



## Effect of Cadmium Toxicity on Different Antioxidant Enzymes in Growing Wheat (*Triticum aestivum* L.) Seedlings

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**Abstract:** Assessing the impact of Cadmium (Cd) on plant cells requires an understanding of the defensive mechanisms and adaptive responses employed by plants to counteract the deleterious effects of Cd toxicity. Wheat seedlings were grown under different concentrations (0  $\mu$ M, 100  $\mu$ M and 500  $\mu$ M) of Cadmium salt (CdCl<sub>2</sub>). The growth of metal-treated seedlings was significantly affected. This study evaluated the ecotoxicological effects of two experimental concentrations of Cadmium (100  $\mu$ M and 500  $\mu$ M) against water control. It has been examined in recent studies that the toxic effects of Cadmium comprising the variables in fresh weight, dry weight and germination percentage of the wheat seedlings, photosynthesis and enzymatic activities in different stresses after seven days of cadmium exposure on roots and shoots of wheat seedling. Different antioxidant enzyme activities such as catalase (CAT), ascorbic acid oxidase (AOX), catechol peroxidase (CPX), superoxide dismutase (SOD), acid and alkaline phosphatase and inorganic pyrophosphatase have been measured. The various enzyme activities were increased in roots treated with high concentrations (500  $\mu$ M) of Cadmium but decreased at low concentrations (100  $\mu$ M) with respect to water control. This result indicated a complex defense mechanism in the root tissues induced by Cadmium. This work provides valuable insights for future research on plant responses to heavy metal stress and advances our understanding of the complex relationship between Cd toxicity and the antioxidant defense system in wheat seedlings.

### Introduction

A non-essential heavy metal called Cadmium (Cd) is very hazardous for living organisms (Bhattacharya, 2015; Aiqing, 2021; Shanmugaraj, 2019). The sources that are anthropogenic, such as phosphate fertilizers, industrial effluences, and sewage sludge, basically increase the pollution of Cadmium in the agricultural systems, especially in the environment, causing a specific reduction in the yield of the crops (Aprile, 2018; Saha, 2017; Cui, 2020). Basically, in plants, the common symptoms of the toxicity of Cadmium include chlorosis, growth inhibition, and necrosis (Dinny, 2019; Surinaidu, 2021). It has to be shown that the toxicity of Cadmium can inhibit translocation and the uptake of minerals. In the nucleus of cells, the toxicity of Cadmium can cause morphological changes (Shanying, 2017). High

level of Cadmium also decreases the assimilation of carbon dioxide (de Araújo, 2017). A higher concentration of Cadmium increases the peroxidation of lipids in the cell membranes (Ahmad, 2016). The main effect of Cadmium toxicity is the alteration of the antioxidant activity of the enzymes (Liu, 2017; Madhu et al., 2022). The Reactive Oxygen Species (ROS), like hydroxyl radical, singlet oxygen and superoxide anion, are vigorously created as a byproduct of aerobic metabolism in various compartments of the cells (Romero, 2019). Recent studies have noticed that in plants, depending on the concentrations, reactive oxygen species basically play a dual role, either in cell functioning like different signalling molecules or in ROS, which is very harmful to the cells and causes mainly oxidative injury, is produced due to heavy metal toxicity. The toxicity of heavy metals



is influenced by environmental pollution. ROS activity may cause the intake of heavy metals, which can accumulate nervous disorders, poisoning and cancer and lead to mortality. So, the amount of the generated reactive oxygen species has maintained a specific physiological phenomenon by the antioxidant enzymes (e.g., catalase, peroxidase, etc.) under a steady state, including salinity, drought and toxicity of metal may cause a substantial rise in the reactive oxygen species levels through disrupting the oxidative homeostasis (Kobeya, 2020; Sabella, 2022). Phosphorus (P) is a very important component for the metabolism of cells in every plant. The primary role of phosphorus is the transfer of energy, and it acts as the modulator of enzymes and transcription of genes; therefore, storage, metabolism and assimilation are of major importance for plant development and growth. The hydrolytic disintegrate of phosphate esters may be carried out by the enzyme phosphatases, which shows vital action in the metabolic regulation, energy metabolism and the broad range signal transduction pathways of cereal plants (Younas, 2020). Inorganic phosphatase is the nutrient which often limits the growth of the plants in the common environment. Inorganic pyrophosphatase catalyses the hydrolysis of pyrophosphate and enhances the pool of phosphate in plants by hydrolyzing inorganic pyrophosphatase (Singla and Garg, 2011; Bhusare, 2018). Inorganic pyrophosphate is a major consequence of many biosynthetic reactions and very much necessary for regulation of various biochemical reactions in the plant tissues (Gutiérrez-Martínez, 2020). Acid phosphatases (orthophosphoric monoester phosphohydrolases) being overlapping and broad specificities of substrate are very much pervasive and the enzymes abundant in various plant species. Acid phosphatases catalyse the non-specific hydrolysis in the ranges of pH 4-6 and show a specific role in metabolism and phosphate supply in plants (Sabella, 2022; Gutiérrez-Martínez, 2020). Equivalently, the enzyme alkaline phosphatases show a vital role in the utilization of phosphomonoesters that are required for the maintenance of the metabolism of cells (Mamun et al., 2018; Rizwan, 2017).

