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Effect of Lead Stress on Morphological and Physiological Features of Wheat (*Triticum aestivum* L.) during Vegetative Stage

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ABSTRACT: The presence of lead (Pb) in agricultural soils caused by human activities has sparked concerns about its harmful effects on plant species. This research aimed to explore how lead affects different physiological factors in wheat (*Triticum aestivum* L.). Wheat seeds were germinated under controlled conditions in petri plates and exposed to varying concentrations (0, 50, 100, 150, 200, 250, and 500 µM) of $Pb(NO_3)_2$. As the Pb concentration increased, there was a gradual decrease in germination percentage, fresh and dry weights, shoot and root lengths, as well as chlorophyll and carotenoid levels compared to the control group. This decline in growth and pigment content is attributed to peroxidative processes triggered by Pb toxicity in the root system. Enzyme analyses showed that exposure to 500 µM Pb resulted in reduced catalase (CAT) and peroxidase (POD) activities, while superoxide dismutase (SOD) activity increased in both shoot and root tissues of wheat seedlings. In conclusion, this study highlights the negative impact of lead on various physiological aspects of wheat growth, including germination, growth inhibition, reduction in pigment content, and disruptions in enzyme activity. These findings underscore the need for effective strategies to alleviate lead-induced stress in agro ecosystems.

Keywords: Chlorophyll, Lead, Catalase, Peroxidase, Superoxide dismutase.

INTRODUCTION

Heavy metal pollution has emerged as a significant global environmental challenge. In the context of soil contamination, zinc (Zn) and cadmium (Cd) also coexist alongside lead (Pb), compounding the issue (Hernandez-Allica *et al.,* 2007). The presence of lead has profound adverse effects on both plant and animal systems. While natural sources contribute to the distribution of Pb in soil, aquatic ecosystems, and the atmosphere, human activities predominantly contribute to environmental Pb pollution. These activities encompass mining, smelting operations, the use of Pb-containing pesticides, paints, fertilizers, and gasoline, as well as the utilization and disposal of Pb-acid batteries, munitions, fusible alloys, and sewage sludge containing Pb (Sharma and Dubey 2005). Industrial areas often show elevated Pb concentrations in cultivated soils, leading to significant reductions in agricultural productivity. Among various heavy metals, lead is the second most detrimental pollutant after arsenic (Pourrut *et al*., 2011). Lead toxicity triggers a series of morpho-physiological and biochemical changes within plant systems, affecting cellular components and organ-level functions. This toxicity results in considerable decreases in seed germination rates, fresh and dry shoot and root biomass, impaired plant growth, and chlorosis. Furthermore, lead's adverse effects extend to cellular division and photosynthetic activities, including a decrease in chlorophyll production due to the hindered absorption of essential nutrients like magnesium (Mg) and iron (Fe) by plants (Pourrut *et al*., 2011). Elevated lead concentrations are linked to cellular apoptosis (Seregin and Ivanov 2001). The impact of lead toxicity on plants varies based on factors such as plant species, lead concentration and soil properties. Notably, the presence of lead stimulates the generation of reactive oxygen species (ROS) and increases the activity of antioxidant enzymes in plants (Mishra *et al*., 2006). Excessive ROS production leads to harmful effects on plant cells, including the inhibition of photosynthetic activity, disruption of ATP synthesis, and DNA damage. The balance between ROS production and the activity of antioxidative enzymes determines the extent of ROS-induced stress in plants facing challenges from heavy metals. Several plant species, including *Triticum aestivum* L. (Ekmekci *et al*., 2009), *Oryza sativa* (Verma and Dubey 2003) and *Brassica juncea* (Zaier *et al*., 2010), have been studied to understand the consequences of lead-induced stress. Hydroponic cultivation methods enable efficient observation and rapid assessment of relative growth and toxicity (Zhivovtovksy *et al*., 2011). However, the harmful effects of soil Pb contamination extend to wheat yield, quality, and overall production. This study aims to clarify the toxicological effects of Pb exposure on various parameters, including germination percentage, fresh and dry weights, root and shoot lengths, total chlorophyll and carotenoid content, as well as the

activities of catalase, peroxidase, and superoxide dismutase.

