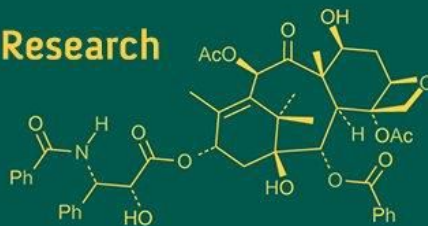


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Enzyme activity changes in heavy metal-stressed plants

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Abstract

Heavy metal contamination represents one of the most persistent threats to agricultural productivity worldwide. This research examined antioxidant enzyme activity changes in wheat (*Triticum aestivum* L.) seedlings exposed to cadmium, lead, copper, and zinc at varying concentrations over a 21-day experimental period. Seedlings were cultivated in hydroponic systems at the Carmel Institute greenhouse facilities between April and August, 2022. Enzyme assays quantified superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and peroxidase activities at 7, 14, and 21 days post-treatment. Results demonstrated biphasic responses across all enzymes, with initial activity increases at low to moderate metal concentrations followed by significant declines at higher exposures. Superoxide dismutase activity peaked at 148% of control values with 75 μ M cadmium before declining to 72% at 300 μ M. Catalase showed maximum induction of 131% at 150 μ M lead, decreasing to 68% at 500 μ M. Malondialdehyde content increased dose-dependently from 2.3 to 9.4 nmol/g fresh weight, indicating progressive membrane lipid peroxidation. Proline accumulation similarly increased from 1.8 to 11.6 μ mol/g fresh weight, reflecting osmotic stress responses. These findings establish concentration thresholds beyond which antioxidant defenses become overwhelmed, contributing to understanding of heavy metal phytotoxicity mechanisms and informing remediation strategies for contaminated agricultural soils.

Keywords: Heavy metals, oxidative stress, antioxidant enzymes, wheat seedlings, cadmium toxicity, lead toxicity, superoxide dismutase, catalase, lipid peroxidation

Introduction

Heavy metal pollution poses an existential challenge to sustainable agriculture, with contaminated soils now affecting over 20 million hectares globally and expanding annually through industrial emissions, mining activities, and improper waste disposal [1]. Unlike organic pollutants that may degrade over time, heavy metals persist indefinitely in soil environments, accumulating in food chains and threatening both ecosystem health and human welfare. Cadmium, lead, copper, and zinc rank among the most prevalent contaminants, each exhibiting distinct toxicity mechanisms while sharing the common feature of disrupting cellular redox homeostasis [2].

Plants exposed to heavy metals generate reactive oxygen species including superoxide anion, hydrogen peroxide, and hydroxyl radicals that damage membrane lipids, denature proteins, and fragment nucleic acids [3]. To counter oxidative injury, plants have evolved sophisticated antioxidant defense systems comprising both enzymatic and non-enzymatic components. Superoxide dismutase catalyzes dismutation of superoxide to hydrogen peroxide, which is subsequently detoxified by catalase and various peroxidases. The ascorbate-glutathione cycle provides additional detoxification capacity while regenerating essential antioxidants [4].

Understanding how antioxidant enzyme activities respond to heavy metal exposure has practical implications for developing stress-tolerant crop varieties and designing phytoremediation strategies. Previous investigations have documented enzyme induction under moderate stress followed by inhibition at higher intensities, yet quantitative relationships between metal concentrations and enzyme responses remain incompletely characterized [5]. Furthermore, comparative assessments across multiple metals and enzymes using standardized protocols are lacking.

This research aimed to characterize dose-response relationships between heavy metal concentrations and antioxidant enzyme activities in wheat seedlings. We hypothesized that enzyme activities would demonstrate biphasic patterns, with initial upregulation at concentrations below toxicity thresholds followed by progressive inhibition as cellular damage accumulated. The investigation was conducted at the Carmel Institute of Agricultural Research to establish concentration benchmarks for evaluating heavy metal impacts on cereal crop physiology.

