

Effects of cadmium, lead, zinc and copper on chlorophyll and protein content of rice (*Oryza sativa* L.)

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Abstract

This study investigated the effects of cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) on some biochemical constituents like chlorophyll, nitrogen and protein levels in rice. A pot experiment was conducted using agricultural loamy soil amended with graded concentrations of Cd, Pb, Zn, and Cu salts, with untreated soil serving as control. Rice leaf samples were collected after 45 days of transplantation in in-situ conditions and analyzed for chlorophyll content. Nitrogen and protein content in rice grains were determined after harvest at ripening. Results revealed significant ($p < 0.05$) reductions in chlorophyll a, chlorophyll b, and total chlorophyll across all treatments, with Cd exerting the most severe inhibitory effect, followed by Pb, Cu, and Zn. Similarly, nitrogen and protein contents declined under metal exposure, with Cd and Pb causing the greatest reductions, while Zn and Cu showed comparatively moderate toxicity. These findings demonstrate that heavy metal stress disrupts pigment biosynthesis, nitrogen metabolism, and protein synthesis in rice, thereby compromising its physiological performance and nutritional quality.

Keywords: Heavy metal, crops, agroecosystem, nitrogen metabolism, protein synthesis and metal stress

Introduction

Heavy metal contamination in agricultural soils is an escalating concern, particularly in regions heavily reliant on industrial activities, urbanization, and agrochemical usage^{1,2}. Among these metals, lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) have emerged as significant pollutants, disrupting soil health and threatening crop productivity³. The toxic effects of these metals often manifest in plant physiological and biochemical processes, including chlorophyll synthesis and protein metabolism, which are critical for plant growth and yield⁴.

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population, is highly sensitive to environmental stressors, including heavy metal contaminated soils⁵. Chlorophyll, essential for photosynthesis, and protein content, critical for nutritional quality, are frequently impaired under metal stress^{6,7}. Lead and cadmium, categorized as non-essential and highly toxic elements, are known to disrupt enzymatic activities and interfere with nutrient uptake. On the other hand, zinc and copper, although essential in trace amounts, become toxic at elevated concentrations, causing oxidative damage and protein denaturation in plants^{8,9}.

Studies have highlighted the detrimental impact of lead and cadmium on chlorophyll synthesis, resulting in chlorosis and reduced photosynthetic efficiency. Cd is

readily absorbed by rice plants, primarily through ion transporters like OsNRAMP5 and OsIRT1, disrupting root cell growth, photosynthesis, and enzyme activity while inducing reactive oxygen species (ROS) production and phytotoxicity¹⁰. Rice mitigates Cd stress via regional sequestration, chelation with phytochelators, and antioxidative mechanisms^{11,12}. Similarly, Pb toxicity impairs rice growth, reproductive development, and photosynthesis by disrupting ATP production, lipid peroxidation, and essential nutrient uptake, but rice deploys detoxification strategies such as Pb sequestration in vacuoles and activation of antioxidants⁶. For instance, Pb competes with essential cations such as calcium (Ca), magnesium (Mg), and potassium (K) for uptake by root cells, resulting in nutrient imbalances and physiological disorders. Pb was shown to replace Mg ions in chlorophyll molecules, directly affecting their stability and function¹³. Conversely, zinc and copper, while necessary for various enzymatic reactions and structural proteins, exhibit dual behavior. Excess zinc has been shown to hinder protein metabolism by disrupting nitrogen assimilation pathways¹⁴. Elevated copper levels lead to lipid peroxidation and protein degradation, exacerbating cellular damage in rice plants¹⁵. Cu stress affects root growth, photosynthesis, and nutrient absorption, with high Cu levels disrupting essential metal homeostasis and chloroplast ultrastructure, reducing

yield and productivity¹⁶. Cu-induced oxidative stress also alters the TCA cycle, with significant down regulation of intermediates like citric and malic acids, severely impairing carbohydrate metabolism and rice growth¹⁷.

Despite these findings, the synergistic or antagonistic interactions of these metals and their individual effects on rice remain largely unexplored. Furthermore, limited research has specifically examined how Pb, Cd, Zn, and Cu collectively influence chlorophyll and protein content in rice. These gaps hinder the development of comprehensive strategies for mitigating heavy metal toxicity in rice-based agroecosystems. The present study is to investigate the effects of lead, cadmium, zinc, and copper on chlorophyll, nitrogen and protein content in rice. Specifically, the main objectives are to:

- (a) Assess the effect of individual metal exposures on chlorophyll a, chlorophyll b, and total chlorophyll content.
- (b) Evaluate the effect of metals on the protein content of rice grains.