MATERIALS AND METHODS

Viable wheat (*Triticum aestivum* L.) seeds, of the PBW 343 variety, were procured from the Krishi Vigyan Kendra (KVK) Nawada, located in the Bihar region. To ensure sterility, the seeds underwent a surface sterilization process involving exposure to a 0.1% sodium hypochlorite solution for duration of 10 minutes. Subsequently, the sterilized seeds were rinsed under a continuous flow of tap water and subjected to a thorough rinse with distilled water, repeated 5 to 6 times. These treated seeds were then germinated on petri plates, with each plate containing seeds exposed to different lead (Pb) concentrations, specifically 0, 50, 100, 150, 200, 250, and 500µM. As a point of reference, control groups were established by dampening filter papers with 10 milliliters of deionised water. All experiments and analyses were conducted utilizing seedlings that had reached a developmental age of 5 days subsequent to germination.

A. Shoot and root length

Shoot and root lengths were quantified subsequent to the application of distinct concentrations of lead nitrate $Pb(NO_3)_2$, encompassing 0, 25, 50, 100, 200, 300, and 500 µM. In this investigation, a total of five randomly chosen seedlings from both the control and treated groups were subjected to analysis. This measurement process was conducted in triplicate for enhanced accuracy and reliability. The assessment of shoot length encompassed the measurement from the base of the culm to the tip of the germinated seedling. Simultaneously, root length was gauged from the junction where the shoot and root interconnect in germinated wheat seedlings. Utilizing a meter scale, the aforementioned measurements were executed. To derive a comprehensive representation, the average length of the five seedlings was computed on a per-plant basis. This value, expressed in centimetres, signifies the cumulative length of both the shoot and root segments of the germinated wheat seedlings.

B. Fresh and Dry weight of shoot and root

Before conducting fresh weight measurements, the seedlings underwent a preliminary cleansing step using deionised water, followed by gentle blotting on Whatman filter paper to remove excess moisture. Subsequently, the biomass was partitioned into distinct root and shoot segments. The measurement of plant fresh weight (FW) for both the shoot and root components was promptly executed utilizing a precision weighing balance. In the pursuit of obtaining dry weight (DW) values, plant samples were subjected to a desiccation process. The desiccation was carried out in a hot air oven at a temperature of 70°C for duration of 48 hours. This controlled drying procedure ensured the removal of all moisture content from the plant samples, ultimately yielding the dry weight measurements.

C. Determination of chlorophyll and carotenoid contents

The quantification of chlorophyll content was executed following the methodology outlined by Wellburn in 1994. Initially, 0.5 grams of leaves, derived from both control and Pb-treated plants, were dissected into minute fragments. These leaf fragments were subjected to an incubation process within 5 milliliters of dimethyl sulfoxide (DMF), the entire setup being covered with aluminium foil to prevent light exposure. The incubation was conducted in darkness at a temperature of 4°C for duration of 24 hours. This ensured the extraction of pigments in an optimal manner. Following the 24-hour dark incubation, the leaf fragments were effectively extracted to retrieve any remaining pigments. The quantification of Chl a, Chl b, and carotenoid contents was carried out through absorbance measurements at wavelengths of 664 nm, 647 nm, and 480 nm, respectively. This absorption analysis was performed utilizing a Perkin-Elmer double-beam spectrophotometer with ultraviolet-visible (UV-Vis) capabilities. The absorbance measurements were made against a background of DMF, which served as the blank reference. Chlorophyll-a, chlorophyll-b, and carotenoid contents were then quantified by using the experimental equations provided by (Wellburn, 1994) . The quantified pigment content was expressed in units of micrograms per gram of fresh weight (µg g^{-1} FW), providing a reliable representation of the pigmentation levels in the plant samples.