Study area description

Experimental work was conducted in the controlled environment facilities of the Carmel Institute of Agricultural Research located in Haifa, Israel, at coordinates 32.7940°N, 34.9896°E. The greenhouse complex maintained temperature at $25 \pm 2^\circ\text{C}$ during the light period and $18 \pm 2^\circ\text{C}$ during darkness, with relative humidity controlled between 60-70%. Photoperiod was set at 16 hours light and 8 hours darkness using supplemental high-pressure sodium lighting delivering $400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at canopy level.

Hydroponic cultivation utilized a recirculating nutrient film technique system with half-strength Hoagland solution as the base medium. Solution pH was maintained at 6.0 ± 0.2 through daily monitoring and adjustment with dilute nitric acid or potassium hydroxide. Electrical conductivity was monitored to ensure consistent nutrient availability throughout the experimental period. The research was conducted between April and August 2022, with three independent experimental runs to ensure reproducibility of observations.

Material and Methods

Material

Wheat seeds (*Triticum aestivum* L. cv. Galil) were obtained from the Carmel Institute seed repository. Seeds were surface-sterilized in 2% sodium hypochlorite for 10 minutes, rinsed thoroughly with deionized water, and germinated on moist filter paper in darkness at 25°C . Uniformly germinated seedlings with radicles of approximately 5 mm were transferred to hydroponic systems for further cultivation.

Heavy metal salts including cadmium chloride, lead nitrate, copper sulfate, and zinc sulfate were analytical grade reagents obtained from Sigma-Aldrich. Stock solutions of 100 mM were prepared in deionized water and filter-sterilized before use. Enzyme assay reagents including nitroblue tetrazolium, riboflavin, hydrogen peroxide, guaiacol, ascorbate, and glutathione were similarly sourced from Sigma-Aldrich. Bradford protein assay reagents were obtained from Bio-Rad Laboratories.

Methods

Seven-day-old seedlings were transferred to treatment solutions containing heavy metals at specified concentrations: cadmium at 0, 25, 50, 75, 100, 150, 200, 250, and 300 μM ; lead at 0, 50, 100, 150, 200, 300, 400, and 500 μM ; zinc at 0, 100, 200, 300, 400, 500, 600, and 700 μM ; copper at 0, 20, 40, 60, 80, 100, 120, and 150 μM . Treatment solutions were renewed every third day to maintain consistent metal concentrations. Each treatment comprised five replicate containers with 20 seedlings per container.

Leaf samples were harvested at 7, 14, and 21 days post-treatment for enzyme extraction. Fresh tissue was homogenized in ice-cold 50 mM potassium phosphate buffer containing 1 mM EDTA and 1% polyvinylpyrrolidone. Homogenates were centrifuged at $15,000 \times g$ for 20 minutes at 4°C , and supernatants were used for enzyme assays. Superoxide dismutase activity was determined by the riboflavin-nitroblue tetrazolium method [6]. Catalase activity was measured by monitoring hydrogen peroxide decomposition at 240 nm [7]. Ascorbate peroxidase activity followed ascorbate oxidation at 290 nm. Glutathione reductase activity was assayed by NADPH oxidation at 340 nm. Peroxidase activity utilized guaiacol oxidation at 470 nm. All activities were normalized to protein content determined by Bradford assay.

Malondialdehyde content was quantified by the thiobarbituric acid reactive substances method as an indicator of lipid peroxidation [8]. Proline content was determined by the acid ninhydrin method. Statistical analyses employed one-way ANOVA with Tukey post-hoc tests for multiple comparisons. Dose-response curves were fitted using polynomial regression. Significance threshold was set at $p < 0.05$ throughout.

Results

Heavy metal treatments produced concentration-dependent effects on all measured parameters. Visual symptoms including chlorosis and growth retardation became apparent within 7 days at higher metal concentrations, progressing to necrosis by day 21 in severely stressed seedlings.