Materials and Methods

Experimental Design and Soil Preparation

Four separate controlled pot experiments with agricultural loamy soil, free from prior contamination was conducted at the Crop field of the Department of Soil Science, University of Chittagong, during mid-January 2022 to June 2022 to evaluate the effects of four heavy metals such as cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) on the chlorophyll and protein content of rice (*Oryza sativa* L.). Soil was collected from the surface layer (0–15 cm), air-dried, and sieved through a 2 mm mesh. Physicochemical properties of the soil, including pH (5.32 gL⁻¹), electrical conductivity (128.1 µscm⁻¹), organic matter (1.41%), CEC (24.24 meq/100g), total nitrogen (0.5%), total phosphorus (0.07%), Cd (0.003 mg kg⁻¹), Pb (0.01 mg kg⁻¹), Zn (10 mg kg⁻¹) and Cu (6 mg kg⁻¹) respectively, were analyzed prior to treatment.

Five treatments consisted of a single heavy metal were added to the soil in the form of its respective salt that is cadmium as cadmium sulfate (3CdSO₄. 8H₂O), lead as lead nitrate [Pb (NO₃)₂], zinc as zinc sulphate (ZnSO₄. 7H₂O), and copper as copper sulphate (CuSO₄. 5H₂O). Metal salts were thoroughly mixed with the soil to obtain Cd levels of 2, 4, 6, 8 and 10 mg kg⁻¹ soil, Pb levels of 20, 40, 60, 80 and 100 mg kg⁻¹ soil, Zn levels of 30, 60, 90, 120 and 150 mg kg⁻¹ soil and Cu levels of 4, 8, 12, 16 and 20 mg kg⁻¹ soil, respectively. A control treatment without heavy metal addition was also

maintained in each experiment. Thus, six treatments were arranged in a randomized complete block design (RCBD) with three replications in each separate experiment.

Planting and Growth Conditions

Pre-germinated rice seeds (*Oryza sativa* L.) of variety BRRI -28 were transplanted into 4 kg capacity plastic pots containing the treated soils. One seedling was planted per pot. The pots were maintained under natural daylight in a research field. Soil moisture was maintained throughout the growth period by regular watering with tap water.

Duration of Exposure and Sampling

Plants were grown for a period about 120 days following transplantation¹⁸. At harvest, the fully expanded leaves were collected, rinsed with distilled water to remove surface contaminants, and immediately subjected to biochemical analyses. Leaf samples were analysed for chlorophyll content in fresh weight basis and rice grain were collected for analyzing nitrogen and protein content in dry weight basis.

Chlorophyll Analysis

Chlorophyll a, chlorophyll b, and total chlorophyll (Chl) contents were determined following the method of Arnon¹⁹ an additional modified by Lichtenthaler and Wellburn²⁰. Fresh leaf tissue (1 g) was homogenized in 100% acetone and centrifuged at 200 rpm for 1 hour and made volume up to 50 ml in the volumetric flask. The absorbance of the supernatant was measured at 645 nm and 662 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800). Chlorophyll contents were calculated using following equations²¹ and expressed as mg g⁻¹ fresh weight.

$$\begin{aligned} Chl - a &= \frac{11.75A_{662} - 2.35A_{645}}{W \times 1000} V \\ Chl - b &= \frac{18.61A_{645} - 3.96A_{662}}{W \times 1000} V \\ Total\ Chl &= (Chl\ a + Chl\ b) \end{aligned}$$

Where, A = absorbance at specific wavelength, V = final volume of chlorophyll extract in 100% acetone, W = fresh weight of leaf tissue, Chl-a = *chlorophyll a*, Chl-b = *chlorophyll b* and Total Chl = *total chlorophyll*.

Total Nitrogen and Protein Analysis

Total nitrogen content was determined using the Kjeldahl digestion method²². Oven-dried 0.5 g ground rice grain was digested with concentrated sulphuric acid in the presence of a digestion catalyst (K₂SO₄: CuSO₄.5H₂O: Se = 100:10:1) and volume up to 100 ml in volumetric flask. The digested samples

were distilled and titrated with dilute acid to quantify nitrogen content, expressed as a percentage of dry weight.

$$\% \text{ Total Nitrogen (TN)} = \frac{(T-B) \times f \times 0.014 \times 100 \text{ ml volume} \times 100}{w \times \text{volume of extract used}}$$

where, T = Sample titration value (mL) of standard H₂SO₄; B = Blank titration value (mL) of standard H₂SO₄; f = strength of H₂SO₄; W = Weight of rice grain in gram.