$Chla = 11.65OD_{664} - 2.69OD_{647}$

$Chlb = 20.81OD_{647} - 4.53OD_{664}$

$Car = (1000 \times OD_{480} - 0.89 \times chla – 52.02\ chlb)/245$

D. Extraction of enzymes

Fresh shoot and root tissues, weighing 100 milligrams each, were subjected to homogenization using a pre-chilled mortar and pestle. This homogenization process occurred under cold conditions to preserve the integrity of the samples. The homogenization was carried out using 5.0 millilitres of extraction buffer, composed of 50 millimolar (mM) phosphate buffer at a pH of 7.5, and supplemented with 1 millimolar (mM) ethylenediaminetetraacetic acid (EDTA).Following homogenization, the resultant mixture centrifuged at a speed of 15,000 rpm for a 15 minutes. This centrifugal step facilitated the separation of components within the homogenate, yielding a supernatant and a pellet. The supernatant, which contained the soluble cellular components of interest, was subsequently employed for the quantification of enzyme activities. Specifically, the activities of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) were assessed using the obtained supernatant. These enzymatic activities offer insights into the antioxidative responses and stress mitigation mechanisms present within the plant tissues.

E. Estimation of Catalase activity

Catalase activity was determined in accordance with the methodology established by (Chance and Maehly 1955). The assessment of catalase activity involved monitoring the reduction in absorbance caused by the breakdown of hydrogen peroxide (H_2O_2) at a wavelength of 240 nanometers (nm). To conduct this assay, a 300 microliter (µl) volume of the enzyme

extract was mixed with 2.2 millilitres (ml) of phosphate buffer at a pH of 6.8, resulting in a final volume of 2.5 ml. The reaction was initiated by the addition of 300 µl of hydrogen peroxide (H_2O_2). The consumption of H_2O_2 was tracked over a 3-minute period, with measurements taken at 1-minute intervals. The reduction in absorbance at 240 nm was observed, reflecting the breakdown of H_2O_2. For accurate and reproducible outcomes, the initial absorbance at 240 nm should be within the range of 0.450 to 0.500, and a decrease in absorbance should be evident upon the addition of the enzyme extract. The extinction coefficient for H_2O_2 was established as 0.0394 millimolar per centimeter ($mM^{-1}cm^{-1}$). The catalase activity was quantified in terms of micromoles of H_2O_2 decomposed per minute per gram of fresh weight (µmoles of H_2O_2 decomposed $min^{-1}g^{-1}$ FW), thereby providing a standardized metric for the catalytic capacity of the enzyme.

F. Estimation of Peroxidase (POD) activity

Peroxidase enzymes facilitate the decomposition of H_2O_2 by utilizing electron acceptors like ascorbate, quinones, and cytochrome C. Their function involves the oxidation of H_2O_2 while concurrently interacting with various electron donors. This electron transfer activity is not confined to a single type of electron donor (Shannon *et al*., 1966). To assess peroxidase activity, a reaction mixture was prepared. This mixture consisted of 500 microliters (µl) of 50 millimolar (mM) guaiacol within a 0.1 M phosphate buffer at a pH of 6.5. Additionally, 200 µl of the enzyme extract and 300 µl of an 800 mM H_2O_2 solution were incorporated into the mixture. A parallel reaction mixture devoid of H_2O_2 served as the blank reference. The commencement of the reaction occurred upon the addition of H_2O_2 to the reaction mixture. Alterations in absorbance were monitored at a wavelength of 470 nanometers (nm) over a 3-minute time frame, with measurements captured at 1-minute intervals. The peroxidase activity was quantified based on the change in absorbance per unit time and expressed as change in absorbance per minute per gram of fresh weight (change in absorbance $min^{-1}g^{-1}$ FW). This metric provided a measure of the peroxidase enzyme's catalytic performance.