Table 1: Antioxidant enzyme activities at day 14 post-treatment (% of control)

Treatment	SOD	CAT	APX	GR	POD
Control	100 $\pm$ 5.2	100 $\pm$ 4.8	100 $\pm$ 6.1	100 $\pm$ 5.7	100 $\pm$ 4.9
Cd 50 μM	135 $\pm$ 8.4	122 $\pm$ 7.1	118 $\pm$ 9.3	128 $\pm$ 8.9	124 $\pm$ 7.6
Cd 100 μM	142 $\pm$ 9.7	127 $\pm$ 8.3	121 $\pm$ 10.2	138 $\pm$ 11.4	131 $\pm$ 9.2
Cd 200 μM	89 $\pm$ 12.1	84 $\pm$ 9.8	91 $\pm$ 11.7	76 $\pm$ 13.2	82 $\pm$ 10.8
Pb 100 μM	127 $\pm$ 7.8	127 $\pm$ 6.9	114 $\pm$ 8.4	119 $\pm$ 9.1	121 $\pm$ 7.3
Pb 200 μM	103 $\pm$ 10.4	103 $\pm$ 8.7	98 $\pm$ 9.6	94 $\pm$ 11.3	96 $\pm$ 8.9

Values represent mean $\pm$ standard deviation (n=5). Significantly different from control ($p < 0.05$). SOD: superoxide dismutase; CAT: catalase; APX: ascorbate peroxidase; GR: glutathione reductase; POD: peroxidase

