, and excessive root damage. Several physiological issues may be indirectly caused by the plant's decreased mineral nutrition (Son et al., 2023).

APX is among the enzymatic components that participate in the metabolism of ascorbate, allowing decomposition of H_2O_2 and consequently regulating its cellular content (Corpas et al., 2024). In this study, activation of the antioxidant system was observed. This is likely to reduce the oxidative stress generated by Cd toxicity. APX enzyme activity was stimulated by low concentrations of cadmium. These results are compatible with the work of Srivashtav et al. (2024), which reported that in castor bean plants, APX is closely related to the Cd dose, and a considerable increase was observed in the roots compared to the leaves after ten days of Cd treatment.

Plants can induce increased synthesis of APX to enhance their antioxidant capabilities to mitigate the adverse effects of Cd. In coffee cells, APX activity was increased at the lowest cadmium concentration (Gomes-Junior et al., 2006). These observations suggest that APX plays a crucial role in regulating the response to oxidative stress. In accordance with studies conducted by Saleh et al. (2020), antioxidant responses of wheat treated with a low concentration of cadmium were stimulated.

On the other hand, a decrease in APX activity at higher concentrations of Cd may occur due to H_2O_2 and its derivatives (Corpas et al., 2024). It was found that APX activity in *Pisum sativum* was not detectable in cells subjected to high concentrations of cadmium (El-Okkiah et al., 2022). Similarly, CAT and APX activity in *Canna orchioides* was significantly reduced by the application of high concentrations of cadmium (20 mg/l) compared to the control (Zhang et al., 2020). APX plays an essential role in eliminating H_2O_2, but its activity is conditioned by metal concentrations, aiming mainly to eliminate H_2O_2 at the source of production (Gutiérrez-Martínez et al., 2020). At the cell wall level, there is competition between two enzymes, class III peroxidase and APX, to convert H_2O_2 into H_2O. In fact, APX uses ascorbate to facilitate this reaction (Loix et al., 2017). Subsequently, the levels of H_2O_2 are controlled by APX and used as a signal to activate lignification through transcription (Shafi et al., 2015). In the same vein, very high concentrations of cadmium can directly activate APX activity. These results agree with the work of Buzduga et al. (2022), which showed that APX only increased after treatment with 500 µM of Cd chloride for 5 days.

It is likely that the reduction in CAT activity in wheat roots exposed to cadmium is due to inhibition of the enzyme caused by cadmium binding to its active site, in line with the mechanisms described by Wang et al. (2015). This highlights the effect of cadmium on the antioxidant system of plants, potentially because cadmium is a divalent cation (Cd^{2+}) that can bind to negatively charged groups or specific ligands present in the active sites of enzymes. The effect of cadmium on catalase activity is complex and non-linear. The concentration of 100 mg/l seems to be the most inhibitory; it is possible that the intervention of other antioxidant enzymes, such as APX, was involved in the response to oxidative stress induced by cadmium, which has been confirmed in this study. Before any obvious signs of damage manifest, cadmium may stimulate or inhibit the activity of a number of antioxidant enzymes (Xu et al., 2014), while higher concentrations do not necessarily cause stronger inhibition. This could indicate mechanisms of adaptation or compensation of the enzyme at very high concentrations, or toxic effects specific to certain concentration ranges. Given the crucial role of APX in the reduction of H_2O_2 to water in a variety of subcellular compartments, and its greater affinity for H_2O_2 than CAT (Li, 2023), it appears to be a key player in stress response.
Toxicity of heavy metals can also lead to the formation of GPX, which is more effective than CAT in eliminating H_2O_2. Thus, the enzymes CAT,

GPX, and APX all play an essential role in eliminating cellular levels of H_2O_2 in plants (Mansoor et al., 2023).

Conclusions

Cadmium exerts adverse effects on wheat root weight by disrupting numerous physiological processes. Understanding these mechanisms is essential to develop effective strategies to reduce cadmium toxicity and improve agricultural production. Inhibition of CAT activity in response to Cd-induced stress is compensated for by APX activity, which appears to be more engaged. Ascorbate peroxidase plays a complex and multifaceted role in wheat's response to cadmium stress. Understanding the molecular mechanisms regulating the activity of this enzyme is necessary to develop effective strategies aimed at improving plant tolerance to heavy metals and preserving the quality of agricultural products.

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