Protein content was estimated by multiplying total nitrogen values by a conventional factor of 6.25, assuming that nitrogen constitutes approximately 16% of plant protein. Percent of protein content was calculated with the following formula on a dry weight basis²³.

$$\text{Protein \%} = \% \text{ Nitrogen} \times 6.25 \text{ (conversion factor)}$$

Statistical Analysis

The significance of differences among the means of the treatments were evaluated by one way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at the significance level of 5%. The statistical analyses were done using Excel, and SPSS

version 20. All data were carefully examined for accuracy and consistency prior to statistical analysis.

Results and Discussion

Chlorophyll content

Figure 1 illustrates the impact of cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) exposure on chlorophyll *a*, chlorophyll *b*, and total chlorophyll content in rice leaves, expressed in mg g⁻¹. Across all treatments, a general decline in chlorophyll content was observed in comparison to the control. Chlorophyll *a* showed the most pronounced reduction under Cd exposure, followed by Pb, Cu, and Zn. Chlorophyll *b* also declined under all heavy metal treatments, with Cd again having the most substantial inhibitory effect. Total chlorophyll content mirrored the trends observed for individual pigments, demonstrating the cumulative stress effect of heavy metals, particularly Cd and Pb. The reductions were statistically significant ($p < 0.05$), indicating that heavy metal exposure, especially Cd, adversely affects chlorophyll biosynthesis in rice.

