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Research Article

Physiological and Biochemical Responses of Two Chili Pepper (*Capsicum annuum* L.) Genotypes to Heavy Metal Stress

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ABSTRACT

Heavy metal contamination adversely affects plant growth by disrupting physiological and biochemical processes. This study evaluated the responses of two chili pepper (*Capsicum annuum* L.) genotypes (Cayenne and ID15) to combined nickel (Ni), lead (Pb), and cadmium (Cd) stress by assessing total leaf chlorophyll, total soluble sugar, and fruit protein contents. Significant differences were observed among genotypes, heavy metal treatments, and most genotype × treatment interactions. Cayenne maintained significantly higher total leaf chlorophyll (2.782 mg g⁻¹ fresh weight) than ID15 (2.532 mg g⁻¹), indicating greater tolerance to heavy metal-induced damage to the photosynthetic apparatus. Chlorophyll content declined progressively with increasing heavy metal concentrations, from 4.129 mg g⁻¹ in the control to 2.115 mg g⁻¹ under severe stress. In contrast, ID15 accumulated slightly higher soluble sugars (2.544%) than Cayenne (2.490%), while sugar content initially increased under low metal stress (2.942%) before declining to 2.087% at the highest metal level, reflecting impaired carbohydrate metabolism under severe toxicity. Genotype had no significant effect on fruit protein content; however, heavy metal treatments significantly reduced protein concentration from 1.362% in the control to 0.880% under the highest stress level, indicating inhibition of nitrogen metabolism and enhanced protein degradation. Significant genotype × treatment interactions further demonstrated contrasting adaptive responses of the two genotypes. Overall, Cayenne exhibited greater tolerance through superior chlorophyll retention, whereas ID15 showed relatively better osmotic adjustment through soluble sugar accumulation. These findings highlight genotype-dependent mechanisms of heavy metal tolerance and provide useful information for selecting chili pepper genotypes suitable for cultivation in metal-contaminated soils.

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Introduction

Chili pepper (*Capsicum annuum* L.), which belongs to the family Solanaceae, is one of the most economically important vegetable and spice crops in the world. Several plants in the Solanaceae family are of agricultural

importance such as tomato (*Solanum lycopersicum*), eggplant (*S. melongena*), and sweet peppers (*C. annuum*). Chili pepper is grown in a wide range of agroecological conditions, with remarkable genetic diversity and adaptability to a wide range of environments (Barboza et

al., 2022). Although chilli pepper is very adaptive, environmental stress has become a common constraint to its productivity and heavy metal contamination of agricultural soils is one of the greatest concerns in the world. Heavy elements like lead (Pb), cadmium (Cd) and nickel (Ni) negatively impact plant growth by disrupting physiological and metabolic processes, affecting photosynthesis, inhibiting electron transport in chloroplast, curtailing production of chlorophyll and production of carbohydrates, finally leading to significant losses in yield (Altaf et al., 2021; Mahdavian et al., 2021).

The high nutritional value, bioactive compounds, and medicinal properties of chili pepper make it an important vegetable and spice crop, as well as a valuable functional and medicinal food (Tripodi, 2024). The fruits are rich in vitamin C, which is a strong antioxidant required for immune system, collagen production, healing of wounds and provides protection against oxidative damage (Barrita and Sánchez, 2013). Chili peppers also are rich in vitamins B6 and K, vital minerals such as calcium, copper, iron, and magnesium and also have an excellent fatty acid profile, with low saturated fatty acids and relatively high unsaturated fatty acids and balanced ratio of omega-3 and omega-6 fatty acids (Zaki et al., 2013). Regular consumption of chili peppers has been linked to the decrease in the risk of cardiovascular diseases, type 2 diabetes, obesity, and certain cancerous diseases such as prostate cancer and as well as a general decrease in death risk (Chakrabarty et al., 2017). However, heavy metal stress in chili plants may significantly reduce the nutritional and medicinal potential of chili fruits due to the accumulation of toxic metals in plants which affect cellular metabolism and increased generation of Reactive Oxygen Species (ROS) causing oxidative damage (Mahdavian et al., 2021).

Heavy metals are generally considered as metallic elements that have a density of more than 5 g cm^{-3} and many of them are not essential for plants and are even toxic in low concentrations. Cadmium (Cd), lead (Pb), nickel (Ni), mercury (Hg), and arsenic (As) are among the most environmentally important pollutants that enter agricultural soils from industrial emissions, mining, application of sewage sludge, irrigation of wastewater, excessive applications of fertilizers and other anthropogenic activities (El-Sappah et al., 2024). These compounds do not degrade and remain a threat to ecosystem and human health due to their persistence and ability to bioaccumulate in plant tissue and into the food

web. Heavy metals can disrupt mineral nutrition by competing with essential nutrients like calcium (Ca), magnesium (Mg), potassium (K), iron (Fe) and zinc (Zn). Cadmium, for instance, interferes with the absorption of iron and zinc resulting in nutrient imbalances and stunted growth of plants (Liu et al., 2021). In addition, heavy metals have a negative effect on chlorophyll biosynthesis and photosynthesis, degrade integrity of cell membranes, damage enzymatic activities, and lead to overproduction of ROS, which causes oxidative stress, lipid peroxidation and cellular damage (Brahim et al., 2024). Redox-active metals like Fe, Cu, Cr and Mn directly catalyze ROS production, while non-redox-active metals like Cd and Pb indirectly induce oxidative stress by impairing antioxidant defense systems and by reducing intracellular antioxidant levels (Neto et al., 2017).