G. Estimation of Superoxide dismutase (SOD) activity

The assessment of superoxide dismutase (SOD) activity was performed following the methodology outlined by (Marklund and Marklund 1974). SOD is responsible for the catalysis of superoxide anion disproportionation, resulting in the formation of hydrogen peroxide (H_2O_2) and molecular oxygen. The experimental procedure involved the creation of a reaction mixture containing specific components. To this end, 2.5 milliliters (ml) of 100 millimolar (mM) Tris HCl buffer (pH 8.2), 100 µl of 6 mM ethylenediaminetetraacetic acid (EDTA), 300 µl of 6 mM pyrogallol solution, and 100 µl of the enzyme extract were combined. The measurement of SOD activity was initiated upon mixing these components. During the assay, changes in absorbance were monitored at a wavelength of 420 nanometers (nm) using a spectrophotometer. This monitoring was conducted over a 3-minute period, with absorbance measurements recorded at 1-minute intervals. The SOD activity was quantified based on the principle that one unit of SOD causes a 50% inhibition of the auto-oxidation of pyrogallol, as observed in the blank reference. The unit of SOD activity was expressed as (unit min^{-1} g^{-1} FW), providing an indicator of the enzyme's catalytic efficiency in inhibiting superoxide anion-mediated reactions.

H. Statistical analysis

The entire experimental procedure was carried out in triplicate, ensuring the acquisition of reliable and representative data. The calculated values were then presented as the mean ± standard error (SE), offering a measure of the central tendency of the data along with an indication of its variability. For statistical analysis, an analysis of variance (ANOVA) was performed utilizing the GraphPad Prism software, version 5.01, developed by GraphPad Software Inc., located in La Jolla, California, USA. The application of ANOVA allowed for the evaluation of significant variations among the experimental groups. Subsequent to the ANOVA, significant differences were denoted by distinct alphabetical letters ($p \leq 0.05$), signifying the presence of statistically significant disparities between specific groups. This practice of differentiating letters helped to clearly communicate the outcomes of the statistical analysis and signify significant distinctions in the experimental results.

RESULTS

A. Effect of Pb on germination and plant growth

The study investigated the influence of escalating lead (Pb) concentrations in the nutrient medium on germination percentage. Notably, higher levels of lead nitrate led to a visible decrease in the germination percentage. Specifically, treatment with 100 µM Pb resulted in an 18.07% reduction in seed germination rate. This reduction became more prominent with 500 µM Pb treatment, causing a significant 56.62% decrease in germination rate compared to the control. Additionally, the lengths of both shoot and root were evaluated five days after initiating treatment. It was observed that as the concentration of lead nitrate increased, both shoot and root lengths gradually declined. Remarkably, treatment with 100 µM Pb led to a 37.64% decrease in shoot length and a 46.36% decrease in root length. In contrast, treatment with 500 µM Pb brought about more substantial reductions, resulting in a 64.11% decrease in shoot length and a 68.59% decrease in root length compared to the control (Fig. 1). The assessment of fresh weight and dry weight of germinated seedlings followed a similar pattern. Treatment with 100 µM Pb led to a reduction of 34.78% in shoot fresh weight and 38.09% in root fresh weight. However, treatment with 500 µM Pb resulted in even more significant reductions, with a 64.78% decrease in shoot fresh weight and a 63.66% decrease in root fresh weight compared to the control (Fig. 1). This trend persisted in the measurements of dry weight. At 100 µM Pb treatment, reductions of 41.64% in shoot dry weight and 51.06% in root dry weight were

observed. With 500 µM Pb treatment, reductions in shoot dry weight and root dry weight were 64.43% and 56.38%, respectively, compared to the control (Fig. 1). Taken together, these findings underscore that the presence of lead in the nutrient medium leads to a significant decrease in germination percentage, shoot and root lengths, as well as fresh and dry weights of germinated seedlings. Higher concentrations of lead amplify these negative effects, highlighting the plant system's sensitivity to increasing lead levels.

