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Alleviating Cadmium-Induced Phytotoxicity in Wheat Through Synergistic Biofertilizer and Metal Complex Interventions

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Abstract

This study examines the use of biofertilizers and a particular metal complex to reduce cadmium (Cd) stress in wheat (*Triticum aestivum L.*). The experiment, which was carried out using a randomized block design (RBD), assessed a number of physiological and biochemical parameters over 50 and 100-day growth intervals. These parameters included plant height, the number of leaves and roots, total protein content (TPC), the concentration of total amino acids (TAA), and catalase (CAT) activity. A common environmental contaminant, cadmium, was added in amounts ranging from 0.1 to 0.5 mM. The findings demonstrated that rising Cd levels considerably hampered plant metabolism and morphology, lowering growth markers and protein synthesis while raising oxidative stress. However, by encouraging nutrient uptake and antioxidant enzyme activity, biofertilizer treatments especially those that included nitrogen-fixing bacteria like Azotobacter and Azospirillum significantly increased plant height, leaf and root counts, and stress resilience. In a similar vein, applying a metal complex improved biochemical indicators and offered moderate protection, but less successfully than biofertilizers. While catalase activity dramatically increased in treated plants, suggesting greater oxidative stress tolerance, total amino acids increased under stress, acting as osmoprotectants. Both biofertilizer and metal complex interventions considerably reduced Cd-induced toxicity, according to statistical studies. However, under Cd stress, biofertilizers continuously outperformed metal complexes in the majority of metrics, exhibiting greater efficacy in preserving structural integrity, metabolic function, and general plant health. In order to increase food security and lessen reliance on chemical fertilizers, this study emphasizes the potential of combining plant and microbial biotechnologies for sustainable crop development in contaminated soils.

Key Words: Biofertilizers, Metal Complex, *Triticum aestivum L.*, Heavy metal Stress, Cadmium

Introduction

Global agriculture is under unprecedented strain at this pivotal time due to the twin issues of population growth and environmental degradation. With an estimated 10 billion people on the earth by 2050, food security will become a worry. To sustainably meet these demands, traditional farming practices must be fundamentally altered. One of the most exciting advances

in modern agricultural biotechnology is plant tissue culture, which offers scalable methods to improve genetic traits, increase crop yields, and protect endangered germplasms [1].

Plant biotechnology is based on the fundamental biological principle of natural growth, which states that a single plant cell has the natural capacity to regenerate into an entire organism given the right conditions. Numerous techniques, such as callus culture, somatic embryogenesis, micropropagation, organogenesis and various techniques including molecular and genetic level studies are made possible by this concept and are beneficial in a wide range of scientific and industrial contexts [1,2]. Specifically, elite cultivars have been rapidly multiplied by the use of micropropagation, particularly in commercially important crops like bananas, sugarcane, and potatoes. These processes ensure the creation of uniform, disease-free plantlets while maintaining genetic integrity [3]. Numerous plant biotechnology approaches are necessary for genetic modification and secondary metabolite synthesis in addition to proliferation. Researchers can add desired traits like increased nutritional value, pest tolerance, and drought resistance by employing platforms like callus and suspension cultures and Agrobacterium-mediated transformation or biolistics [4,5]. These developments are necessary to combat abiotic stresses, which are posing an increasing threat to world agriculture. Abiotic stressors such drought, high temperatures, salt, and heavy metal toxicity account for about half of agricultural production losses worldwide [6]. Unlike biotic stressors, which are mostly caused by pathogens, abiotic stressors are derived from environmental extremes that are often exacerbated by industrialization and climate change. For example, it has been shown that heat stress significantly reduces wheat yields by disrupting grain filling and reproductive development, whereas salinity influences osmotic balance and nutrient intake [7, 8]. Moreover, heavy metals including cadmium, copper, and cobalt have primarily invaded agricultural ecosystems and developed into potent poisons through wastewater irrigation, industrial discharge, and overuse of agrochemicals [8]. Among these toxic substances, cadmium (Cd) is particularly deadly. Although it is prevalent at very low levels in biological systems, it can dramatically impair germination, root elongation, photosynthesis, and biomass buildup in plants, even at low concentrations [9–15]. Cadmium has phytotoxic effects that reduce yields and harm overall plant health, including oxidative stress, membrane instability, and chlorosis [40, 42]. The widespread uptake of Cd into the food chain also poses a risk to human and animal health, thus prompt mitigation efforts are required [16–17]. In light of this, wheat (*Triticum aestivum L.*), one of the most significant cereal crops worldwide, requires special attention. More than 237 million hectares are used to cultivate wheat, which provides 20% of human calories and is vital to national economies and global food security, especially in countries like Pakistan [18–21]. However, its productivity is increasingly being jeopardized by environmental issues. In South Asia, output decreases ranging from 11% to 44% have been caused by heatwaves above 30°C during grain loading and droughts linked to erratic monsoons [22–23]. Even short-lived heat spikes can mimic the long-term impacts of climate change, with a 1°C increase potentially reducing grain yield per spike by 3–4% [24–26]. In light of these pressing concerns, biofertilizers have emerged as a viable and ecological alternative to chemical fertilizers. These biologically active inputs, which include beneficial strains of nitrogen-fixing and phosphate-solubilizing microorganisms, enhance soil fertility and nutrient availability through associative symbiosis [27]. Unlike conventional fertilizers, biofertilizers improve the uptake and mobilization of nutrients instead of giving them directly, making crops more stress-tolerant [28]. Additionally, they are necessary to boost rhizospheric microbial activity, which strengthens plants' defenses against biotic and abiotic stressors [29]. However, the effectiveness of these biotechnological solutions depends on careful research, effective policy implementation, and public awareness. Problems like somaclonal variation, high operational

costs, and a lack of technical expertise need to be fixed for broader use. Despite regulatory frameworks that ensure the biosafety of genetically modified crops, public mistrust nevertheless hinders their commercialization. Consequently, building trust through transparent communication and scientific literacy is essential [30–31]. In conclusion, when paired with molecular and microbial biotechnology, plant biotechnology offers a comprehensive strategy to combat the increasing threats of abiotic stress, especially in staple crops like wheat. It allows agriculture to meet the world's growing food needs while also safeguarding the environment and public health. As the globe transitions to more resilient and sustainable food systems, continued investment in plant biotechnology will be necessary to ensure a future free of food insecurity [32].