The heavy metal tolerance of plants is highly genotype dependent, as a result of differences in antioxidant capacity, metal uptake, osmotic adjustment, sequestration, and detoxification mechanisms. Thus, it is crucial to identify the genotypes with improved physiology and biochemical tolerance to develop climate-resilient cultivars which can be grown in polluted soils to ensure sustainable agricultural production under growing environmental contamination.

The present study was designed to assess the impact of lead, cadmium and nickel contamination on some physiological and biochemical characteristics of two chili pepper cultivars, Cayenne and ID15. In particular, the study evaluated the variations in chlorophyll contents, soluble sugar concentrations and protein contents of fruits under heavy metal stress, compared to the tolerance of the two genotypes and analyzed the interactions between the genotype and heavy metal stress in order to find the genotype which is more physiologically and biochemically resilient under contaminated soil condition.

Materials and Methods

Experimental site and plant materials

The experiment was carried out using two chili pepper genotypes (Cayenne and ID15) under heavy metal stress at the experimental field in Kirkuk, Iraq (35.5292° N , 44.3773° E) from 15 March up to 1 November 2025. The study was designed to test the effect of lead, cadmium and nickel on some of the physiological and biochemical characteristics such as total chlorophyll contents of leaves, total soluble sugar contents and total protein content of fruits.

Experimental design and treatments

The experiment was conducted using a $3 \times 3 \times 3$ full factorial arrangement to investigate the individual and interactive effects of nickel (Ni), lead (Pb), and cadmium (Cd) on the physiological and biochemical responses of chili pepper. Three concentrations of each heavy metal were evaluated, resulting in 27 treatment combinations, and an additional untreated control was included, giving a total of 28 treatments (Table 1).

The experimental factors consisted of nickel at 40, 60, and 80 mg kg⁻¹ soil (designated as Ni₁, Ni₂, and Ni₃, respectively); lead at 80, 120, and 160 mg kg⁻¹ soil (Pb₁, Pb₂, and Pb₃, respectively); and cadmium at 3.0, 4.5, and 6.0 mg kg⁻¹ soil (Cd₁, Cd₂, and Cd₃, respectively). All possible combinations of these factor

levels were generated to obtain the 27 heavy metal treatments. An untreated control (T28), in which no Ni, Pb, or Cd was added to the soil, was included for comparison (Table 1).

The experiment was established in a Randomized Complete Block Design (RCBD) with three replications per treatment. Each experimental unit was amended with the designated heavy metal combination and planted with the experimental chili genotypes. The factorial arrangement enabled the evaluation of the main effects of Ni, Pb, and Cd concentrations as well as their two-way and three-way interactions, thereby providing a comprehensive assessment of the combined influence of these heavy metals on plant growth and physiological and biochemical traits.

Table 1. Treatment combinations and concentrations of nickel, lead, and cadmium applied in the experimental study

Treatment	Combination	Ni (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Cd (mg kg ⁻¹)
T1	Ni ₁ Pb ₁ Cd ₁	40	80	3
T2	Ni ₁ Pb ₁ Cd ₂	40	80	4.5
T3	Ni ₁ Pb ₁ Cd ₃	40	80	6
T4	Ni ₁ Pb ₂ Cd ₁	40	120	3
T5	Ni ₁ Pb ₂ Cd ₂	40	120	4.5
T6	Ni ₁ Pb ₂ Cd ₃	40	120	6
T7	Ni ₁ Pb ₃ Cd ₁	40	160	3
T8	Ni ₁ Pb ₃ Cd ₂	40	160	4.5
T9	Ni ₁ Pb ₃ Cd ₃	40	160	6
T10	Ni ₂ Pb ₁ Cd ₁	60	80	3
T11	Ni ₂ Pb ₁ Cd ₂	60	80	4.5
T12	Ni ₂ Pb ₁ Cd ₃	60	80	6
T13	Ni ₂ Pb ₂ Cd ₁	60	120	3
T14	Ni ₂ Pb ₂ Cd ₂	60	120	4.5
T15	Ni ₂ Pb ₂ Cd ₃	60	120	6
T16	Ni ₂ Pb ₃ Cd ₁	60	160	3
T17	Ni ₂ Pb ₃ Cd ₂	60	160	4.5
T18	Ni ₂ Pb ₃ Cd ₃	60	160	6
T19	Ni ₃ Pb ₁ Cd ₁	80	80	3
T20	Ni ₃ Pb ₁ Cd ₂	80	80	4.5
T21	Ni ₃ Pb ₁ Cd ₃	80	80	6
T22	Ni ₃ Pb ₂ Cd ₁	80	120	3
T23	Ni ₃ Pb ₂ Cd ₂	80	120	4.5
T24	Ni ₃ Pb ₂ Cd ₃	80	120	6
T25	Ni ₃ Pb ₃ Cd ₁	80	160	3
T26	Ni ₃ Pb ₃ Cd ₂	80	160	4.5
T27	Ni ₃ Pb ₃ Cd ₃	80	160	6
T28	(Control)	0	0	0

Determination of total chlorophyll content

Total chlorophyll contents were measured according to protocol described by Abbas and Abbas (1992). Fresh leaf samples were homogenized in 80% (v/v) acetone and the homogenates were filtered before spectrophotometric analysis. Absorbance of the extract was determined in UV-Visible spectrophotometer at 645 and 663 nm.