B. Photosynthetic Parameters chlorophyll and carotenoid

Chlorophyll content serves as a valuable indicator of heavy metal toxicity within plant systems. In this study, we examined the impact of lead (Pb) on various photosynthetic parameters, including chlorophyll-a (chl a), chlorophyll-b (chl b), total chlorophyll, and carotenoid content, in wheat seedlings cultivated under different lead concentrations. Our investigation revealed notable reductions in photosynthetic parameters across all Pb-treated plants. Specifically, significant reductions were observed in both chl a and chl b content. At a concentration of 100 µM Pb, chl a content declined by 30.59%, and chl b content by 31.01%, compared to the control. The reductions became even more pronounced with higher Pb concentrations, with chl a content decreasing by 63.99% and chl b content by 68.69% in leaves of seedlings treated with 500 µM Pb, as compared to the control (Fig. 2). Furthermore, the comprehensive assessment of photosynthetic pigments indicated that total chlorophyll and carotenoid content also experienced substantial decreases. At 100 µM Pb treatment, there were reductions of 30.76% in total chlorophyll content and 25.31% in carotenoid content, compared to the control. These reductions intensified with increasing Pb concentration, resulting in total chlorophyll content decreasing by 64.81% and carotenoid content decreasing by 74.72% in leaves of seedlings treated with 500 µM Pb, in comparison to the control. Collectively, these findings underscore the sensitivity of photosynthetic parameters to lead toxicity in wheat seedlings. The reduction in chlorophyll content, including chl a, chl b, total chlorophyll, and carotenoids, indicates the disruptive impact of Pb on the plant's photosynthetic processes, thus highlighting chlorophyll content as an effective biomarker for heavy metal stress in plants.

C. Effect of Pb on Catalases (CAT), Peroxidase (POD) and Superoxide dismutases (SOD)

Upon exposure to lead (Pb), there was a noticeable increase in the activity of scavenging enzymes, specifically catalase (CAT) and superoxide dismutase (SOD), in both root and shoot tissues (Fig. 3). The impact of Pb on catalase activity is demonstrated in Figure 3. A treatment of 100 µM Pb led to a reduction of 23.98% in CAT activity in the shoot and 29.29% in the root. Meanwhile, treatment with 500 µM Pb resulted in even more substantial reductions, with a decrease of 52.02% in shoot CAT activity and 60.1% in root CAT activity compared to the control (Fig. 3). Contrastingly, the activity of peroxidase (POD) exhibited a significant decrease as the Pb concentration increased in wheat seedlings (Fig. 3). Treatment with 100 µM Pb led to a decrease of 23.62% in shoot POD activity and 30.23% in root POD activity. The effects were more pronounced at a higher Pb concentration, with 500 µM Pb treatments leading to a reduction of 66.87% in shoot POD activity and 72.86% in root POD activity compared to the control (Fig. 3). The activity of superoxide dismutase (SOD) displayed an opposite trend, with a significant increase in response to higher Pb concentrations in wheat seedlings (Fig. 3). Treatment with 100 µM Pb resulted in an increase of 49.18% in shoot SOD activity and 26.43% in root SOD activity. At 500 µM Pb treatment, the increase was even more prominent, with a rise of 121.3% in shoot SOD activity and 68.03% in root SOD activity compared to the control (Fig. 3). These observations collectively indicate the activation of antioxidant defense mechanisms in response to lead-induced oxidative stress. The enhanced activities of CAT and SOD, along with the decrease in POD activity, highlight the intricate interplay of enzymatic responses that plants employ to counteract the detrimental effects of heavy metal toxicity, specifically lead stress.

DISCUSSION

Lead (Pb) stands out as one of the most phytotoxic metals present in agricultural soils, with its concentration continually rising due to human activities. This study aimed to explore the impact of lead nitrate $Pb(NO_3)_2$ on the germination of wheat seedlings. Similar to previous research by Mesmar and Jaber (1991); Lamhamdi *et al*., (2011); Kumar *et al*., (2018). We observed a decrease in germination percentage as a result of Pb exposure. Higher concentrations of lead were found to infiltrate the embryo during imbibitions, causing a delay in germination in wheat seedlings. This phytotoxicity of lead is attributed to damage to the light harvesting centre, inhibition of enzyme activity, and interference with photosynthetic pigments. Our findings indicate that Pb treatment at 100 µM resulted in an 18.07% reduction in seed germination, while treatment with 500 µM Pb led to a more significant decrease of 57.69%. The growth of wheat seedlings, as measured by plant length, was detrimentally affected by Pb. Notably, both shoot and root lengths were significantly reduced in Pb-treated seedlings compared to the control (Fig. 1). Treatment with 100 µM Pb caused a reduction of 37.64% in shoot length and 46.36% in root length, while treatment with 500 µM Pb led to more pronounced decreases of 64.11% in shoot length and 68.59% in root length (Fig. 1). This observed pattern aligns with the findings of Abdul (2010); Kaur *et al*., (2012), whose studies on maize and wheat respectively, under Pb-contaminated conditions, also indicated reductions in root growth. The deleterious impact of Pb was further evident in terms of both fresh and dry weights of germinated seedlings, with higher Pb concentrations corresponding to gradual declines. Notably, the most substantial decreases were observed