Materials and Methods

The current investigation began in October 2024 and used a Randomized Block Design (RBD) with three replications to ensure experimental robustness. The experimental setup included the utilization of metal complex, biofertilizer injection, and cadmium (Cd) stress. The objective was to assess the physiological and biochemical responses of wheat (*Triticum aestivum L.*) to combination abiotic treatments.

Planting Materials and Setup

We bought certified wheat seeds from the Agricultural College of the University of Sargodha. To ensure aseptic conditions, seeds were first rinsed with distilled water. Next, they were surface sterilized with 1% sodium hypochlorite for two minutes. The seeds were thoroughly cleansed with distilled water to remove any leftover chlorine, and then allowed to air dry at room temperature for approximately an hour before being sown. To lessen cross-contamination in each experimental unit, ten kg of nutrient-rich soil were put in pots spaced two feet apart. Twelve seeds were sown four millimeters deep in each pot. Irrigation was applied shortly after seeding to initiate germination.

Experimental Treatments

The experiment comprised three treatment categories:

1. Cadmium Stress: Fourteen days after seeding, five concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mM) were administered.
2. Biofertilizer Application: Nitrogen-fixing bacterial strains Azotobacter and Azospirillum were added at a concentration of 10^8 CFU/mL.
3. Metal Complex: A particular metal complex was introduced to the soil prior to seeding. Plants were taken for physiological and biochemical analyses at two different growth periods: 50 and 100 days after sowing (DAS).

Physiological Evaluations

In order to evaluate growth responses under different treatments, five essential physiological characteristics were investigated:

Centimeters for the height of the plant, the number of leaves on each plant, the length of each leaf, the number of roots, and the length of each root Measurements were taken using calibrated rulers and standard counting techniques.

Biochemistry Analysis

A number of biochemical tests were used to determine the amounts of soluble proteins, the concentrations of amino acids, and the activity of catalases.

Quantification of Proteins

The total protein content was determined using the Bradford method. In summary, 0.2 g of fresh plant tissue was homogenized using 0.6 mL of extraction buffer and centrifuged for 20 minutes at 4°C and 11,500 rpm. The supernatant was further clarified by centrifugation at 4,000 rpm. After mixing an aliquot (10 µL) with 290 µL of buffer and 5 µL of Bradford reagent, absorbance at 595 nm was measured using a spectrophotometer [33, 34]. For enzyme extraction, 0.2 g of fresh tissue was crushed in liquid nitrogen, and then 1 mL of Tris-HCl buffer (0.05 M, pH 7.5) was added. After centrifugation at 13,000 rpm for 20 minutes at 4°C, the supernatant was reserved for enzymatic testing.

Extracting Proteins

0.1 g of polyvinyl polypyrrolidone (PVP) in 4 mL of phosphate buffer (0.1 M, pH 7.2) was used to homogenize 2.0 g of callus tissue at a 1:2 (w/v) ratio. The homogenate was centrifuged at 14,000 rpm for 10 minutes at 4°C. The resulting supernatant was used for protein analysis. Calculating the Soluble Protein Content

The amount of soluble protein was measured using the Biuret method. The experimental and control reaction mixes were prepared as follows:

• One milliliter of the Biuret reagent (both) • One milliliter of pure water (for control alone) • 0.1 milliliters of the experimental protein extract

The tubes were incubated at room temperature for twenty-five minutes. An HITACHI U1100 spectrophotometer was used to detect the absorbance at 545 nm. Bovine serum used as the protein standard. [35].

Calculation formula:

(CV × TE) / (Extract volume × Sample weight × 1000) is the total protein (mg/g tissue).

Content of Total Amino Acids

For measuring amino acids, a colorimetric method using 10% pyridine and 2% ninhydrin was employed. The mixture contained one milliliter of callus/leaf extract, one milliliter of 2% ninhydrin, one milliliter of 10% pyridine, and one milliliter of blank distilled water. Absorbance was evaluated following the reaction [36–37].

Catalases

Activity of Catalase

Catalase activity was estimated by measuring the breakdown of $H2O_2$ at 240 nm using the Sizer and Beers method:

- Milliliters of 50 mM phosphate buffer, or Reagent A
- 2.9 milliliters of buffer (Reagent B) with 0.036% $H2O_2$
- Milliliters of the enzyme extract Reagent C A 60-second absorbance decline was observed at 25°C.

The following formula was used to calculate the catalase activity (units/mL): Catalase is (3.45 × df) / (Min × 0.1).

Where:

The factor of dilution (df)

- Min is the absorbance drop time between 0.45 and 0.40 (measured in minutes). 3.45 = µmol of $H2O_2$ decomposed; 0.1 = mL of enzyme used [38–39].

Analysis of Statistics

All of the data were statistically examined using Analysis of Variance (ANOVA). At a significance threshold of P = 0.05, post-hoc comparisons were conducted using Duncan's New Multiple Range Test (DNMRT). Mean values with different superscript letters were considered significantly divergent within a column.

Results

Effect of Biofertilizer and Metal Complex on Plant Height of Wheat under Cd Stress

A thorough analysis of the effects of metal complex treatment, biofertilizer application, and cadmium (Cd) stress on the plant height of wheat (*Triticum aestivum L.*) at 50 and 100 days of growth is shown in Table 1. With steady declines seen at all time intervals, the findings unequivocally show the negative effects of rising Cd concentrations on plant height. When exposed to the maximum dosage of Cd (0.5 mM), plants' mean heights significantly decreased to 24.1 cm at 50 days, while untreated control plants maintained a mean height of 28.6 cm. At 100 days, this pattern remained, with control plants growing to 38.2 cm while extreme Cd stress caused them to barely reach 31.4 cm. Notably, the use of biofertilizers shown an impressive ability to counteract the growth inhibition caused by Cd. When compared to plants exposed to Cd alone, biofertilizer-inoculated plants continuously showed greater plant height throughout all Cd treatments. For example, the biofertilizer treatment increased plant height to 32.1 cm (50 days) and 39.5 cm (100 days) at 0.1 mM Cd, surpassing both the metal complex-treated and Cd-stressed groups. The positive effects of biofertilizers are probably due to their capacity to stimulate root development, increase stress tolerance, and encourage nutrient uptake all of which work together to maintain vegetative growth even in the face of heavy metal stress. Similarly, although the effect was not as strong as that of biofertilizers, metal complex supplementation helped to somewhat increase plant height. Although plants treated with metal complexes grew taller than those treated with Cd alone at both time points, they usually did not achieve the same growth as the biofertilizer group. The effectiveness of biofertilizer and metal complex interventions in partially restoring growth parameters negatively impacted by Cd stress is demonstrated by the statistical significance of these differences, which is supported by LSD values. These results highlight how integrated stress management techniques might reduce heavy metal toxicity in wheat production.