## Material and Methods

### Seed collection and treatment

Wheat (*Triticum aestivum* L.) seeds cv. DBW-187 obtained from the Bidhan Chandra Krishi Vishwavidyalaya, Nadia, West Bengal, were sterilized using sodium hypochlorite (5.0%, v/v) and well cleaned. In Petri dishes (φ10cm) lined with filter paper containing varying concentrations (0, 100, and 500 μM) of cadmium chloride (CdCl<sub>2</sub>) exclusively, procured from Merck,

India, 15 seeds were dispersed for every treatment. After 48 hours at 30 ± 2°C in a dark and humid germinator, the seeds were subjected to a 16-hour photoperiod (260 μmol m<sup>-2</sup> s<sup>-1</sup> PFD) for seven days. The seedlings were taken for the subsequent investigations after seven days. The cadmium concentrations reported are equivalent to relevant soil and ambient conditions.

### Calculate the germination percentage

Different germination parameters were calculated according to the following formula (Qayyum et al., 2017).

$$\text{Mean germination time (MGT)} = \frac{\sum Dn}{\sum n} \dots\dots\dots (1)$$

Where 'D' is the total number of days calculated from the beginning of germination, and 'n' is the total number of germinated seeds on the day 'D'.

Calculation of the Germination index (GI) was based by using this formula (Bhusare, et al., 2018)

$$\text{GI} = \frac{\sum (Gt / Tt)}{\dots\dots\dots} \dots\dots\dots (2)$$

Where 'Gt' is the total number of the germinated seeds on that day and 'Tt' is the total number of days up to the day.

The germination percentage was measured by using the following formula (Baycu et al., 2017; Głowacka et al., 2019).

$$\text{GP} = (\text{Number of germinated seed} / \text{Total seed}) \times 100 \dots\dots (3)$$

### Morphological studies and biomass measurement

The harmful effects of Cadmium were noted after seven days, and the lengths of the developing wheat seedlings' shoots and roots were measured. Following harvest, the wheat seedlings were weighed once for each treatment and kept at -40°C for biochemical analysis. Furthermore, ten seedlings from each treatment were taken out and weighed. The shoots and roots of each set were separated, and the plant parts were dried in an oven for two days at 100°C. The shoots and roots of each group were isolated, and the fragments were dehydrated in an oven for duration of two days at a temperature of 100°C followed by three days at a temperature of 80°C (Almuwayh, 2021; Gubrelay et al., 2015; Ambrosino et al., 2021). After the allotted time, the seeds' dry weights were measured, and the variations in fresh weight (water content) and dry weight between the subsequent treatment sets were noted.

### Determination of chlorophyll and carotenoid contents

Seven days old wheat leaves old were used to compute the concentrations of chlorophyll-a, chlorophyll-b, and total chlorophyll (Arnon, 1949). Using a JASCO V-630 spectrophotometer, the chlorophyll concentrations were initially determined spectrophotometrically at 645 and 663 nm after 1 g of fresh wheat leaves were extracted along with 25 ml of 80% acetone (v/v). The amount of

chlorophyll was stated as milligrams (mg) of chlorophyll present per gram of fresh weight.

The contents of carotene and xanthophyll were determined using spectrophotometric technique by Davies' (1965). 20–25 ml of cyclohexane and a coloured acetone alkaline solution were combined in a separating funnel. The hexane layer was cleaned with 20 millilitres of distilled water. Xanthophylls and carotene were extracted three times from the top hexane layer using 20 millilitres of 90% methyl alcohol (v/v). With a JASCO V-630 spectrophotometer, the absorbance values at 425 nm and 450 nm, respectively, were used to quantify the concentrations of carotene and xanthophylls. The following results were reported in terms of optical density  $g^{-1}fw$ .

#### Estimation of antioxidant enzymatic activity

The extraction of enzymes was carried out at a temperature of 4°C. The plant sample weighing 1 gm was crushed with 5.0 milliliters of pre chilled 0.1 M sodium phosphate buffer (pH 7.4). The homogenate mixture underwent centrifugation at a speed of 12,000 g for duration of 10 minutes. The resulting liquid above the sediment, known as the supernatant, was often utilized for measuring the enzyme's activity.

#### Catalase activity

The catalase activity (CAT; EC 1.11.1.6) was measured by determining the quantity of potassium permanganate ( $KMnO_4$ ) consumed in relation to hydrogen peroxide ( $H_2O_2$ ) (Casper and Laccoppe, 1968). The reaction consisted of 1-2 ml of 3% hydrogen peroxide (v/v), 0.5 ml of 0.1 M sodium phosphate buffer at pH 7.4, and 1.0 ml of enzyme extract. The reaction was inhibited by adding 3.0 ml of 10% sulfuric acid ( $H_2SO_4$ ) (v/v) after incubation. The remaining volume of hydrogen peroxide was titrated using a 0.02(N) solution of potassium permanganate. The enzyme's activity was quantified as the number of milligrams (mg) of hydrogen peroxide decomposed per milligram (mg) of protein per hour (h).