The concentration of total chlorophyll was estimated by the following equation:

$$\text{Total chlorophyll (mg L}^{-1}\text{)} = 20.2(A_{645}) + 8.02(A_{663})$$

Where A_{645} and A_{663} are the absorbance values obtained at 645 and 663 nm respectively.

The chlorophyll concentration was then converted to fresh weight using the equation below:

$$\text{Total chlorophyll (mg 100 g}^{-1}\text{ FW)} = (\text{mg L}^{-1}/1000) \times (100/\text{Fresh sample weight (g)})$$

Where FW = Fresh weight.

$$\text{Total soluble sugars (\%)} = \left(\frac{\text{Sugar concentration obtained from the standard curve} \times \text{Final extract volume}}{\text{Sample weight} \times 1000} \right) \times 100$$

If the concentration of the sugar is presented in mg mL⁻¹.

Determination of total protein content

The total protein content of fruits of the chili pepper was measured by the Folin-Lowry colorimetric method of Schacterle and Pollak (1973), modified from Lowry et al. (1951). The extracts of the fruit tissues were prepared and 0.1 mL of each extract was added to 0.9 mL of distilled water to make 1 mL. Folin reagent (5 mL) was then added and the reaction mixture was incubated in a water bath for 5 min at 55°C.

The absorbance was measured after incubation at 650 nm, with the help of a UV-Visible spectrophotometer. Protein concentration was determined by using the calibration curve with known concentrations of Bovine serum albumin (BSA) and expressed as a percentage of fresh fruit weight.

Statistical analysis

The experiment was conducted using a Randomized Complete Block Design (RCBD) with a factorial arrangement. Statistical analyses were performed using SAS software (Statistical Analysis System). A two-way analysis of variance (ANOVA) was used to evaluate the effects of genotypes, heavy metal treatments, and the genotype $\times$ treatment interaction. The significance of differences among treatment means was determined using Duncan's Multiple Range Test (DMRT) at the 5% probability level ($P \leq 0.05$), according to (Al-Rawi and Khalaf Allah, 1980).

Determination of total soluble sugar content

The total soluble sugar content was measured by the phenol-sulfuric acid method used by Joslyn (1970). Fresh chili pepper fruits were randomly picked and blended in 80 mL (80% (v/v) ethanol). The homogenate was heated at 60°C in a water bath for 30 min and then centrifuged at 3,000 rpm for 15 min. The supernatant was taken and the final volume was made up to 25 mL with perchloric acid. To 1 mL of extract, 1 mL 5% phenol solution was mixed and then 5 mL of concentrated sulfuric acid was added. The absorbance was measured after complete color development at 490 nm using UV-Visible spectrophotometer.

Standard curve for glucose was prepared by using known concentration of glucose and then soluble sugar concentration of each sample was calculated by using the standard curve.

The percentage of total soluble sugars was calculated as:

Results and Discussion

Effect of genotypes, heavy metal levels, and their interaction on total leaf chlorophyll content

The total leaf chlorophyll contents significantly varied between the two chili pepper genotypes as shown in Figure 1. The highest chlorophyll concentration was found in Cayenne genotype while ID15 genotype had the lowest concentration (2.532mg/gfw). This difference might be due to the genotype-dependent differences in tolerance to heavy metal stress. The better ability of Cayenne genotype to maintain high chlorophyll levels indicates its ability to maintain chloroplast integrity, to exclude metal accumulation or to neutralize metal-induced oxidative damage.

Heavy metal treatments also had significant effect (Figure 2). The highest total chlorophyll concentration (4.129 mg g⁻¹ fresh weight) was observed in the control treatment (T28 = Ni₀Pb₀Cd₀), while the chlorophyll concentration decreased with the increase of heavy metal concentration. The lowest value of 2.115 mg g⁻¹ fresh weight was recorded for T26 (Ni₃Pb₃Cd₂ = 80, 160, and 4.5 mg kg⁻¹ soil for Ni, Pb, and Cd, respectively). Disruption of photosynthetic machinery such as chloroplast ultrastructure, thylakoid membrane integrity and the impairment of Photosystem II activity caused by heavy metal concentrations is likely responsible for the reduction of chlorophyll content, thereby reducing the rate of

chlorophyll biosynthesis and increasing the rate of chlorophyll degradation. The results are consistent with those obtained by Sana et al. (2024).