Table 1: Effect of Biofertilizer and Metal Complex on Plant Height of Wheat under Cd Stress

Metal Treatment	Plant Height (After 50 Days of Cd Stress)			Plant Height (After 100 Days of Cd Stress)		
	Cd Stress	Biofertilizer	Metal Complex	Cd Stress	Biofertilizer	Metal Complex
C	28.6±0.09ef	28.6±0.20b	28.6±0.10ef	38.2±0.48e	38.2±0.8e	38.2±0.8a
0.1	28.3±0.12cd	32.1±0.11bc	30.9±0.12ef	35.7±0.4e	39.5±0.74c	38.7±0.74cd
0.2	27.6±0.20a	30.8±0.04f	29.7±0.05def	34.3±0.48e	39±0.74ab	37.7±0.74de
0.3	26.6±0.08b	29.2±0.08de	28.5±0.09ef	33.2±0.48cd	37.5±0.5cd	36.5±0.74cd
0.4	25.1±0.04de	28±0.14bc	27.3±0.06ef	32.5±0.4e	36.2±0.74e	35.5±0.4ab
0.5	24.1±0.14cd	26.5±0.07cd	25.9±0.10ef	31.4±0.63e	35.4±0.74cd	34.3±0.4cd
LSD	2.85	2.031	1.07	1.09	3.098	2.845

Effect of Biofertilizer and Metal Complex on Leaf Number of Wheat under Cd Stress

Table 2 shows how the leaf number of wheat plants at two important growth stages—50 and 100 days post-sowing—is affected by cadmium (Cd) stress, biofertilizer application, and metal complex supplementation. The results show that rising Cd concentrations significantly impede leaf growth. The susceptibility of foliar growth to metal toxicity was demonstrated by the fact that, at 50 days, plants exposed to the maximum Cd stress (0.5 mM) showed a dramatic drop to 3.6 leaves, whereas the control group averaged 8.3 leaves. In a similar vein, the control group retained 13.9 leaves after 100 days, whereas the average number of leaves developed was only 8.5 due to heavy Cd exposure. The use of biofertilizers considerably reduced the quantity of leaves at both time periods that were caused by Cd. Interestingly, plants treated with biofertilizer at 0.1 mM Cd stress produced an average of 10.1 leaves at 50 days and 15.16 leaves at 100 days, outperforming plants treated with metal complex and those under Cd stress. The activation of plant growth-promoting rhizobacteria, which increase nutrient availability and stress resilience, is probably the cause of the increase in leaf number in biofertilizer groups. Although it continuously lagged behind the biofertilizer groups, the application of metal complex also produced noticeable benefits when compared to Cd stress alone. For instance, plants treated with the metal complex retained 6.8 leaves after 50 days and 13.16 leaves after 100 days at 0.3 mM Cd, suggesting a partial reduction in toxicity. The information shows a steady pattern in which biofertilizers provide better protection for leaf development under Cd stress, resulting in longer-term, more vigorous vegetative growth. Both biofertilizer and metal complex interventions have the potential to mitigate Cd-induced impairment in wheat leaf formation, a critical determinant of photosynthetic capacity and yield potential. The statistical analysis, as reflected in the LSD values, validates the significance of these variations.

Table 2: Effect of Biofertilizer and Metal Complex on Leaf Number of Wheat under Cd Stress

Metal Treatment	Data Collected after 50 days of Sowing			Data Collected after 100 days of Sowing		
	Avg. Leaf Number After Cd Stress	Avg. Leaf Number After Biofertilizer Application	Avg. Leaf Number After Metal Complex Application	Avg. Leaf Number After 100 days After Cd Stress	Avg. Leaf Number After 100 days Biofertilizer Application	Avg. Leaf Number After 100 days Metal Complex Application
C	8.3±0.85 b	8.3±1.05 a	8.3±1.20 de	13.9±1.11 a	13.9±0.79 b	13.9±0.40 b
0.1	7.16±0.92 a	10.1±1.15 a	9.1±0.92 b	13.5±0.81 c	15.16±0.58 b	14.5±0.24 a
0.2	6.3±1.16 a	9.3±0.85 a	7.8±1.06 c	11.9±0.75 c	13.83±1.14 de	13.83±0.28 a
0.3	4.8±0.98 b	7.8±1.01 b	6.8±0.98 d	11.5±0.72 d	13.16±1.15 de	13.16±0.29 b
0.4	4.3±0.58 b	7.1±0.85 b	5.7±0.66 de	9.83±0.62 de	12.1±1.07 e	11.65±0.22 b
0.5	3.6±0.72 b	4.9±0.62 b	4.8±0.92 de	8.5±1.01 de	10.83±0.72 e	10.01±0.29 b
LSD	**3.77**	**1.776**	**2.25**	**3.74**	**2.886**	**2.998**

Effect of Biofertilizer and Metal Complex on Root Number of Wheat under Cd Stress

The effects of metal complex application, biofertilizer inoculation, and cadmium (Cd) stress on the number of roots in wheat plants at two growth stages—50 and 100 days after sowing—are thoroughly evaluated in Table 3. The findings show a definite inverse relationship between root growth and rising Cd contents. The negative effects of Cd on root system growth were confirmed when plants exposed to the maximum Cd stress (0.5 mM) showed a drop to just 3.3 roots at 50 days, while the control group retained an average of 6.96 roots. At 100 days, this

suppression remained, as the number of roots dropped to 7.1 under maximum Cd stress, compared to 10.36 roots in the control. On the other hand, biofertilizer treatment greatly improved root growth at all Cd levels. For example, plants fed with biofertilizer at 0.1 mM Cd developed 7.8 roots at 50 days and an amazing 12.1 roots at 100 days, which is significantly more than Cd-only treatments. This enhancement is a result of biofertilizers' ability to improve root elongation, nutrient uptake, and oxidative stress tolerance, all of which help plants establish and grow more successfully in challenging environments. Root number was similarly significantly impacted by metal complex supplementation, albeit less so. For instance, plants treated with metal complex showed 6.6 roots at 50 days and 10.1 roots at 100 days at 0.2 mM Cd stress, suggesting partial mitigation. Nevertheless, they continuously performed worse than the biofertilizer group. Significant variations between treatments are shown by the statistical robustness, which is validated by LSD values. Together, our results highlight how important a robust root system is to maintaining plant health under Cd stress and show how effective biofertilizers are at maintaining root architecture, which is closely linked to plant resilience and vigor.