#### Ascorbic acid oxidase activity

Ascorbic acid oxidase activity (AOX; EC 1.10.3.3) was measured in wheat leaves that were seven days old (Olliver, 1967). The procedure used one to two millilitres of enzyme extract, three millilitres of sodium phosphate buffer (0.1 M, pH 7.4), and one or two millilitres of ascorbic acid (0.025 w/v). Titrated with a 2,6-diphenylphenolindophenol (DCPIP) solution, the reaction was stopped after 30 minutes of incubation with 5 millilitres of 10% tetracarboxylic acid (TCA) (w/v). The difference between the sample and blank reading showed

the activity of AOX and expressed as milligram ascorbic acid decomposed  $mg^{-1} protein h^{-1}$ .

#### Catechol peroxidase activity

The spectrophotometric measurement of catechol peroxidase (CPX; EC 1.11.1.7) activity was conducted using a JASCO V-630 spectrophotometer. The reaction comprised 1-2 milliliters of 10% hydrogen peroxide (v/v), 5.0 milliliters of 0.1M sodium phosphate buffer (pH 7.4), 1.0 milliliters of 0.5% catechol (w/v), and 1.0 milliliters of enzyme extract. The absorbance readings of the reaction mixtures were recorded at 420 nm following a 1-minute incubation period. The activity of the enzyme was expressed in terms of change in  $\Delta OD_{420} mg^{-1} protein min^{-1}$  (Chance and Maehly, 1955).

#### Superoxide dismutase activity

The enzymatic function of Superoxide dismutase (SOD; EC 1.15.1.1) was assessed using the nitro blue tetrazolium (NBT) technique, as described by Giannopolitis and Ries (1977). The reaction mixture consisted of 2.5 ml of Tris-HCl buffer (80 mM) with a pH of 7.5, which contained 10.8 mM Tetramethyl ethylenediamine (TEMED) and 0.12 mM Ethylene diamine tetraacetic acid (EDTA). Additionally, it included 0.1 ml of  $3.3 \times 10^{-1}$  % Bovine serum albumin (BSA), 0.1 ml of a 1 ml solution of 0.6 mM riboflavin, 1 ml of a 6 mM nitroblue tetrazolium (NBT) solution, and 0.1 ml of enzyme extract. The introduction of riboflavin initiates the reaction. Subsequently, the glass tubes were agitated and positioned within a fluorescent light with an intensity of  $60 mmol m^{-2} s^{-1}$ . The response was prolonged for 10 minutes and terminated upon the cessation of the light source. The measurement of absorbance was conducted at a wavelength of 560 nm. The control and blank were conducted using identical procedures, except that the enzyme and illumination were omitted, respectively. Under the specified experimental circumstances, a single unit of SOD refers to the quantity of enzyme that results in about 50% inhibition of NBT reduction. The enzyme activity was quantified as EU (Enzyme Units) per milligram of protein per minute.

#### Acid phosphatase activity

The plant materials were homogenized in a sodium acetate buffer with a concentration of 0.1 M and a pH of 4.8. The homogenization process required the addition of 5  $\mu M$  cysteine. Subsequently, the mixture was centrifuged at a speed of 10000 rpm for duration of 15 minutes at a temperature of 40°C. The supernatants were collected and subjected to heating at a temperature of 70°C for a duration of 5 minutes in the presence of 3 mM Calcium chloride ( $CaCl_2$ ), resulting in the inactivation of  $\beta$ -amylase activity. The reaction consisted of a 0.1 M

sodium acetate buffer with a pH of 4.8, 1% soluble starch in a 0.15 M sodium chloride (NaCl) solution, and 1.0 ml of enzyme extract. The total final volume of the reaction was 4.0 ml. Subsequently, the incubation was subjected to a temperature of 30°C for duration of 5 minutes. Subsequently, the process was inhibited by the introduction of 6 M hydrochloric acid. 0.5 ml of a solution containing 0.2% I<sub>2</sub> in 2% KI, known as IKI solution, was added to a conical flask containing 1 ml of aliquots. The total volume was adjusted to 25 ml by adding distilled water, and the absorbance was measured at a wavelength of 660 nm. The enzyme activity is quantified as the amount of starch hydrolyzed per gram of fresh weight per minute, expressed in micrograms (µg) (Xiao et al., 2021; Haider et al., 2021).

### Alkaline phosphatase activity

The total procedure used for the assay of the enzyme, alkaline phosphatase, was the same as acid phosphatase, but there was an exception. For enzyme extraction and incubation, 100 mM sodium bicarbonate buffer (pH 10.0) was mainly used.

### Inorganic pyrophosphatase activity

Roots and shoots were homogenized in 5.0 ml of 0.1 M glycine- NaOH buffer (pH 8.8) to assay inorganic pyrophosphatase. After complete centrifugation the total supernatant was dialyzed. The assay mixture contained 10.0 µmol glycine-NaOH buffer (pH 8.8), 4.0 µmol Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 10.0 µmol MgCl<sub>2</sub> and enzyme in the volume of 2.0 ml. After incubation at 30°C for 20 mins, the reaction was terminated by adding 0.5-1 ml of 30% trichloroacetic acid. According to the method of Subbarow and Fiske (1925), if any precipitate formed, then that was removed by centrifugation, and the supernatant was mainly used for the determination of Pi. The specific activity of the enzyme was defined as nkat mg<sup>-1</sup> protein (Xiao et al., 2021; Haider et al., 2021).