at the 500 µM Pb concentration. Comparable outcomes were reported by (Mesmar and Jaber 1991; Lamhamdi *et al*., 2011) in studies involving wheat seedlings subjected to lead stress.

Moreover, the levels of photosynthetic pigments, encompassing total chlorophyll and carotenoid content, emerged as sensitive indicators of lead toxicity in plants. Our investigation revealed a reduction in total chlorophyll and carotenoid content at the 500 µM Pb treatment when compared to the control group. Similar trends were noted by (Yang *et al*., 2011; Kaur *et al*., 2015; Kumar *et al*., (2018). Mobin and Khan, 2007) in their research involving wheat and *Brassica juncea* exposed to Pb and cadmium stress respectively.

The study also unveiled that Pb-induced stress led to a significant decrease in the activity of catalase (CAT) and peroxidase (POD) enzymes in germinated wheat seedlings. Furthermore, variations were detected in the defense mechanisms, particularly in CAT, POD, and superoxide dismutase (SOD) activities. Reduced CAT activity suggested its limited protective role against Pb toxicity in wheat roots, a finding consistent with the observations of Kaur *et al*. (2012) in wheat and Verma and Dubey (2003) in rice. Conversely, the upregulation of POD activity is a common response of higher plants to exposure to toxic metals. Our research noted increased POD activity at the 500 µM Pb concentrations, aligning with the observations of (Van Assche *et al*., 1988; Chakravarty and Srivastava 1997) across various species exposed to toxic Pb levels. Furthermore, SOD activity exhibited an increase in response to Pb exposure in wheat seedlings. The role of this enzyme in defending against reactive oxygen species (ROS) was corroborated by the findings of (Nareshkumar *et al*., 2015) emphasizing its significance in mitigating oxidative stress.

In conclusion, this study underscores the adverse effects of Pb on diverse aspects of wheat seedling growth and physiology, including germination, seedling length, weight changes, and alterations in photosynthetic pigments and enzymatic activities related to antioxidant defense mechanisms. The results underscore the susceptibility of wheat seedlings to lead toxicity and offer insights into the intricate responses plants employ to counter the detrimental consequences of heavy metal stress.

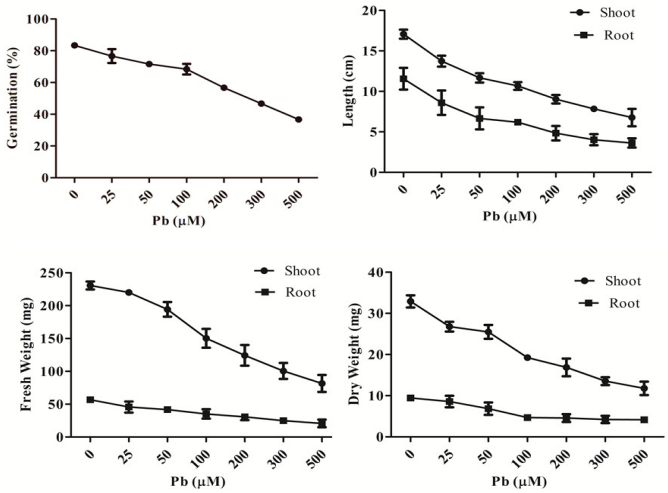

Fig. 1. Effect of different concentrations of Pb on germination percentage, shoot and root length, fresh and dry weight of germinated wheat seedling. Data represent the mean (±SE) was calculated from three replications for each treatment. All values are significantly different at p≤0.05 applying post hoc Tukey's test.