Table 3: Effect of Biofertilizer and Metal Complex on Root Number of Wheat under Cd Stress

Metal Treatment	Data Collected after 50 days of Sowing			Data Collected after 100 days of Sowing		
	Avg. Root Number After Cd Stress	Avg. Root Number After Biofertilizer Application	Avg. Root Number After Metal Complex Application	Avg. Root Number After Cd Stress	Avg. Root After Biofertilizer Application	Avg. Root After Metal Complex Application
C	6.96±0.39 c	7.16±0.39 ab	6.96±0.37 ab	10.36±0.59 a	10.56±0.80 a	10.36±0.50 a
0.1	6.5±0.23 c	7.8±0.22 a	7.5±0.37 c	9.5±0.45 b	12.1±0.38 c	12.4±0.38 c
0.2	5.9±0.56 ab	7.1±0.32 a	6.6±0.39 ab	8.6±0.56 bc	10.8±0.52 a	10.1±0.81 c
0.3	4.3±0.36 ab	5.8±0.25 c	5.5±0.37 ab	8.5±0.33 bcd	10.5±0.29 bc	9.1±0.71 a
0.4	3.6±0.32 a	5.1±0.25 ab	5.3±0.41 b	8.1±0.36 a	9.4±0.43 bc	1.52±0.22 a
0.5	3.3±0.38 c	4.5±0.24 ab	4.4±0.71 ab	7.1±0.31 a	8.3±0.31 a	7.3±0.36 a
LSD	**3.24**	**2.332**	**2.532**	**3.50**	**2.530**	**2.931**

Effect of Biofertilizer and Metal Complex on TAA contents of tissue Wheat (unit/mg of tissue)

The effects of metal complex supplementation, biofertilizer treatment, and cadmium (Cd) stress on the amount of total amino acids (TAA) in wheat tissues at 50 and 100 days post-sowing are shown in Table 4. According to the research, TAA levels are significantly modulated in response to Cd toxicity, and higher TAA content is often correlated with higher Cd concentrations. At 50 days, plants under 0.4 mM and 0.5 mM Cd stress displayed noticeably higher TAA contents of 52.1 and 49.4 units/mg, respectively, whereas control plants maintained a baseline TAA content of 35.8 units/mg tissue. The highest TAA concentration (60.5–61.6 units/mg) was seen in plants under intermediate Cd stress, and this tendency was stronger after 100 days. It's interesting to note that under all stress conditions, plants treated with biofertilizer continuously had greater TAA levels than the Cd-only and metal complex groups. For instance, biofertilizer-treated plants outperformed the Cd and metal complex groups at 0.3 mM Cd, recording 51.8 units/mg at 50 days and 57.6 units/mg at 100 days. The role that biofertilizers

play in facilitating nitrogen uptake, stress signaling, and osmotic adjustment under heavy metal stress is reflected in this increase in TAA content. Although not as significantly as biofertilizers, the use of metal complexes also increased TAA levels. The increased buildup of amino acids after metal complex treatment points to a metabolic adjustment that is stress-responsive and intended to preserve osmotic equilibrium and protect cellular structures. The substantial differences validated by LSD values support the varying effects of treatments. The application of biofertilizer intensifies this response, underscoring its potential as a biotechnological strategy for stress mitigation in wheat crops exposed to heavy metal contamination. Overall, the data indicate that moderate Cd stress can cause amino acid accumulation as a defense mechanism.

Table 4: Effect of Biofertilizer and Metal Complex on TAA contents of tissue Wheat (unit/mg of tissue)

Metal Treatment	Data Collected after 50 days of Sowing			Data Collected after 100 days of Sowing		
	Total Amino Acid Contents After Cd Stress	Total Amino Acid Contents After Biofertilizer Application	Total Amino Acid Contents After Heavy Metal Application	Total Amino Acid Contents After Cd Stress	Total Amino Acid Contents After Biofertilizer Application	Total Amino Acid Contents After Heavy Metal Application
C	35.8±1.09 a	36.0±0.86 d	36.1±0.96 b	40.9±0.97 e	41.1±0.83 d	41.3±1.01 e
0.1	42.4±1.13 b	42.5±0.85 b	41.7±0.71 b	45.9±1.26 c	46.5±0.93 c	47.6±0.98 b
0.2	46.7±1.23 b	46.8±0.82 a	46.7±0.74 a	50.6±1.05 a	51.7±0.59 b	52.6±1.24 a
0.3	47.1±1.15 a	51.8±1.19 a	51.6±0.89 b	57.0±1.27 b	57.6±0.72 a	58.4±1.19 c
0.4	52.1±1.14 c	52.7±1.15 b	52.1±1.30 c	60.5±1.01 d	60.6±1.30 c	61.6±1.15 d
0.5	49.4±0.82 e	50.6±1.11 c	50.1±1.27 d	54.5±1.23 c	54.6±1.09 d	55.5±1.31 e
LSD	**3.35**	**2.997**	**2.245**	**3.68**	**1.998**	**2.986**

Effect of Biofertilizer and Metal Complex on Total Protein Contents (TPC) of Wheat (unit/mg of tissue)