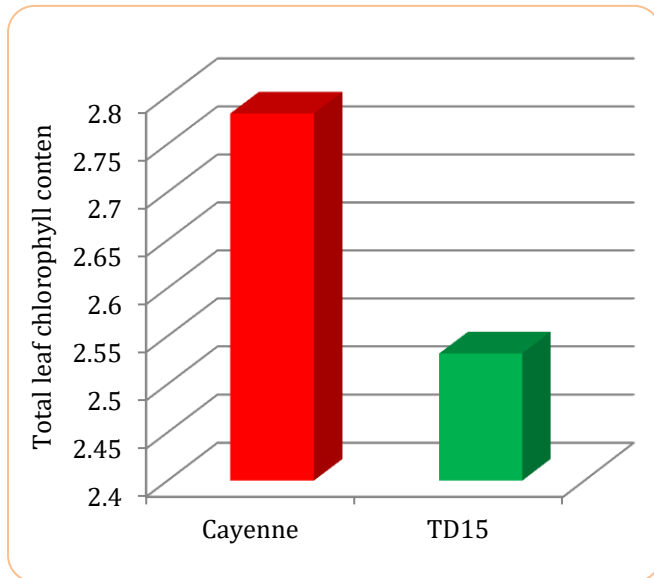


Figure 1. Total leaf chlorophyll content of two chili pepper genotypes under heavy metal stress.

There was also a significant genotype $\times$ treatment interaction (Figure 3). The Cayenne genotype recorded the highest total chlorophyll content (4.405 mg g⁻¹ fresh weight) under the control treatment (T28) while ID15

genotype recorded the lowest value (1.945 mg g⁻¹ fresh weight) under T26 treatment. The significant decrease in chlorophyll level in response to higher levels of heavy metals might be due to the damage to the chloroplasts, decrease in the activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), decrease in CO₂ fixation, and ultimately inhibition of carbon assimilation which lead to impaired photosynthetic activity. Similar findings have been reported by Ali et al. (2024), Zulfiqar et al. (2024), Usman et al. (2025) and Wang et al. (2026).

Effect of genotypes, heavy metal levels, and their interaction on total soluble sugar content

Genotypic differences in total soluble sugar contents were significant as revealed in the results given in Figure 4. The mean soluble sugar content for the ID15 genotype was found to be 2.544% while Cayenne genotype recorded mean soluble sugar content of 2.490%. The enhanced sugar accumulation in ID15 by retaining photosynthetic efficiency and carbon assimilation could be due to its greater ability to withstand the heavy metal stress. Soluble sugars also play a key role as osmoprotectants for osmotic adjustment, water retention at cellular level and maintenance of metabolic activity during heavy metal stress. Thus, higher levels of their accumulation show more efficient adaptive response of ID15 to heavy metal toxicity. These results are in line with those of Altaf et al. (2022).