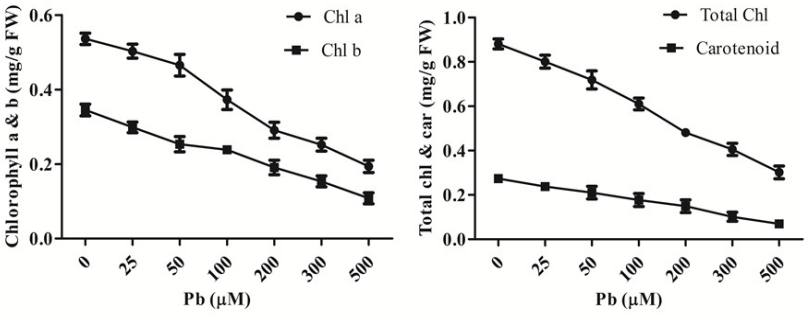

Fig. 2. Effect of different concentration of Pb on chlorophyll a & b, total chlorophyll and carotenoid content of germinated wheat seedling. Data represent the mean (±SE) was calculated from three replications for each treatment. All values are significantly different at p≤0.05 applying post hoc Tukey's test.

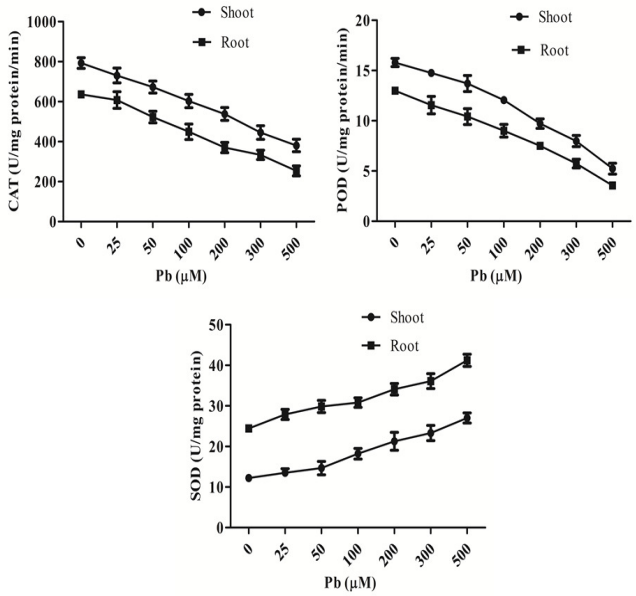

Fig. 3. The impact of varying concentrations of Pb on the enzymatic activities of CAT (catalase), POD (peroxidase), and SOD (superoxide dismutase) in germinated wheat seedlings was examined. The data represent the mean values (± standard error, SE) obtained from three separate replications for each treatment. All recorded values exhibited statistically significant differences at the $p \leq 0.05$ level, as determined by applying the post hoc Tukey's test.

CONCLUSION AND FUTURE SCOPE

The current study has revealed that subjecting wheat seedlings to varying lead treatments results in a range of metabolic disruptions. These include reductions in germination rate, plant length, biomass, and photosynthetic pigment content, all indicative of the presence of lead-induced toxicity in the seedlings. In response to this toxicity, there was an observed increase in the activities of antioxidant enzymes, accumulation. These responses appear to serve as protective mechanisms against the harmful effects of lead exposure. Notably, the root system of the seedlings demonstrated greater vulnerability to lead toxicity compared to the shoot. Given the insights gained from this research, future experiments could be directed towards identifying specific molecules or compounds with the potential to mitigate the toxic impact of lead. By exploring novel strategies or substances that can counteract the detrimental effects of lead, researchers could contribute to the development of effective measures for safeguarding plants against heavy metal toxicity. These investigations could offer valuable solutions for managing the challenges posed by lead pollution and its adverse effects on plant systems.

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Conflict of Interest. None.

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