### Measurement of cadmium concentration

Total cadmium contents were measured from Cadmium-treated shoots and roots of wheat after the growth of seven days by digestion of acid for 500 mg oven-dried (70°C for 3 days and then 100°C for 2 days) samples. Then the dried samples over the hot plate using 7.0 ml of Nitric acid (65%) and 2.0 ml of H<sub>2</sub>O<sub>2</sub>. An atomic absorption spectrophotometer was used to determine the cadmium concentrations of the sample with a flow injection hydride generation system (Perkin Elmer, Analyst 700), using standard cadmium solution to prepare

a standard curve. This study used analytical grade solvents for assay (Gubrelay et al., 2015).

### Statistical analysis

The following experiments were imposed with 3 replicates in a fully randomized design, with every replication carrying out an average of 15 seeds on a single Petri dish. The significant differences and data among the mean values were correlated by the descriptive statistics (± SE). Every data was analyzed using ANOVA, and 'P < 0.05' was considered a significant level in every statistical test (Bhusare et al., 2018).

## Results

### Effect of Cadmium (Cd) on germination percentage

Cadmium becomes very much toxic to plants' growth and metabolism. The germination percentage was highly affected at different concentrations of CdCl<sub>2</sub> treatments in wheat seedlings (var. DBW-187). The present study has shown that the mean germination time, germination rate and germination index were inhibited by increasing treatments of cadmium salt (Table 1).

**Table 1. Analysis of variance of treatment (T) and their interactions (G×T) for the Mean germination time (MGT), germination index (GI), and germination rate (GR) on the germination of 7 days old (var. DBW-187) of wheat (*Triticum aestivum* L.) seedlings. The values are means of five replicates ± SE. Values are not significantly different compared to only cadmium treatment P ≤ 0.05.**

Treatment	MGT (day)	GI	GR (%)
Control (0 µM)	18.0 ± 0.18	14.44 ± 0.09	92.3 ± 0.12
CdCl <sub>2</sub> (100 µM)	12.5 ± 0.13	11.89 ± 0.06	74.8 ± 0.09
CdCl <sub>2</sub> (500 µM)	12.1 ± 0.09	9.6 ± 0.05	70.9 ± 0.07

### Effect of Cadmium (Cd) on morphological parameters and biomass content

The exposure of Cadmium to wheat growth generally caused a significant reduction in the lengths of root and shoot (Figure 1, Table 2). On root, the inhibitory effect was more specific than on shoot. The length of the root was decreased significantly in 100 µM and 500 µM cadmium treatments, which were 27% and 63% reduction. Comparatively, the reduction in shoot length was lower, amounting to 14% and 35% reduction in 100 µM and 500 µM cadmium treatments.



**Figure 1.** Effect of different concentrations of cadmium chloride ( $\text{CdCl}_2$ ) on the growth of seven-day-old wheat seedlings (*Triticum aestivum* L.) cv. DBW-187.

**Table 2.** Effect of different concentrations (0, 100, 500  $\mu\text{M}$ ) of  $\text{CdCl}_2$  on shoot and root length of 7 days old (var. DBW187) wheat (*Triticum aestivum* L.) seedlings. The values are means of five replicates  $\pm$  SE. Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .

Treatment	Shoot length (cm)	Root length (cm)
Control (0 $\mu\text{M}$ )	0.14 $\pm$ 0.061	0.11 $\pm$ 0.059
$\text{CdCl}_2$ (100 $\mu\text{M}$ )	0.12 $\pm$ 0.046	0.08 $\pm$ 0.053
$\text{CdCl}_2$ (500 $\mu\text{M}$ )	0.09 $\pm$ 0.051	0.04 $\pm$ 0.025

#### Accumulation of heavy metal in wheat seedlings

There was an increase in the levels of total cadmium contents both in the shoot and root of seven-day-old wheat seedlings treated with various Cadmium (cd) concentrations. In short, the accumulation of Cadmium at 100  $\mu\text{M}$  and 500  $\mu\text{M}$  was increased by 99% and 100%, respectively. In the root, the concentrations of Cadmium were increased by 98% and 99%, respectively, compared to water control (Table 3).

**Table 3.** Measurement of cadmium different concentrations (0, 100, 500  $\mu\text{M}$ ) of  $\text{CdCl}_2$  on 7 days old (var. DBW187) wheat (*Triticum aestivum* L.) seedlings. The values are means of five replicates  $\pm$  SE. Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .

Treatment	Cadmium concentration ( $\text{mg L}^{-1}$ )	
	Shoot	Root
Control (0 $\mu\text{M}$ )	0.0003 $\pm$ 0.0002	0.0006 $\pm$ 0.0002
$\text{CdCl}_2$ (100 $\mu\text{M}$ )	0.03 $\pm$ 0.02	0.0583 $\pm$ 0.011
$\text{CdCl}_2$ (500 $\mu\text{M}$ )	0.21 $\pm$ 0.04	0.86 $\pm$ 0.064

#### Effect of Cadmium on photosynthetic pigments

Under the increasing concentrations of cadmium (Cd) treatments, there was a linear decrease in chlorophyll-a, chlorophyll-b, total chlorophyll, xanthophyll and carotene. On an average, the pigment contents decreased by 50% for chlorophyll-a, 76% for chlorophyll-b, 70% for total chlorophyll, (Table 4) and 66% for xanthophylls, 84% for carotene compared to water control (Table 5).

**Table 4.** Effect of different concentrations (0, 100, 500  $\mu\text{M}$ ) of  $\text{CdCl}_2$  on Chlorophyll a, Chlorophyll b and total Chlorophyll contents of 7 days old (var. DBW-187) wheat (*Triticum aestivum* L.) seedlings. The values are means of five replicates  $\pm$  SE. Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .

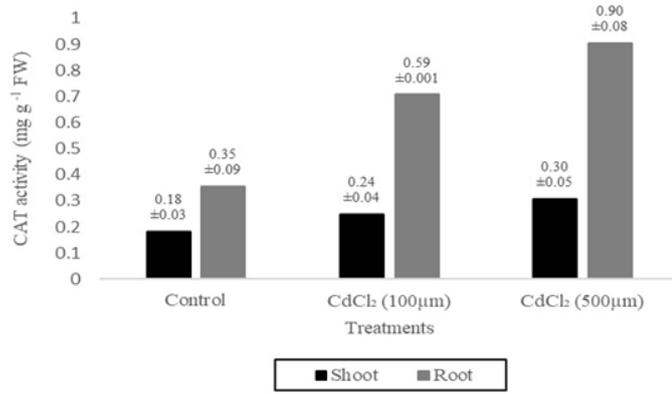
Treatment	Chl a ( $\text{mg g}^{-1}\text{FW}$ )	Chl b ( $\text{mg g}^{-1}\text{FW}$ )	Total Chl ( $\text{mg g}^{-1}\text{FW}$ )
Control (0 $\mu\text{M}$ )	0.028 $\pm$ 0.018	0.09 $\pm$ 0.021	0.118 $\pm$ 0.051
$\text{CdCl}_2$ (100 $\mu\text{M}$ )	0.023 $\pm$ 0.014	0.06 $\pm$ 0.029	0.0884 $\pm$ 0.030
$\text{CdCl}_2$ (500 $\mu\text{M}$ )	0.019 $\pm$ 0.009	0.049 $\pm$ 0.018	0.068 $\pm$ 0.021

**Table 5.** Effect of different concentrations (0, 100, 500  $\mu\text{M}$ ) of  $\text{CdCl}_2$  on carotene and xanthophyll contents of 7 days old (var. DBW-187) wheat (*Triticum aestivum* L.) seedlings. The values are means of five replicates  $\pm$  SE. Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .

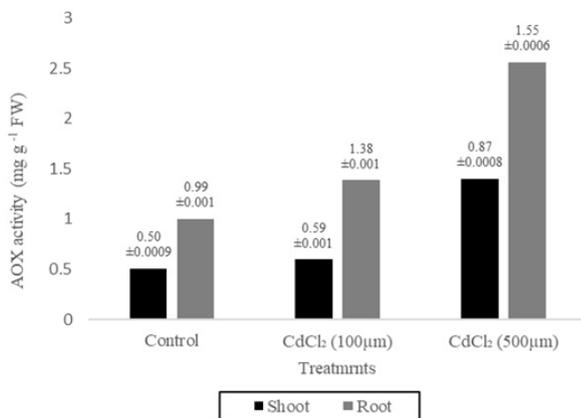
Treatment	Carotene ( $\text{g}^{-1}\text{FW}$ )	Xanthophyll ( $\text{g}^{-1}\text{FW}$ )
Control (0 $\mu\text{M}$ )	0.0060 $\pm$ 0.0028	0.0030 $\pm$ 0.0015
$\text{CdCl}_2$ (100 $\mu\text{M}$ )	0.0041 $\pm$ 0.0013	0.0022 $\pm$ 0.0010
$\text{CdCl}_2$ (500 $\mu\text{M}$ )	0.0029 $\pm$ 0.0009	0.0018 $\pm$ 0.0008