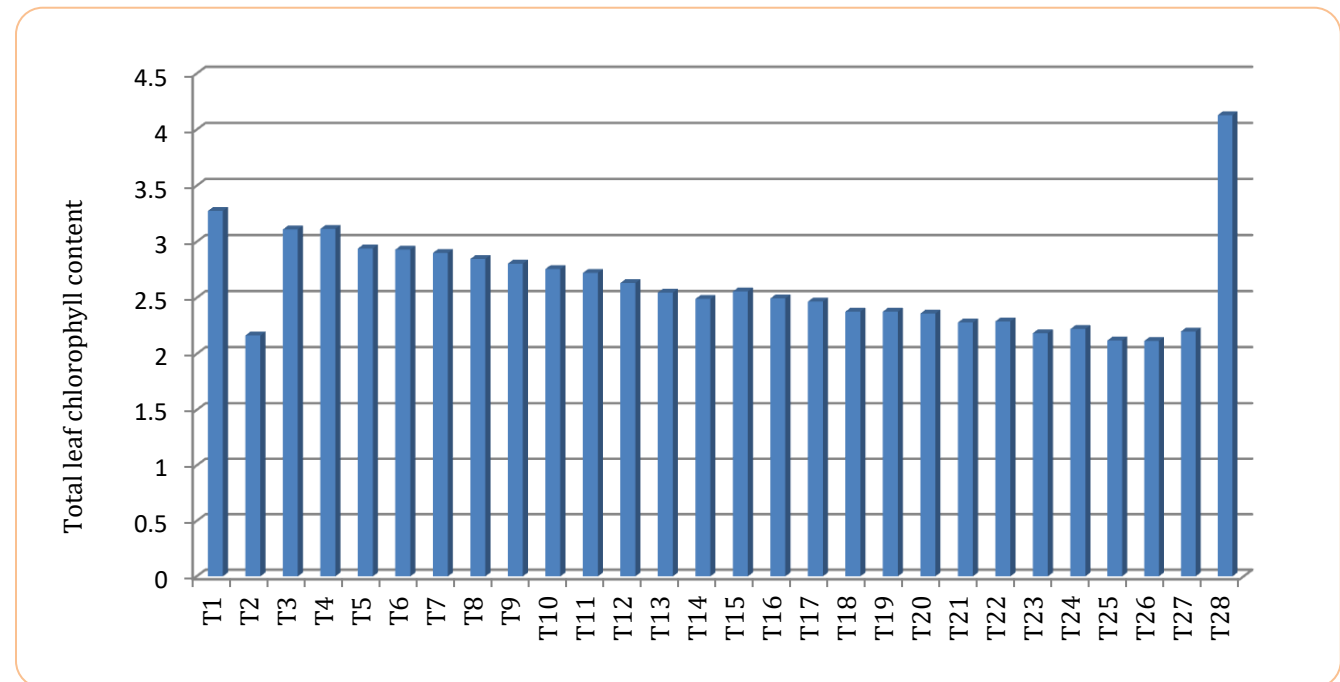


Figure 2. Effect of heavy metal treatments (Ni, Pb, and Cd) on total leaf chlorophyll content of chili pepper.

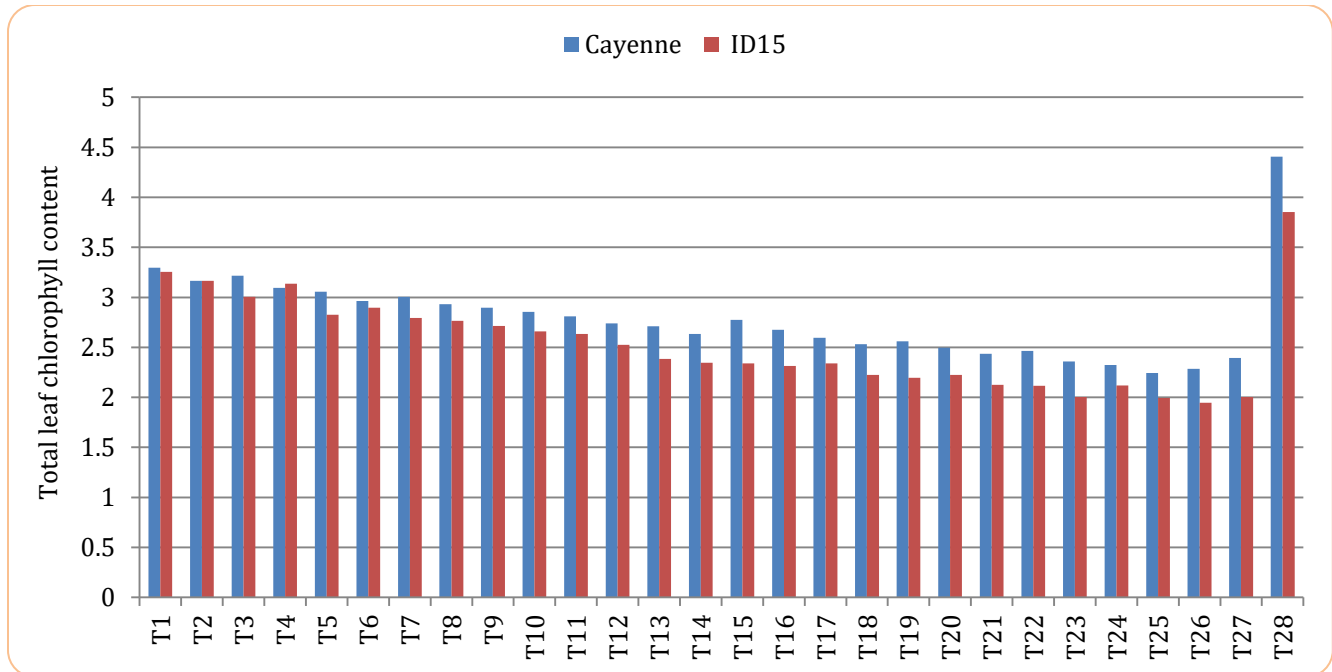


Figure 3. Interactive effects of chili pepper genotypes and heavy metal treatments on total leaf chlorophyll content (mg g⁻¹ fresh weight).

Total soluble sugar content was also greatly influenced by heavy metal treatments (Figure 5). The highest sugar concentration (2.942%) was observed under T1 (Ni₁Pb₁Cd₁; 40, 80, and 3 mg kg⁻¹ soil), followed by T2 (2.922%) and T3 (2.912%). Afterwards, the soluble sugar content decreased gradually with the increase of heavy metals up to the lowest level (2.087%) in T27 (Ni₃Pb₃Cd₃; 80, 160 and 6 mg kg⁻¹ soil). This decrease in sugar levels at higher concentrations of heavy metals, resulting in photosynthesis inhibition, might be linked to the inhibition of RuBisCO activity, disruption of the electron transport of the chloroplast and degradation of chlorophyll, which eventually reduce the carbohydrate biosynthesis. The results are consistent with those obtained by Altaf et al. (2021).