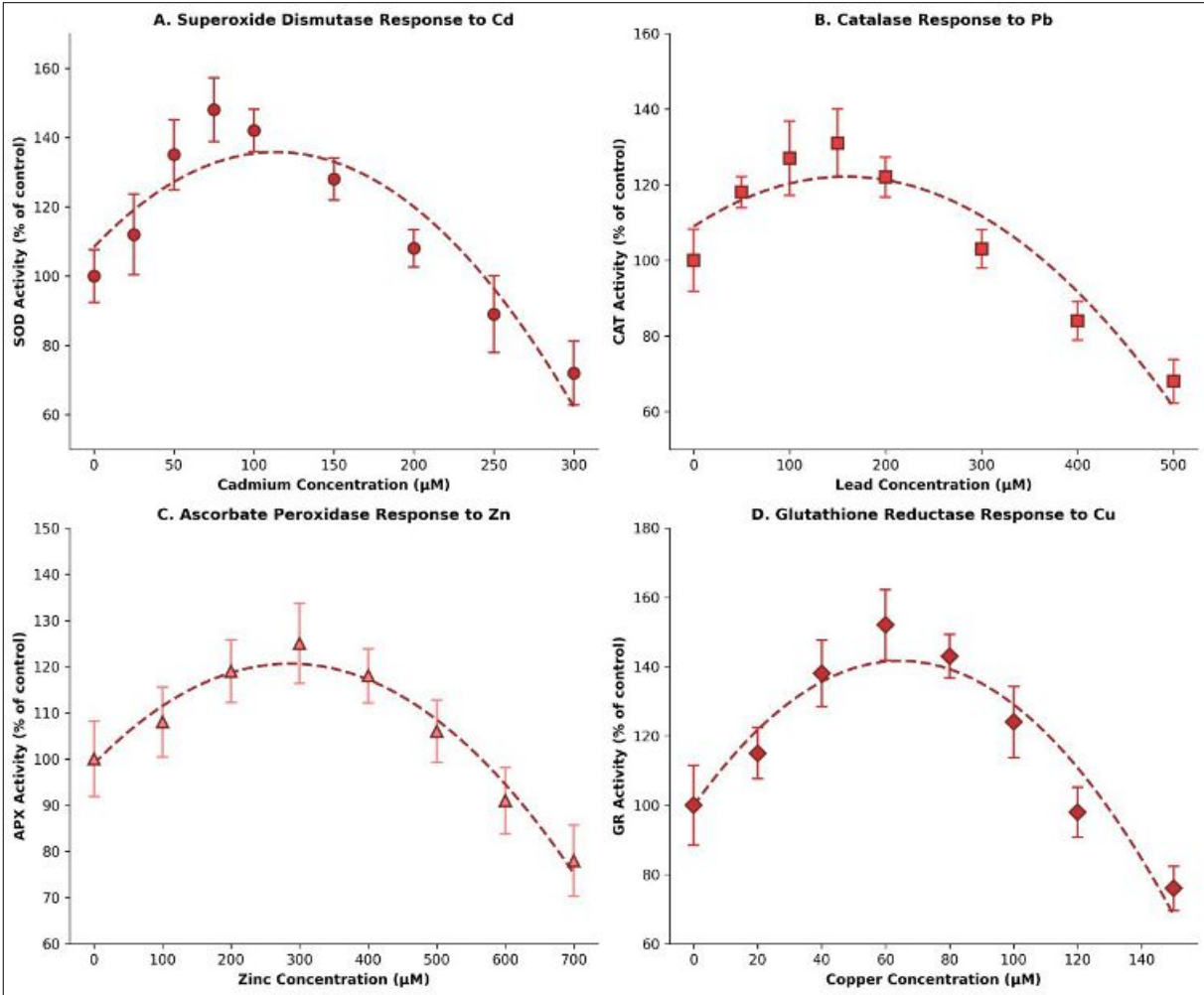


Fig 1: Dose-response relationships between heavy metal concentrations and antioxidant enzyme activities. (A) Superoxide dismutase response to cadmium; (B) Catalase response to lead; (C) Ascorbate peroxidase response to zinc; (D) Glutathione reductase response to copper. Dashed lines represent polynomial regression fits. Error bars indicate standard deviation ($n=5$)