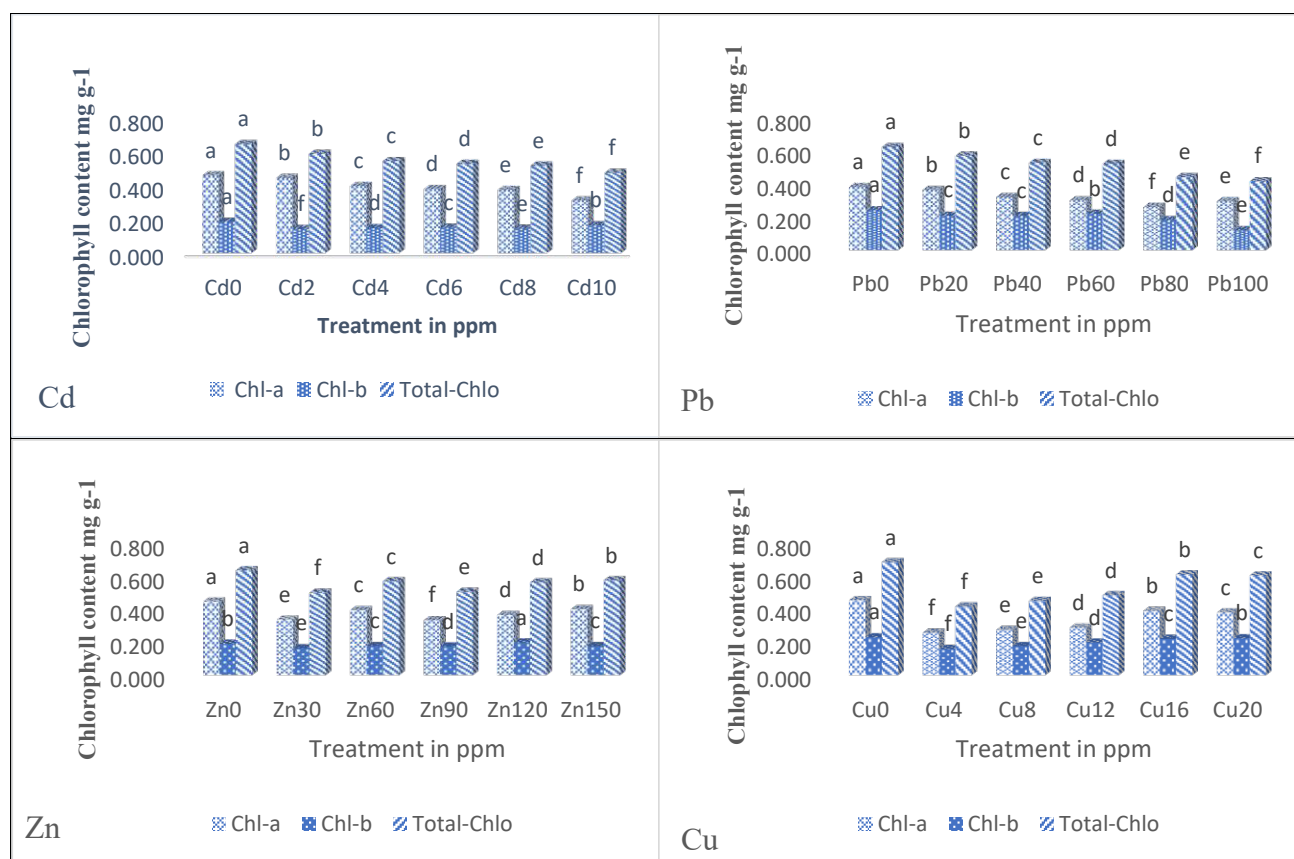


Figure 1. Effect of heavy metals (Cd, Pb, Zn and Cu) exposure on chlo-a, chlo-b and total chlo (mg g⁻¹) content in rice. Bars having same letter(s) are not significantly different among treatments by DMRT ($p \leq 0.05$). [chlo-a = chlorophyll *a*, chlo-b = chlorophyll *b*, total chlo = total chlorophyll].

The observed decrease in chlorophyll content under heavy metal stress suggests a disruption in photosynthetic efficiency and pigment biosynthesis. Cadmium and lead, known for their phytotoxicity, likely interfere with chloroplast structure and enzymatic activity responsible for pigment formation^{24, 25}. The significant decline in chlorophyll *a* under Cd exposure might be attributed to the inhibition of protochlorophyllide reductase or enhanced chlorophyll degradation pathways²⁶. These findings are consistent with earlier studies by Rai *et al.*²⁷ who reported that Cd and Pb reduce chlorophyll content in various cereal crops, impairing photosynthesis and growth. The relatively lower reduction observed under Zn and Cu

could be due to their dual role as both essential micronutrients and toxicants at higher concentrations²⁷.

Total Nitrogen Content

The total nitrogen content (%) in rice plants subjected to Cd, Pb, Zn, and Cu exposure (Figure 2). A consistent reduction in nitrogen content was recorded across all treatments relative to the control. The maximum decline in nitrogen content was observed under Cd treatment, followed closely by Pb. Zn and Cu also caused a measurable decline, but the reductions were less severe than those caused by Cd and Pb. All reductions were statistically significant ($p < 0.05$), suggesting a systemic disruption of nitrogen assimilation or translocation under heavy metal stress.