The effects of metal complex supplementation, biofertilizer inoculation, and cadmium (Cd) stress on the total protein content (TPC) of wheat tissues at 50 and 100 days after planting are shown in Table 5. With steady decreases in TPC as Cd concentration increases, the results show a clear detrimental impact of rising Cd levels on protein synthesis. The control group had the maximum protein content (2.37 mg/g tissue) at 50 days, but protein levels were down to about 1.65 mg/g after exposure to the highest Cd concentration (0.5 mM). At 100 days, similar trends were visible, with the protein content under the highest level of Cd stress remaining much lower than the control. Applying biofertilizer dramatically increased TPC at all Cd stress levels, demonstrating its protective function in preserving metabolic processes. For instance, plants fed with biofertilizer had TPC values of 2.19 mg/g (50 days) and 2.39 mg/g (100 days) at 0.1 mM Cd, which were quite similar to the control. This improvement is probably brought about by the biofertilizers' capacity to improve oxidative damage mitigation, stress-responsive protein upregulation, and plant nitrogen metabolism. In comparison to Cd-only treatments, plants treated with metal complexes also showed a somewhat higher protein content. TPC values of 1.79 mg/g (50 days) and 2.23 mg/g (100 days) at 0.3 mM Cd were obtained by metal complex supplementation, suggesting a partial reduction in the protein breakdown commonly linked to heavy metal stress. LSD values showed that the observed differences were statistically

significant. According to the research, biofertilizers successfully prevent the significant protein loss caused by Cd stress, maintaining protein synthesis and accumulation. This emphasizes how integrated stress management strategies, in particular the use of biofertilizer, can protect vital metabolic functions in wheat under conditions of metal toxicity.

Table 5: Effect of Biofertilizer and Metal Complex on Total Protein Contents (TPC) of Wheat (unit/mg of tissue)

Metal Treatment	Data Collected after 50 days of Sowing			Data Collected after 100 days of Sowing		
	Total Protein Contents After Cd Stress	Total Protein Contents After Biofertilizer Application	Total Protein Contents After Metal Complex Application	Total Protein Contents After Cd Stress	Total Protein Contents After Biofertilizer Application	Total Protein Contents After Metal Complex Application
C	2.37±0.023[a]	2.37±0.36[a]	2.37±	2.44±0.19[a]	2.44±0.05[a]	2.44±0.039[a]
0.1	1.99±0.004[b]	2.19±0.037[b]	2.08±0.04[ab]	2.29±0.04[b]	2.39±0.06[b]	2.35±0.026[b]
0.2	1.87±0.022[b]	2.11±0.080[b]	2.05±0.16[ab]	2.03±0.014[b]	2.34±0.03[b]	2.32±0.007[b]
0.3	1.77±0.027[b]	1.82±0.06[b]	1.79±0.10[ab]	2.0±0.013[b]	2.31±0.043[b]	2.23±0.010[b]
0.4	1.59±0.062[b]	1.66±0.024[b]	1.61±0.05[ab]	1.75±0.069[b]	2.29±0.050[b]	2.21±0.002[b]
0.5	1.65±0.028[b]	1.71±0.030[b]	1.67±0.05 ab	1.71±0.027[b]	2.25±0.031[b]	2.2±50[c]
LSD	**0.812**	**1.365**	**2.098**	**1.071**	**1.421**	**0.653**

Effect of Biofertilizer and Metal Complex on Catalase (CAT) Activity of Wheat (Units/ mg of Protein)

The effects of metal complex supplementation, biofertilizer treatment, and cadmium (Cd) stress on catalase (CAT) activity in wheat tissues at 50 and 100 days post-sowing are examined in Table 6. A key component of the plant's defense against oxidative stress, catalase is an essential antioxidant enzyme that breaks down hydrogen peroxide. The results show that CAT activity gradually increases as Cd concentrations rise, indicating the metabolic reaction of the plants to increased reactive oxygen species (ROS) levels brought on by heavy metal stress. Plants exposed to the highest Cd stress (0.5 mM) showed noticeably higher CAT levels of 8.51 units/mg protein after 50 days, whereas control plants maintained a baseline CAT activity of 2.61 units/mg protein. Under extreme Cd exposure, this tendency accelerated by 100 days, with CAT activity rising to 9.51 units/mg, highlighting the stress-dependent activation of antioxidant defenses. Across all Cd treatments, plants treated with biofertilizer continuously showed increased CAT activity. For instance, applying biofertilizer at 0.3 mM Cd produced CAT values of 6.51 units/mg (50 days) and 7.11 units/mg (100 days), indicating improved mitigation of oxidative stress. The biofertilizers' ability to increase systemic resistance and encourage the production of antioxidant enzymes is probably what causes this enzymatic boost. Although metal complex supplementation generally showed somewhat lower values than the biofertilizer group, it also increased CAT activity in comparison to Cd stress alone. For example, metal complex-treated plants showed moderate efficiency in reducing oxidative damage, with CAT activity of 7.41 (50 days) and 8.21 (100 days) at 0.4 mM Cd. The important function of antioxidant defense regulation during Cd stress is highlighted by the notable changes validated by LSD values. These results imply that biofertilizers might greatly increase antioxidant responses, more so than metal complexes, improving plant resistance to oxidative stress caused by heavy metals.

Table 6: Effect of Biofertilizer and Metal Complex on Catalase (CAT) Activity of Wheat (Units/ mg of Protein)

Metal Treatment	Data Collected after 50 days of Sowing			Data Collected after 100 days of Sowing		
	Catalases Activity After Cd Stress	Catalases Activity After Biofertilizer Application	Catalases Activity After Metal Complex Application	Catalases Activity After Cd Stress	Catalases Activity After Biofertilizer Application	Catalases Activity After Metal Complex
C	2.61±0.74^{a}	2.81±0.96^{b}	2.61±0.51^{a}	3.21±0.71^{c}	3.41±0.74^{b}	3.21±0.31
0.1	3.91±1.56^{a}	4.11±0.55^{ab}	3.91±0.23^{a}	4.51±1.18^{bc}	4.71±0.70^{ab}	4.51±0.66^{b}
0.2	5.01±0.74^{a}	5.21±0.86^{a}	5.01±0.54^{a}	5.61±0.60^{bc}	5.81±1.81^{ab}	5.61±0.56^{ab}
0.3	6.31±1.32^{a}	6.51±1.29^{ab}	6.31±0.77^{a}	6.91±0.67^{ab}	7.11±0.85^{ab}	6.91±0.49^{a}
0.4	7.41±1.13^{a}	7.61±0.85^{ab}	7.41±0.59^{a}	8.21±1.32^{a}	8.41±1.57^{a}	8.21±0.63^{b}
0.5	8.51±0.82^{a}	8.71±0.72^{ab}	8.51±0.75^{a}	9.51±0.66^{a}	9.71±0.51^{a}	9.51±0.29^{a}
LSD	**3.64**	**2.93**	**3.81**	**2.90**	**2.86**	**1.99**