#### Effect of Cadmium (Cd) on antioxidant activities of enzyme

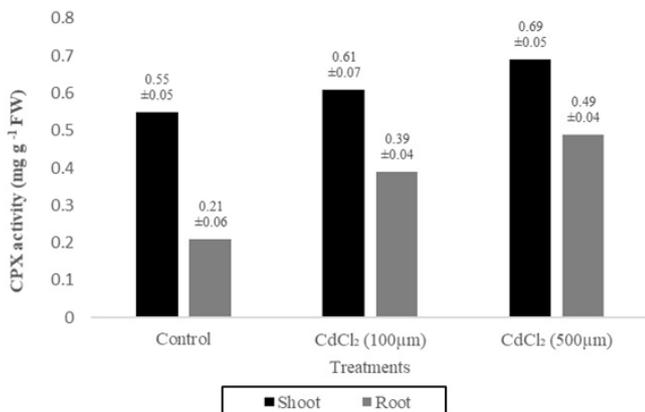
The activities of various antioxidant enzymes such as SOD, AOX, CPX and CAT significantly changed in all Cadmium-treated wheat seedlings. On average, the catalase (CAT) activity was increased by 64% in shoot and 102% in root of wheat seedlings in response to cadmium treatments over water control (Figure 2). On average, ascorbic acid oxidase (AOX) activity increased by about 56% in the shoot and 64% in the root sample compared to water control (Figure 3). Again, on average, it was noted that the activity of catechol peroxidase (CPX) was enhanced in the wheat seedlings by 30% in the shoot sample and 102% in the root sample (Figure 4) compared to the untreated water control. On average, the activity of Superoxide dismutase (SOD) was increased by 46% in the shoot and 50% in the root sample compared to the untreated water control (Figure 5).



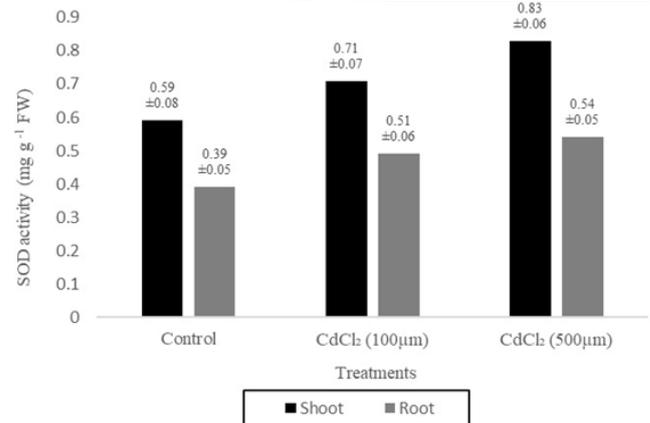
**Figure 2.** Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 μM) on CAT enzyme activity of 7-day-old wheat seedlings (DBW-187) variety of *Triticum aestivum* L. Data are expressed as mean values ± SE (n =5). Values are not significantly different compared to on.



**Figure 3.** Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 μM) on AOX enzyme activity of 7 days old (var. DBW-187) wheat (*Triticum aestivum* L.) seedlings. Data are expressed as mean values ± SE (n =5). Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .



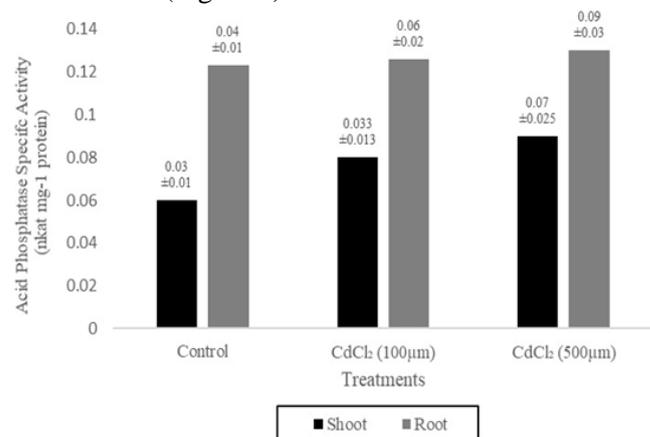
**Figure 4.** Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 μM) on CPX enzyme activity of 7-day-old wheat seedlings (DBW-187) variety of *Triticum aestivum* L. Data are expressed as mean values ± SE (n =5). Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .



**Figure 5.** Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 μM) on SOD enzyme activity of 7 days old wheat seedlings (DBW-187) variety of *Triticum aestivum* L. Data are expressed as mean values ± SE (n =5). Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .

### Acid phosphatase activity

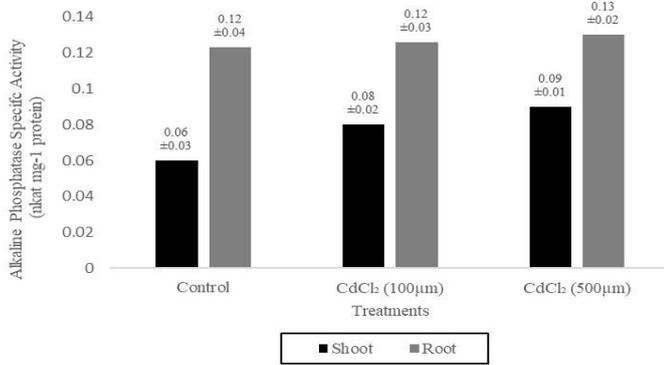
On seven-day-old wheat seedlings, the toxicity of Cadmium (Cd) showed the decreased activity of acid phosphatase in both shoot and root. The enzyme activity was raised by 9% and 57% in shoots at 100 μM and 500 μM of cadmium treatments, respectively. Meanwhile, in the roots, under the same concentrations of cadmium, about 33% and 55% increases in the activity of enzymes were recorded (Figure 6).