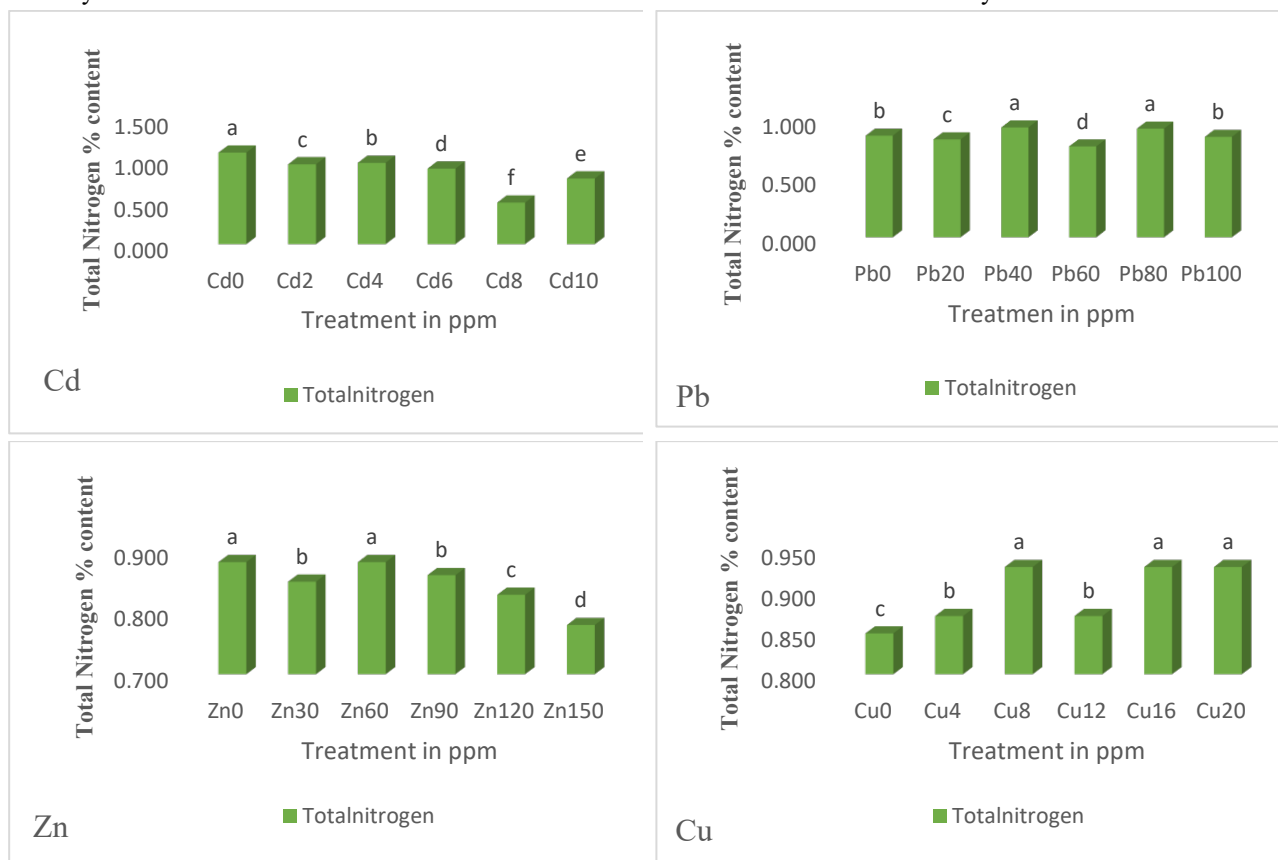


Figure 2. Effect of heavy metals (Cd, Pb, Zn & Cu) exposure on total nitrogen (%) content in rice. Bars having same letter(s) are not significantly different among treatments by DMRT ($p \leq 0.05$).

Nitrogen is a critical macronutrient involved in amino acid and protein synthesis. The marked reduction in nitrogen content, especially under Cd and Pb stress, could be linked to impaired nitrate reductase activity and root uptake mechanisms^{28, 29}. Cadmium is particularly

notorious for causing root membrane damage, thereby limiting the plant's ability to absorb nutrients efficiently.

The results are in alignment with the work of Lebrazi *et al.*³⁰ who demonstrated that Cd toxicity limits nitrogen metabolism in rice by down regulating key enzymes and

transporters. Similarly, Pb can inhibit nitrogen assimilation enzymes and displace essential cations, exacerbating nutritional deficiencies³¹. Zinc and copper, despite being essential for plant metabolism, become toxic at elevated levels and can disrupt nitrogen transport and metabolism^{32, 33}. However, the relatively lower decline observed under Zn and Cu treatments might reflect a threshold effect, where toxicity is less pronounced due to their functional roles in enzymatic reactions.

Protein Content

The percentage of protein content in rice exposed to various heavy metals data is presented in figure 3. A downward trend in protein content was evident for all treatments compared to the control. The most substantial decrease was associated with Cd exposure, followed by Pb, Cu, and Zn. The protein content declined significantly ($p < 0.05$), with Cd and Pb showing the most adverse effects.