The interaction between heavy metal treatments and genotypes had significant effect on total soluble sugar content of chili pepper plants (Figure 6). In the Cayenne genotype, T1 had the highest sugar level of 2.945% while in ID15, the same was true for T2 with a sugar content of 2.945%. However, severe heavy metal stress caused a significant decrease in sugar content. In the Cayenne genotype, treatments T25 (Ni₃Pb₃Cd₁), T26 (Ni₃Pb₃Cd₂), and T27 (Ni₃Pb₃Cd₃) recorded 2.070%, 2.045%, and 2.080%, respectively. This indicates that ID15 has more effective physiological mechanisms to

maintain carbohydrate metabolism under heavy metal stress, which might be due to the improvement of photosynthetic efficiency and the improvement of antioxidant resistance system to reduce oxidative stress. The same findings have been reported by Yang et al., (2024).

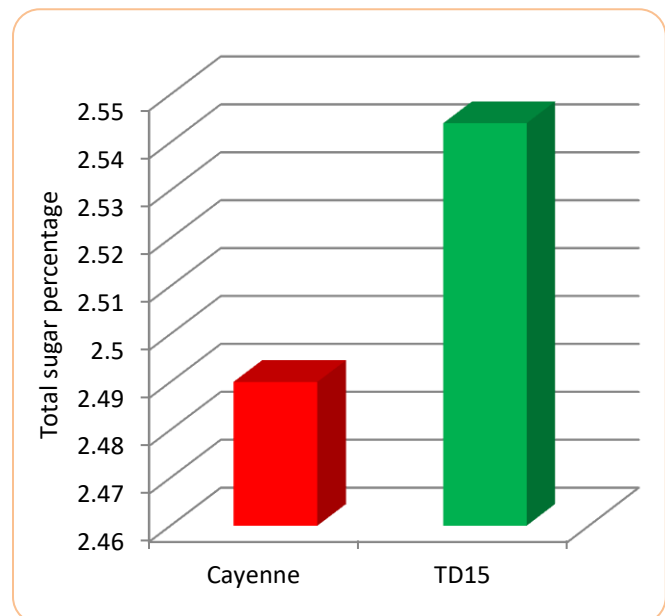


Figure 4. Genotypic variation in total soluble sugar content of chili pepper under heavy metal stress.

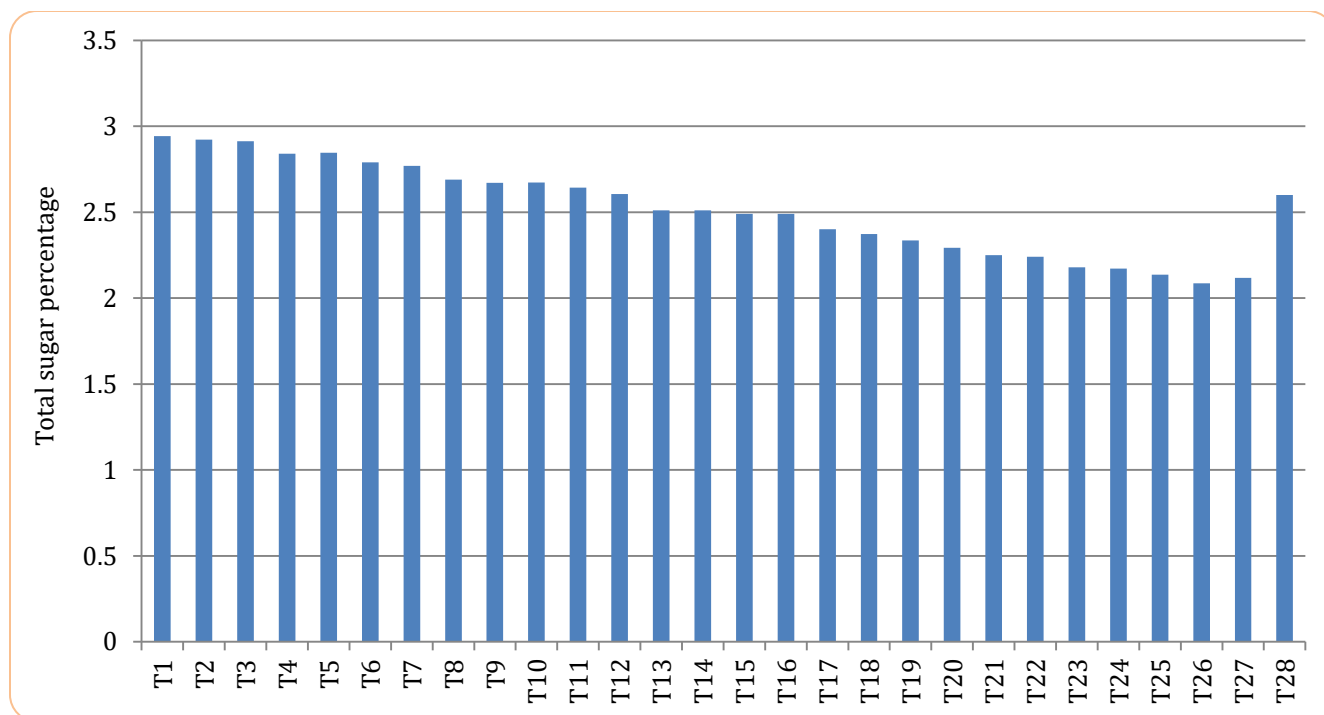


Figure 5. Effect of combined Ni, Pb, and Cd treatments on total soluble sugar content (%) in chili pepper.