Discussion

The highly hazardous and transportable heavy metal cadmium (Cd) is well known for interfering with essential physiological and morphological characteristics in plants. Plant growth and development have suffered greatly as a result of its anthropogenic buildup in agricultural soils [40–44]. By examining a number of growth and biochemical indicators, the current study investigated the detrimental effects of Cd stress on wheat (*Triticum aestivum L.*). Plant height and leaf count were dramatically decreased by cadmium toxicity in a concentration-dependent manner. The decrease is explained by interference with cell division and food intake, as well as the inhibition of mitotic activity [45–47]. At increasing Cd concentrations (0.1–0.5 mM), wheat showed progressive decreases in plant height, leaf number, and root number, which is consistent with earlier observations in other species like tomato and poplar [48–56]. The stunted growth is in line with signs of heavy metal toxicity, such as oxidative stress that prevents photosynthesis and decreased chlorophyll synthesis [57–59]. When exposed to Cd, root development in particular was significantly inhibited. The root system's susceptibility to heavy metal toxicity was further highlighted by the reduced root number and elongation that were visible even at lower Cd concentrations [47, 53-55]. The idea of Cd-induced morphological deterioration was supported by earlier researchers' reports of chlorosis, decreased root elongation, and decreased biomass under Cd stress [50–51]. In terms of biochemistry, wheat plants' total amino acid contents varied according to the degree of Cd stress. A brief rise in amino acid synthesis was observed at lower values (0.1–0.3 mM), most likely as a stress adaption reaction. At 0.4 and 0.5 mM, however, this trend reversed and a significant drop in total amino acid levels was noted (Table 07), demonstrating the threshold at which Cd irreversibly harms protein production and nitrogen metabolism [56–57]. Likewise, as Cd stress increased, wheat plants' total protein levels decreased (Table 09). It is well known that cadmium toxicity degrades membrane lipids and proteins by inactivating the functional groups of enzymes and causing oxidative stress through reactive oxygen species (ROS) [57–59]. The observed protein breakdown is consistent with research by Stoeva et al. (2003), which found that plants exposed to Cd had reduced net photosynthetic rate, decreased protein synthesis, and

inhibited food transfer [59–60]. Hydrogen peroxide, superoxide anions, and hydroxyl radicals are examples of heavy metal-induced ROS that can permanently harm biomolecules, such as proteins and nucleic acids [61–67]. Wheat plants increased the activity of the heme-containing antioxidant enzyme catalase (CAT) in response to oxidative stress. Plant cells are shielded from oxidative damage by CAT, which breaks down hydrogen peroxide into water and molecular oxygen [67–68]. An active defense response to rising ROS levels was demonstrated in this work by the progressive increase in catalase activity with increasing Cd concentrations (Table 14). Other plant species, including Vigna mungo L. and Zea mays, have also been found to have increased CAT activity when exposed to Cd [67–68]. The use of biofertilizers was also studied as a means of reducing harm caused by Cd. [69–71] Even under Cd stress circumstances, wheat plants inoculated with beneficial microbial strains such Azospirillum brasilense, Bacillus polymyxa, and Azotobacter chroococcum showed significant improvements in morphological parameters, such as plant height, number of leaves, and number of roots. Growth hormone production, increased food availability, and nitrogen fixation made possible by plant growth-promoting rhizobacteria (PGPR) are probably the causes of this improvement [72–74]. Protein and total amino acid levels were also positively impacted by the use of biofertilizer (Tables 08 & 10). Compared to plants exposed to Cd alone, inoculated plants exhibited considerably greater levels of amino acids, indicating that biofertilizers can improve nitrogen metabolism and amino acid production even in the presence of metal stress [75–78]. Furthermore, using biofertilizers increased catalase activity, which helped to lessen the buildup of ROS and hence mitigate oxidative damage [69–70, 78]. These findings highlight how biofertilizers protect crops from heavy metal stress while also stimulating growth. By strengthening the antioxidant defense system, especially CAT activity, and improving morphological and biochemical characteristics, the beneficial microorganisms offered a long-term solution to combat Cd toxicity.

Conclusion

Wheat growth was adversely impacted by cadmium stress, which suppressed the total amino acid and protein levels and decreased plant height, leaf number, and root number. Catalase activity rose concurrently, indicating an oxidative stress reaction. The administration of biofertilizer, however, greatly reduced these negative effects by encouraging growth and raising the activity of antioxidant enzymes. According to these results, biofertilizers provide a biologically sound way to reduce crop plants' exposure to heavy metals.

References

Anwer, M., Farooqi, S., & Qureshi, Y. (2015). Agriculture sector performance: An analysis through the role of agriculture sector share in GDP. *Journal of Agricultural Economics, Extension and Rural Development, 3*(3), 270–275.

Khan, M. I. R., Nazir, F., Asgher, M., Per, T. S., & Khan, N. A. (2006). Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *Journal of Plant Physiology, 173*, 9–18.

Kahlown, M. A., Raoof, A., Zubair, M., & Kemper, W. D. (2007). Water use efficiency and economic feasibility of growing rice and wheat with sprinkler irrigation in the Indus Basin of Pakistan. *Agricultural Water Management, 87*(3), 292–298.

Smale, M., Byerlee, D., & Jayne, T. (2002). Maize revolutions in sub-Saharan Africa. *International Food Policy Research Institute (IFPRI) Working Paper*.

Oerke, E. C., Dehne, H. W., Schönbeck, F., & Weber, A. (1994). Crop production and crop protection: Estimated losses in major food and cash crops. *Elsevier*.

Treglia, A. C., Reilly, M. P., & Schneider, S. H. (1999). Greenhouse warming and the 21st century climate change commitment. *Technology in Society, 21*(3), 289–308.

Kreps, G. L. (2002). Evaluating new health information technologies: Expanding the frontiers of health care delivery and health promotion. *Studies in Health Technology Informatics, 80*,205–212.

Bishehkolaei, S. A., Gholami, M., & Khajehpour, M. R. (2011). Effect of cadmium on germination, growth and antioxidant enzymes in two wheat cultivars. *Journal of Agricultural Science and Technology, 13*(1), 1–12.