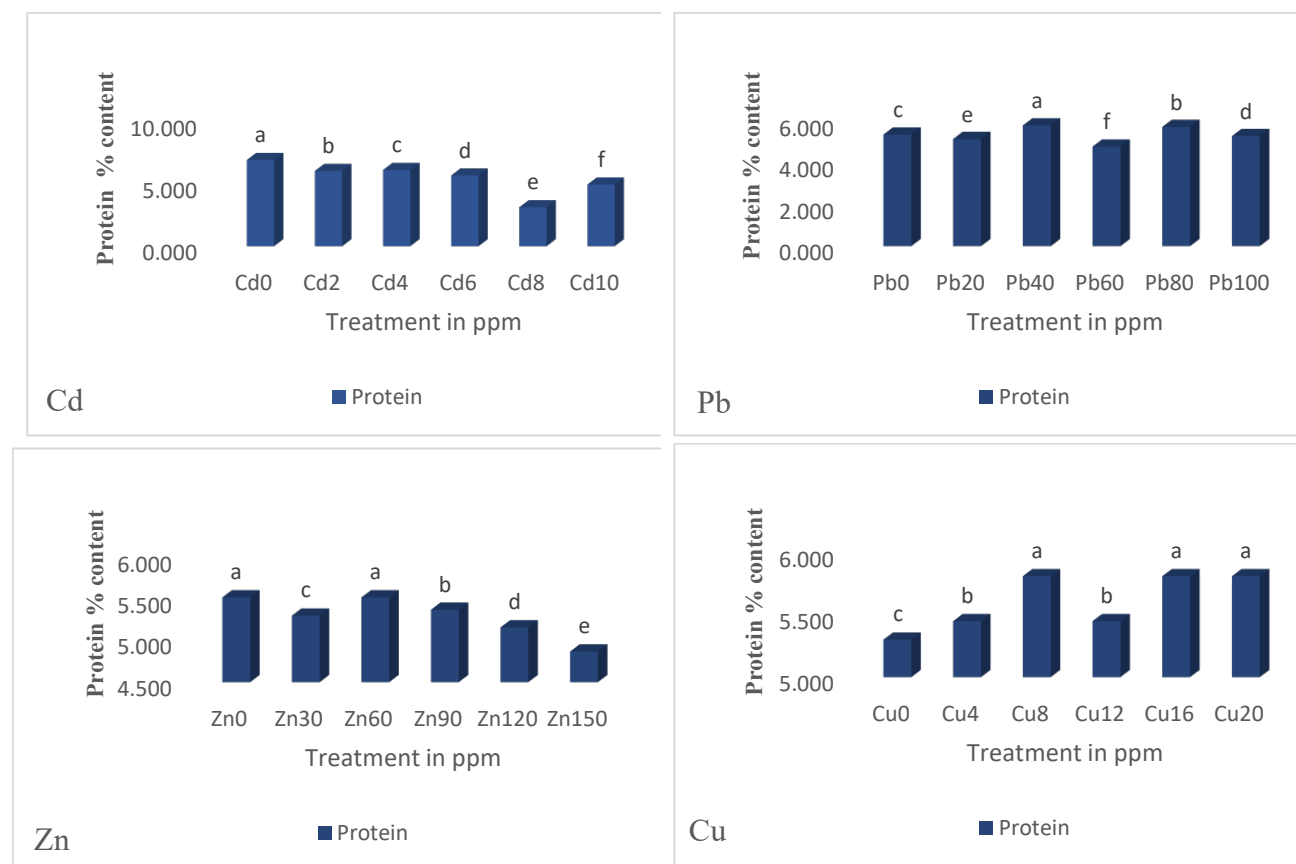


Figure 3. Effect of heavy metals (Cd, Pb, Zn and Cu) exposure on protein (%) content in rice.

Bars having same letter(s) are not significantly different among treatments by DMRT ($p \leq 0.05$).

Protein synthesis is directly influenced by nitrogen availability and metabolic function. The decline in protein content under heavy metal exposure reflects a combination of impaired nitrogen uptake, altered gene expression, and disrupted cellular machinery. Cadmium and lead are known to inhibit ribosomal function and amino acid biosynthesis, thereby affecting protein assembly^{34, 31}.

The parallel decline in nitrogen and protein contents suggests that heavy metal toxicity compromises the nitrogen-to-protein conversion pathway. The result is supported by findings from Hasan *et al.*³⁵ who observed reduced protein levels in rice and wheat under similar metal stress conditions. The relatively lesser reduction in protein under Zn and Cu treatments may reflect the metals' role in maintaining enzymatic functions at lower concentrations, although chronic exposure remains detrimental^{36, 33}.

Overall, the decline in protein content under heavy metal stress raises serious concerns for nutritional quality and food security in contaminated areas.

Conclusion

This research highlights the detrimental effects of Cd, Pb, Zn, and Cu on key biochemical parameters in rice, including chlorophyll content, nitrogen concentration, and protein levels. Cadmium emerged as the most toxic metal, significantly impairing pigment biosynthesis and nitrogen metabolism. Lead followed a similar trend, while Zn and Cu exhibited moderate toxicity. These findings underscore the importance of monitoring heavy metal contamination in agricultural soils, particularly in rice-growing regions exposed to industrial and urban runoff. From a physiological and agronomic perspective, such contamination can compromise crop quality and yield, with downstream effects on human health and food systems. Therefore, it is recommended to evaluate long-term field responses to metal contamination under varied agronomic practices.

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