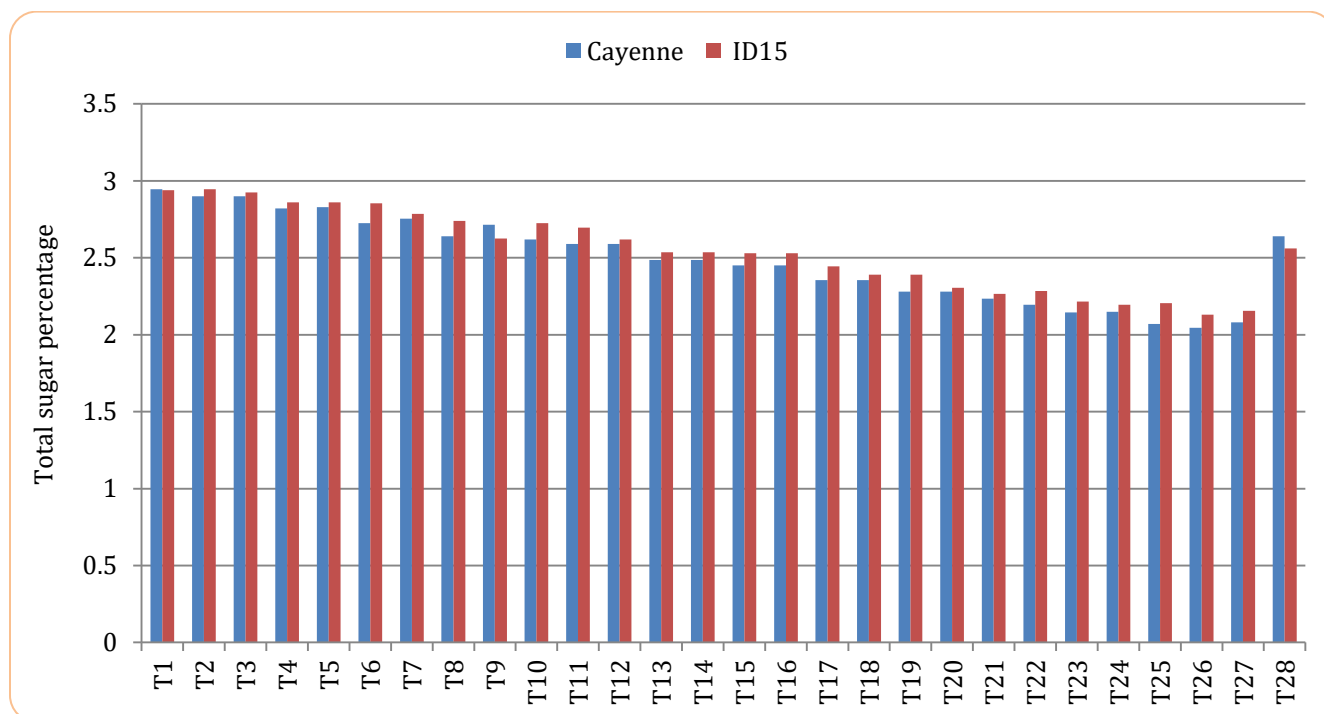


Figure 6. Effect of heavy metal treatments and genotype interactions on total soluble sugar contents of chili pepper plants.

Effect of genotypes, heavy metal levels, and their interaction on fruit protein content

As shown in the results given in the Figure 7, the genotypes did not significantly affect the fruit protein

content. The mean protein content for the Cayenne genotype was 1.087% while the mean protein content for ID15 was 1.082%. The lack of genotypic differences could be due to the similarities between the two

genotypes in terms of their genetic background and physiological response to the heavy metal stress.

Heavy metal treatments, on the other hand, had a significant effect on fruit protein content (Figure 8). The protein concentration decreased gradually with increasing levels of heavy metal with the lowest value (0.880%) observed for the treatment T27 ($\text{Ni}_3\text{Pb}_3\text{Cd}_3$; 80, 160, and 6 mg kg^{-1} soil), while the highest value (1.362%) was obtained for the control treatment T28 ($\text{Ni}_0\text{Pb}_0\text{Cd}_0$). This decrease is probably because of the inhibitory effect of the heavy metals on nitrogen metabolism, especially on the activity of nitrate reductase (NR), thus preventing the synthesis of amino acids and proteins. Furthermore, proteins have sulphhydryl (-SH) groups that easily interact with heavy metals, leading to enzyme inactivation, structural changes and increased protein degradation (Al-Rubaie, 2021).

There was a significant genotype $\times$ heavy metal interaction for fruit protein (Figure 9). The genotype ID15 had the lowest protein content (0.868%) under T22 ($\text{Ni}_3\text{Pb}_2\text{Cd}_1$; 80, 120 and 3 mg kg^{-1} soil), while the control treatment (T28) had the highest protein content (1.380%). This decrease in protein accumulation in the presence of high levels of heavy metals might be due to both direct inhibition of protein biosynthesis and/or

indirect increase in oxidative stress leading to increased protein oxidation and degradation. These results are similar to those obtained by Cheng et al. (2025).