**Figure 6.** Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 μM) on Acid phosphatase enzyme activity (var. DBW-187) of 7 days old wheat seedlings *Triticum aestivum* L. Data are expressed as mean values ± SE (n =5). Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .

### Alkaline phosphatase activity

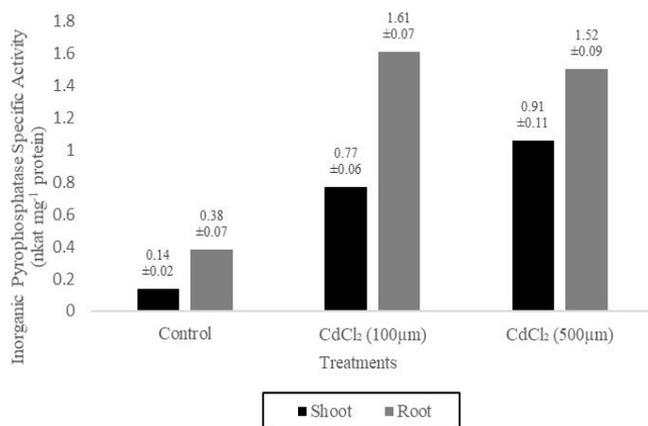
In this cultivar of wheat, very little increase in the activity of alkaline phosphatase was detected in response to 100 μM and 500 μM cadmium in roots, on average, which was 8 % over the untreated water control. While in shoots, the activity of alkaline phosphatase was increased by 25% and 33% at 100 μM and 500 μM treatments of Cadmium, respectively (Figure 7).



**Figure 7. Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 µM) on alkaline phosphatase enzyme activity (var DW-187) of 7-day-old wheat seedlings (*Triticum aestivum* L.). Data are expressed as mean values ± SE (n=5). Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .**

### Inorganic pyrophosphatase activity

In the seven-day-old wheat seedlings, inorganic pyrophosphatase activity was increased in both the shoot and root on exposure to Cadmium. On average, 151 % and 167 % increases in enzyme activity in root and shoot were recorded, respectively, compared to the untreated water control (Figure 8).



**Figure 8. Effect of different CdCl<sub>2</sub> concentrations (0, 100, 500 µM) on Inorganic pyrophosphatase enzyme activity (var DBW-187) of 7-day-old wheat seedlings (*Triticum aestivum* L.). Data are expressed as mean values ± SE (n=5). Values are not significantly different compared to only cadmium treatment  $P \leq 0.05$ .**

### Discussion

In modern studies, the application of different concentrations of Cadmium to wheat seedlings showed adverse consequences in the growth and metabolism of this plant. The growth of seven-day-old wheat seedlings gradually declined when they were exposed to Cadmium-induced stress. Cd is one of the most noxious, toxic, and harmful heavy metals that frequently become available in the climate (Sabella et al., 2022). Due to its toxic nature on living beings, it is not required for plant nutrition or

soil (Abbas et al., 2018). It causes remarkable changes in morphological and different biochemical parameters of wheat (*Triticum aestivum* L.), which was examined in the current study (Haider et al., 2021).

On exposure to Cadmium toxicity, the germination percentage was significantly affected. Due to the increasing concentration of Cadmium, the MGT, GI, and GR levels decreased. Germination-related parameters were hindered, particularly by increased cadmium stress (Karagöz and Dursun, 2020; Shiyu et al., 2020). The reduction in seed germination may be due to a decline in enzymatic activities, causing reduced or delayed seed germination, resulting in reduced production of ATP (Abbas et al., 2018; Almuwayh, 2021).

The exposure of Cadmium (Cd) significantly harmed wheat seedlings' development and normal growth. The rising concentrations of Cadmium (Cd) in different treatments (0, 100 and 500 µM) generally reduce both the root and shoot lengths. The rate of shoot length inhibition is weaker than root length inhibition. Under cadmium treatment of 500 µM, the roots were fully damaged with the browning of tissues. In response to the exposure of Cadmium (Cd), several workers have reported a reduction in the length of roots in different species of plants (de Araújo et al., 2017). The cadmium toxicity basically reduced the mitotic division of meristematic cells, resulting in the decrease of dry biomass and increased diameter of the root (Abbas et al., 2018).

Equivalent to the reduction in seedling growth, the activity of photosynthesis is very much affected by Cadmium (Cd) treatments in wheat seedlings. With the rising concentrations of Cadmium (Cd), both Chl-b and Chl-a contents in the leaves of wheat seedlings decreased along with the leaves of pale green colouration. On cadmium (Cd) exposure, the other necessary photosynthesis pigments, like xanthophyll and carotene, also decreased in wheat seedlings (Muradoglu et al., 2015). In the early work, increasing concentrations of arsenic (As) in rice leaf resulted in an alteration of the shape of chloroplast with the bending of concaving membrane and alter in accumulation along with the partial destruction and flow of assimilates leading to the decrease of chlorophyll contents (Liu et al., 2017). With the help of Chl-a, higher plants produce carbohydrates. So, the contents of Chl-a are directly linked with the production of carbohydrates. Therefore, it may be concluded that due to the toxicity of arsenic (As), the reduction of yield and growth might be partially reduced chlorophyll content in the leaf of rice (Głowacka et al., 2019).