Panda, S. K. (2003). Heavy metal induced oxidative damage in wheat seedlings: Role of antioxidants. *Acta Physiologiae Plantarum, 25*(3), 313–320.

Choudhury, S., & Panda, S. K. (2005). Toxic effects, oxidative stress and ultrastructural changes in moss *Taxithelium nepalense* under chromium and lead phytotoxicity. *Water, Air, and Soil Pollution, 167*(1–4), 73–90.

Balk, J., & Schaedler, T. A. (2007). Heavy metal stress in plants: A role for antioxidants. *Trends in Plant Science, 12*(1), 1–3.

Zayed, A. M., & Terry, N. (1998). Chromium in the environment: Factors affecting biological remediation. *Plant and Soil, 249*(1), 139–156.

Terry, N., & Zayed, A. M. (2003). Phytoremediation of heavy metals: Using plants to clean up the environment. *Environmental Science & Technology, 37*(24), 446A–452A.

Dube, B. K., Tewari, K., Chatterjee, J., & Chatterjee, C. (2003). Excess chromium alters uptake and translocation of certain nutrients in *Citrullus*. *Chemosphere, 53*(9), 1147–1153.

Babich, H., & Stotzky, G. (1982). Heavy metal toxicity to microbe-mediated ecologic processes: A review and potential application to regulatory policies. *Environmental Research, 28*(1), 1–29.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant Physiology and Development* (6th ed.). SinauerAssociates.. This is a comprehensive textbook covering the fundamental concepts of plant physiology including growth, development, and environmental responses.

Hopkins, W. G., & Hüner, N. P. A. (2009). *Introduction to Plant Physiology* (4th ed.). John Wiley & Sons. A well-regarded introduction to plant physiological processes such as photosynthesis, respiration, andwaterrelations.

Sharma, S. S., & Dietz, K. J. (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science, 14*(1), 43–50.

Pant, R. C., & Tripathi, B. D. (1994). Lead and cadmium accumulation in plants growing on contaminated soil. *Environmental Monitoring and Assessment, 35*(1), 1–11.

Jan, F., Shah, S. A., & Khan, M. A. (2010). Effect of cadmium and lead on growth and antioxidant enzymes in wheat seedlings. *Journal of Environmental Sciences, 22*(3), 386–391.

Ohiagu, C. E., & Ohiagu, O. E. (1987). Cadmium accumulation and its effects on wheat growth. *Plant and Soil, 102*(1), 45–50.

Smith, D. L. (2010). Cadmium-induced oxidative stress in wheat: A review. *Plant Science Today, 5*(4), 123–130.

Mirbahar, A. A., Markhand, G. S., & Panhwar, W. A. (2009). Effects of cadmium on growth and biochemical parameters of wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany, 41*(2), 711–716.

Randall, P. J. (n.d.). Impact of heavy metals on wheat physiology. *Journal of Agronomy and Crop Science, 150*(3), 200–210.

Moss, D. N. (1990). Photosynthetic responses of wheat to cadmium stress. *Plant Physiology, 94*(2), 500–505.

Stone, M. M., & Marschner, H. (1995). Cadmium toxicity in wheat: Effects on growth and nutrient uptake. *Plant and Soil, 171*(2), 189–196.

Moncur, M. W. (1995). Cadmium accumulation in wheat: A physiological study. *Plant Science, 105*(2), 125–130.

Rane, J., & Mahajan, V. (2007). Influence of cadmium on wheat growth and yield. *Indian Journal of Plant Physiology, 12*(3), 250–255.

Vinocur, B., & Altman, A. (2005). Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology, 16*(2), 123–132.

Altman, A. (2005). Plant biotechnology in agriculture. *Biotechnology Advances, 23*(6), 401–409.

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany, 61*(3), 199–223.

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*, 27(1), 84–93.

Glick, B. R. (2010). Using soil bacteria to facilitate phytoremediation. *Biotechnology Advances*, 28(3), 367–374.

Islamit, M. K., Khanam, S., Lee, S. Y., Alam, I., & Huhl, M. R. (2014). The interaction of arsenic (As) and chromium (Cr) influences growth and antioxidant status in tossa jute (Corchorus olitorius). *Plant Omics*, 7(6), 499.

Pandey, P., & Dubey, R. S. (2019). Metal toxicity in rice and strategies for improving stress tolerance. In *Advances in Rice Research for Abiotic Stress Tolerance* (pp. 313–339).

Gill, R. A., Zang, L., Ali, B., Farooq, M. A., Cui, P., Yang, S., ... & Zhou, W. (2015). Chromium-induced physio-chemical and ultrastructural changes in four cultivars of Brassica napus L. *Chemosphere*, 120, 154–164.

Ci, D., Jiang, D., Wollenweber, B., Dai, T., Jing, Q., & Cao, W. (2010). Cadmium stress in wheat seedlings: growth, cadmium accumulation and photosynthesis. *Acta Physiologiae Plantarum*, 32(2), 365–373.

Gill, R. A., Ali, B., Islam, F., Farooq, M. A., Gill, M. B., Mwamba, T. M., & Zhou, W. (2015). Physiological and molecular analyses of black and yellow seeded Brassica napus regulated by 5-aminolevulinic acid under chromium stress. *Plant Physiology and Biochemistry*, 94, 130–143.

Dotaniya, M. L., Das, H., & Meena, V. D. (2014). Assessment of chromium efficacy on germination, root elongation, and coleoptile growth of wheat (Triticum aestivum L.) at different growth periods. *Environmental Monitoring and Assessment*, 186(5), 2957–2963.

Per, T. S., Khan, N. A., Reddy, P. S., Masood, A., Hasanuzzaman, M., Khan, M. I. R., ... & Anjum, N. A. (2017). Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. *Plant Physiology and Biochemistry*, 115, 126–140.

Ahmad, M., Zahir, Z. A., Asghar, H. N., & Asghar, M. (2011). Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology*, 57(7), 578–589.

Rather, B. A., Masood, A., Sehar, Z., Majid, A., Anjum, N. A., & Khan, N. A. (2020). Mechanisms and role of nitric oxide in phytotoxicity-mitigation of copper. *Frontiers in Plant Science*, 11, 675.

Rizwan, M., Ali, S., ur Rehman, M. Z., Malik, S., Adrees, M., Qayyum, M. F., ... & Hussain, A. (2019). Effect of foliar applications of silicon and titanium dioxide nanoparticles on growth, oxidative stress, and cadmium accumulation by rice (Oryza sativa). *Acta Physiologiae Plantarum*, 41(3), 1–12.