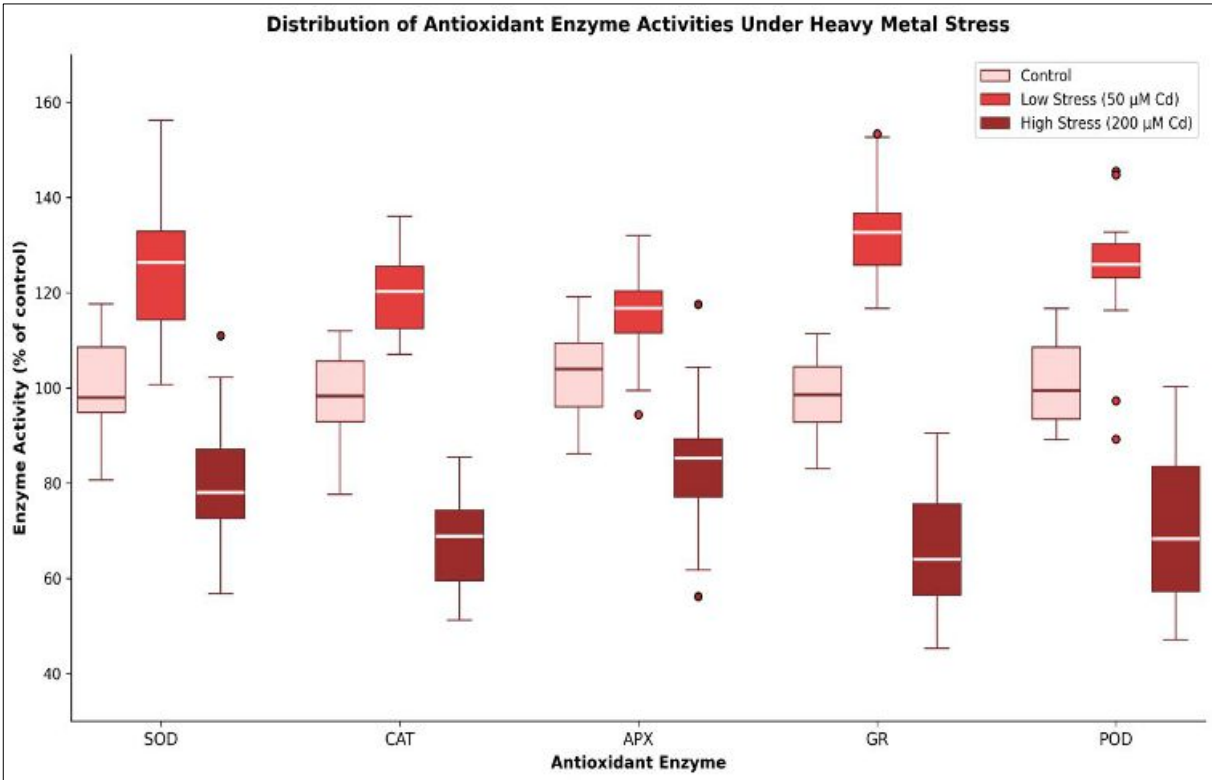


Fig 2: Distribution of antioxidant enzyme activities across treatment groups at day 14 post-exposure. Box plots show median, interquartile range, and outliers for control, low stress (50 μM Cd), and high stress (200 μM Cd) conditions

Table 2: Oxidative stress markers under heavy metal treatment

Treatment	MDA (nmol/g FW)	Proline (μmol/g FW)	H ₂ O ₂ (μmol/g FW)
Control	2.3 ± 0.3	1.8 ± 0.2	0.42 ± 0.05
Cd 50 μM	4.1 ± 0.5	4.2 ± 0.5	0.68 ± 0.08
Cd 100 μM	6.8 ± 0.7	7.3 ± 0.8	0.94 ± 0.11
Cd 200 μM	9.4 ± 0.9	11.6 ± 1.2	1.38 ± 0.16
Pb 100 μM	3.8 ± 0.4	3.6 ± 0.4	0.61 ± 0.07
Pb 200 μM	5.9 ± 0.6	6.1 ± 0.7	0.87 ± 0.10

Values represent mean ± standard deviation (n=5). Significantly different from control (*p*<0.05). MDA: malondialdehyde; FW: Fresh Weight