Under normal conditions, wheat varieties showed the lowest enzymatic activity. The common antioxidant enzymes that prevent oxidative damage in plants are catalase (CAT), catechol peroxidase (CPX), ascorbic acid oxidase (AOX) and superoxide dismutase (SOD). CAT is an enzyme with a heme-containing tetramer that presents in the peroxisomes and converts  $H_2O$  and  $O_2$  from  $H_2O_2$ , a product containing high toxicity whose decomposition is very important (Abbas et al., 2018). Due to this ability, the enzyme catalase has maximum turnover rates compared to other enzymes (Bhusare et al., 2018).

In modern studies, inside cadmium (Cd) treated wheat seedlings, the increasing activity of SOD has been observed. Superoxide dismutase is one of the major superoxides ( $O_2^-$ ) scavenger and creates a first line of defence against the injury of the cells due to reactive superoxides is basically converted to  $H_2O_2$  (Gutiérrez-Martínez. et al., 2020). Excess hydrogen peroxide ( $H_2O_2$ ), which is a very highly reactive oxidizing agent, is scavenged by catalase (CAT). Catechol peroxidase (CPX) is an active oxygen-scavenging enzyme by which the oxidation of a reducing co-substrate is done with the degradation of hydrogen peroxide ( $H_2O_2$ ) (Xiao et al., 2021).

Ascorbic acid oxidase (AOX) is basically a copper (Cu) containing enzyme available in the fractions of cell wall and cytoplasm; in the presence of oxygen, AOX oxidizes ascorbic acid, producing dehydro-ascorbic acid and water. AOX becomes very active in protecting the cells of plants during Cd stress. It was concluded that very high levels of heavy metals damaged the integrity and structure of the cell wall and interfered with other associated activities of metabolism (Nyoni et al., 2020).

Alkaline phosphatase (EC.3.1.3.11) and Acid phosphatase (ortho-phosphoric monoester phosphohydrolases, EC.3.1.3.2) are a widespread enzymatic group that are widely distributed in plants, nonspecifically which catalyses hydrolysis of different types of phosphate ester in an alkaline and acidic environment (Ismael et al., 2019) which enable an adequate phosphorus (P) level maintained by the plants (Qayyum et al., 2017). Due to various metal toxicities, acid phosphatase activity in plants has been altered (Gubrelay et al., 2015). The requirement and toxicity of metallic ions for acid phosphatase activity were higher in the shoot than in the root.

In spite of that, under in vitro phosphate application, acid phosphatase activity was much reduced than in non-phosphate treatment. In the case of alkaline phosphatase, a variable activity behaviour has been noticed in growing wheat seedlings with increasing cadmium treatment.

Increased activity of alkaline phosphatase in the root, whereas in the shoot, a declining activity was observed in the present study. The enzyme Alkaline phosphatase is a very much inducible enzyme that synthesises the lower concentration of external Pi (Hossain et al., 2021).

Inorganic pyrophosphatase (EC.3.6.1.1) catalyses pyrophosphate synthesis and hydrolysis, which is a very simple compound with an energy-rich phosphor anhydride bond. In plant cells, it is very much essential for the regulation of various biochemical reactions (Shanmugaraj et al., 2019). In present studies, the inhibition of inorganic pyrophosphatase activity both in the shoot and root of Cadmium-stressed wheat seedlings was observed. From earlier studies, the inhibition of some metals like  $Co^{2+}$  and  $Cu^{2+}$  has been shown to cause pyrophosphatase activity (Hossain et al., 2021).

### Conclusion

Under hydroponic conditions, current findings demonstrated that all Cd concentrations (0, 100, 500  $\mu$ M) significantly impacted biomass, growth and photosynthetic machinery of the wheat seedlings. Increasing cadmium levels adversely affected the length of root and shoot, germination percentage, and biomass content of the tested wheat genotype. It is possible to conclude that carbohydrate content, total protein content, and photosynthetic activities of wheat plant species might be affected by specific concentrations of cadmium chloride ( $CdCl_2$ ). The openness of wheat (*Triticum aestivum* L.) to different groupings of  $CdCl_2$  brings about an expansion in toxicity and redox potential and soluble phosphatase action. Compared to the root and shoots of *Triticum aestivum* L., the roots contained more Cadmium than the shoots because of the direct uptake via a membrane transport channel. In soils contaminated with heavy metals, *Triticum aestivum* L. can be utilized as a heavy metal accumulator.

Recent studies showed phosphate's combating role in Cadmium toxicity by raising the cytoplasmic phosphate concentration. Higher phosphate concentrations are generally down-regulated with phosphate, cadmium plasma membrane transporter and vastly fight with cadmium chloride for the biochemical processes where cadmium is released for phosphate. Hence, phosphate-enriched fertilizers being used in soil contaminated with heavy metals can cause improvement in plant health condition and production, but phosphorus concentration in fertilizers might be very attentively examined and considered.

## Conflict of Interest

The authors assert that they have no conflicts of interest pertaining to the publishing of this work.

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