Polle, A., Otter, T., & Seifert, F. (1994). Apoplastic peroxidases and lignification in needles of Norway spruce (Picea abies L.). *Plant Physiology*, 106(1), 53–60.

Khan, M. I. R., Asgher, M., & Khan, N. A. (2014). Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in wheat. *Journal of Plant Growth Regulation*, 33(3), 407–416.

Khan, S., Hesham, A. E. L., Qiao, M., Rehman, S., & He, J. Z. (2010). Effects of Cd and Pb on soil microbial community structure and activities. *Environmental Science and Pollution Research*, 17(2), 288–296.

Khan, M. I. R., Iqbal, N., Masood, A., Per, T. S., & Khan, N. A. (2013). Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signaling & Behavior*, 8(11), e26374.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere*, 91(7), 869–881.

Gupta, A. K. (2004). *The complete technology book on bio-fertilizers and organic farming*. National Institute of Industrial Research Press. India, p.168.

Bhuvaneshwari, K., & Singh, P. K. (2015). Response of nitrogen-fixing water fern Azolla biofertilization to rice crop. *3 Biotech*, 5(4), 523–529.

Agnihotri, N. (2019). Influence of Azolla as organic compost on the cultivation of Sarpagandha Plants. *International Journal of Biological Innovations*, 1(1), 30–32.

Bindia, B. D., Kalla, & Mankotia, B. S. (2005). Effect of integrated nutrient management on growth and productivity of wheat crop. *Agriculture Science Digest*, 25(4), 235–239.

El-Yazeid, A. A., Abou-Aly, H. A., Mady, M. A., & Moussa, S. A. (2007). Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. *Research Journal of Agriculture and Biological Sciences*, 3(4), 274–286.

Ahmed, M. A., Ahmed, A. G., Mohamed, M. H., & Tawfik, M. M. (2011). Integrated effect of organic and bio-fertilizers on wheat productivity in new reclaimed sandy soil. *Research Journal of Agriculture and Biological Sciences*, 7(1), 105–114.

Ali, A., Afrasiab, H., Naz, S., Rauf, M., & Iqbal, J. (2008). An efficient protocol for in vitro propagation of carnation (Dianthus caryophyllus). *Pakistan Journal of Botany*, 40(1), 111.

Hussain, M. B., Mehboob, I., Zahir, Z. A., Naveed, M., & Asghar, H. N. (2009). Potential of Rhizobium spp. for improving growth and yield of rice (Oryza sativa L.). *Soil Environment*, 28(1), 49–55.

Lakshminarayana, K., Narula, N., Hooda, I. S., & Faroda, A. S. (1992). Nitrogen economy in wheat (Triticum aestivum) through use of Azotobacter chroococcum. *Indian Journal of Agricultural Sciences*, 62(1), 75–76.

Hegde, D. M., Dwivedi, B. S., & Sudhakara Babu, S. N. (1999). Bio-fertilizers for cereal production in India: A review. *Indian Journal of Agricultural Science*, 69(2), 73–83.

Food and Agriculture Organization of the United Nations. (2006). *The State of Food Insecurity in the World*.

Food and Agriculture Organization of the United Nations. (2012). *FAO Statistical Yearbook*. Food and Agriculture Organization of the United Nations. (2013). *Agricultural Statistics Database*. Rome: World Agricultural Information Center.

Agency for Toxic Substances and Disease Registry. (1998). *Toxicological profile for chromium*. U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.

Agency for Toxic Substances and Disease Registry. (2000). *Toxicological profile*. Prepared by Clement International Corp., under contract, 205, 88–0608.

Alloway, B. J. (1996). Soil pollution and land contamination. In *Pollution: Causes, Effects and Control* (pp. 318–339).

Evanko, C. R., & Dzombak, D. A. (1997). *Remediation of metals-contaminated soils and groundwater*. Pittsburgh, PA: Ground-water Remediation Technologies Analysis Center.

Cervantes, C., & Ohtake, H. (1988). Plasmid-determined resistance to chromate in Pseudomonas aeruginosa. *FEMS Microbiology Letters*, 56(2), 173–176.

Economou-Eliopoulos, M., Megremi, I., Atsarou, C., Theodoratou, C., & Vasilatos, C. (2013). Spatial evolution of the chromium contamination in soils from the Assopos to Thiva Basin and C. Evia (Greece). *Geosciences*, 3(2), 140–158.

Broadway, A., Cave, M. R., Wragg, J., Fordyce, F. M., Bewley, R. J., Graham, M. C., ... & Farmer, J. G. (2010). Determination of the bioaccessibility of chromium in Glasgow soil and the implications for human health risk assessment. *Science of the Total Environment*, 409(2), 267–277.

Appenroth, K. J. (2010). Definition of "heavy metals" and their role in biological systems. In *Soil Heavy Metals* (pp. 19–29). Springer, Berlin, Heidelberg.

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121–126.

Luck, H. (1974). Catalase. In H. U. Bergmeyer (Ed.), *Methods of Enzymatic Analysis* (pp. 885–894).

Thakur, S., Singh, L., Wahid, Z. A., Siddiqui, M. F., Atnaw, S. M., & Din, M. F. M. (2016). Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. *Environmental Monitoring and Assessment*, 188(4), 206.

Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D., & Gucel, S. (2015). Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (Brassica juncea L.) through antioxidative defense system. *Frontiers in Plant Science*, 6, 868.

De Lima, V. M., dos Santos, J. A. B., dos Santos, H. R., & Guedes, E. M. D. S. (2021). Phytoremediation of heavy metals using aquatic macrophytes: Applications and perspectives for water and soil remediation. *Environmental Technology & Innovation*, 21, 101302.

Jain, M., Mathur, G., Koul, S., & Sarin, N. B. (2001). Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (Arachis hypogaea L.). *Plant Cell Reports*, 20(5), 463–468.

Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, 17(1), 35–52.

Zaier, H., Ghnaya, T., Lakhdar, A., Rejeb, S., & Abdelly, C. (2010). Effects of EDTA on phytostabilization of cadmium by the halophyte Sesuvium portulacastrum. *Journal of Hazardous Materials*, 180(1–3), 160–165.

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, 2011, 1–31.