Heavy Metal-Induced Oxidative Stress Response in Plants

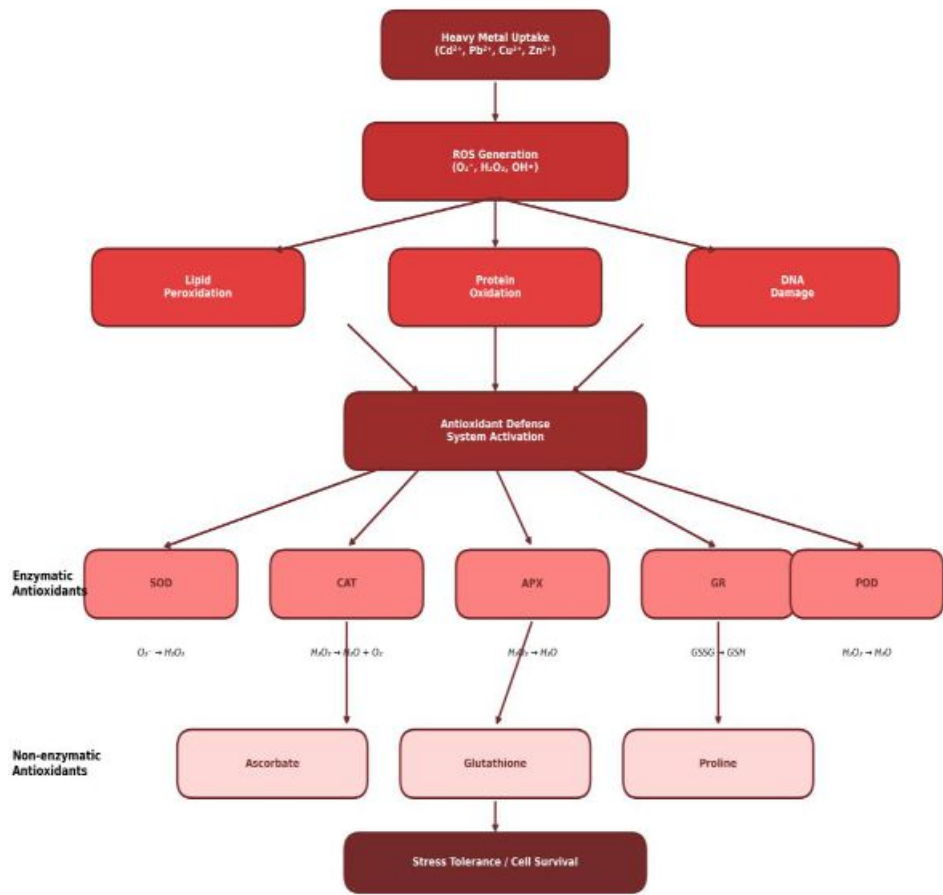


Fig 3: Schematic representation of heavy metal-induced oxidative stress response pathway in plants. Heavy metal uptake triggers reactive oxygen species generation leading to cellular damage. Antioxidant defense systems including enzymatic (SOD, CAT, APX, GR, POD) and non-enzymatic (ascorbate, glutathione, proline) components are activated to mitigate oxidative injury

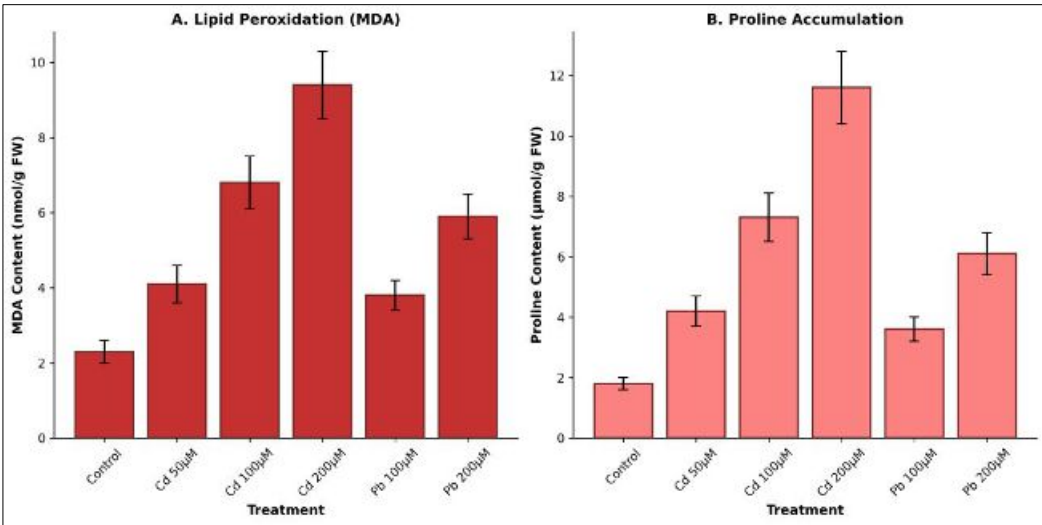


Fig 4: Oxidative stress markers in wheat seedlings under heavy metal treatment. (A) Malondialdehyde content indicating lipid peroxidation; (B) Proline accumulation reflecting osmotic stress response. Error bars represent standard deviation (n=5)

Comprehensive interpretation

The enzyme activity data consistently demonstrated biphasic response patterns across all metals and enzymes examined. At low to moderate metal concentrations, enzyme activities increased significantly above control levels, indicating successful induction of antioxidant defenses in response to oxidative stress. However, at higher concentrations, activities declined below control values, suggesting that accumulated damage had compromised enzyme function or reduced enzyme synthesis capacity.

The correlation between enzyme inhibition and increased malondialdehyde content supports interpretation that high metal concentrations cause membrane damage that impairs antioxidant enzyme function. Similarly, progressive proline accumulation indicates osmotic stress responses paralleling oxidative stress, reflecting the multifaceted nature of heavy metal toxicity.

Discussion

The biphasic enzyme responses observed in this research align with the hormesis concept whereby low-dose stressors stimulate protective responses while higher doses cause damage [9]. The initial enzyme induction represents successful stress perception and signal transduction activating antioxidant gene expression. The subsequent decline at higher concentrations reflects either direct enzyme inactivation by metal binding or reduced protein synthesis capacity as cellular machinery becomes damaged. Cadmium emerged as the most potent inducer of oxidative stress among metals tested, consistent with its known ability to generate reactive oxygen species through multiple mechanisms including displacement of iron from proteins and inhibition of antioxidant enzymes [10]. The threshold concentration for SOD decline at approximately 100 μM cadmium corresponds with concentrations reported to cause visible phytotoxicity symptoms in cereals.

The coordinated induction of multiple antioxidant enzymes suggests activation of common transcription factors responsive to oxidative stress signals. Plant cells possess various ROS-sensing mechanisms that activate defense gene expression through pathways involving mitogen-activated protein kinases and transcription factors of the NAC and WRKY families [11]. The parallel increases across enzyme classes support involvement of such coordinated regulatory mechanisms.

Progressive accumulation of malondialdehyde despite enzyme induction indicates that antioxidant capacity becomes insufficient to prevent membrane damage at higher metal concentrations. Lipid peroxidation represents a self-propagating chain reaction that can overwhelm enzymatic defenses once initiated [12]. The threshold nature of this transition has implications for establishing permissible contamination limits in agricultural soils.

Limitations

Several limitations should be considered when interpreting these findings. First, the hydroponic cultivation system, while enabling precise metal concentration control, differs from soil conditions where metal bioavailability depends on complex interactions with organic matter, clay minerals, and competing ions. Responses in field-grown plants may differ quantitatively from those observed here.

Second, the research examined individual metals in isolation, whereas contaminated soils typically contain

multiple metals that may interact synergistically or antagonistically. Future investigations should address combined metal exposures to better represent field conditions. Third, only one wheat cultivar was examined, and genetic variation in stress tolerance may influence response patterns in other varieties or species.

Conclusion

This research establishes quantitative dose-response relationships between heavy metal concentrations and antioxidant enzyme activities in wheat seedlings. The biphasic response pattern observed consistently across metals and enzymes reveals a threshold phenomenon where protective responses give way to toxicity. Superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and peroxidase all demonstrated initial induction at moderate stress levels followed by decline at higher concentrations.

The concentration thresholds identified provide benchmarks for evaluating heavy metal impacts on cereal crop physiology. For cadmium, enzyme activities peaked near 75-100 μM before declining, suggesting this range represents the upper limit of successful stress adaptation. Malondialdehyde accumulation above this threshold indicates membrane damage that compromises cellular integrity.

These findings have practical implications for managing contaminated agricultural lands. Concentrations below identified thresholds may permit successful crop cultivation with activated but not overwhelmed defense systems, while higher contamination levels require remediation before agricultural use. The enzyme activity parameters measured here could serve as biomarkers for assessing site suitability following remediation efforts.

Future research should extend these observations to field conditions with complex soil matrices and multiple metal exposures. Comparison across wheat cultivars and other cereal species would identify genetic resources for developing heavy metal-tolerant varieties suited to marginal lands. Integration of enzyme activity measurements with transcriptomic analyses would clarify regulatory mechanisms underlying the adaptive responses characterized here.

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Contributions

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