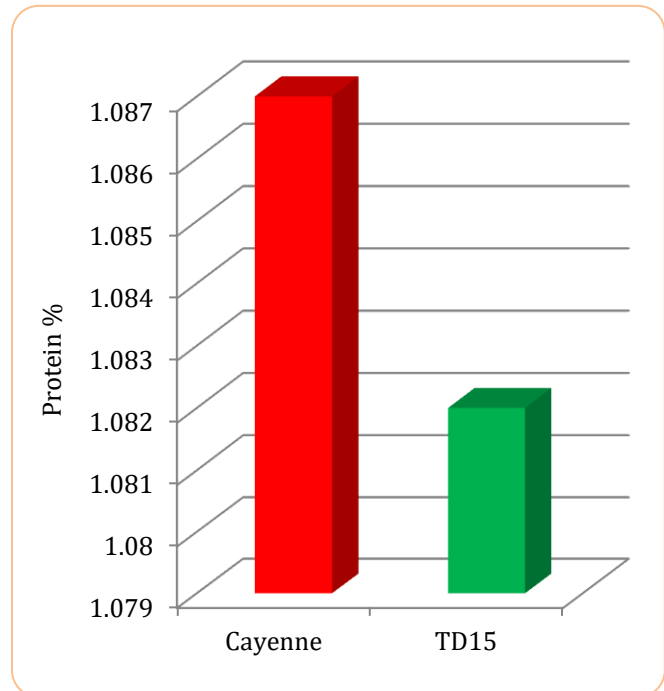


Figure 7. Effect of chili pepper genotypes on fruit protein content (%).

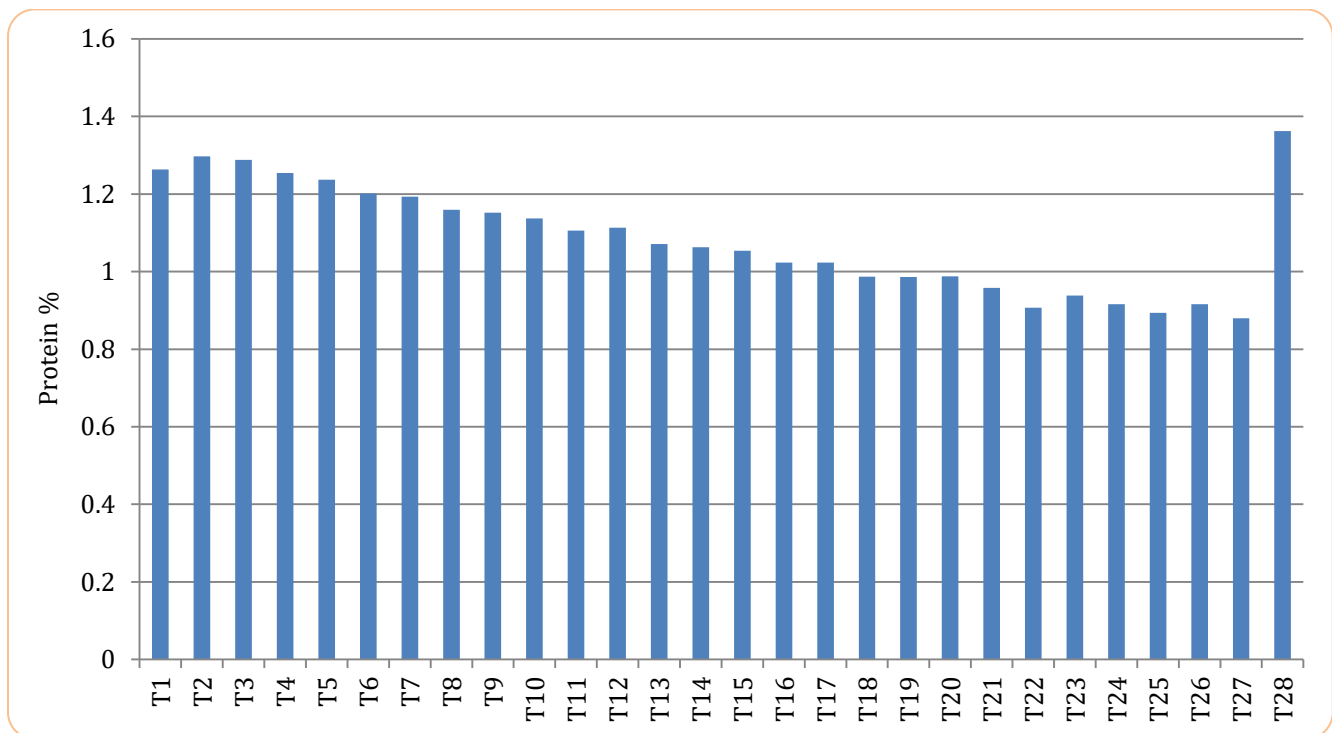


Figure 8. Effect of combined nickel, lead, and cadmium treatments on fruit protein content of chili pepper.

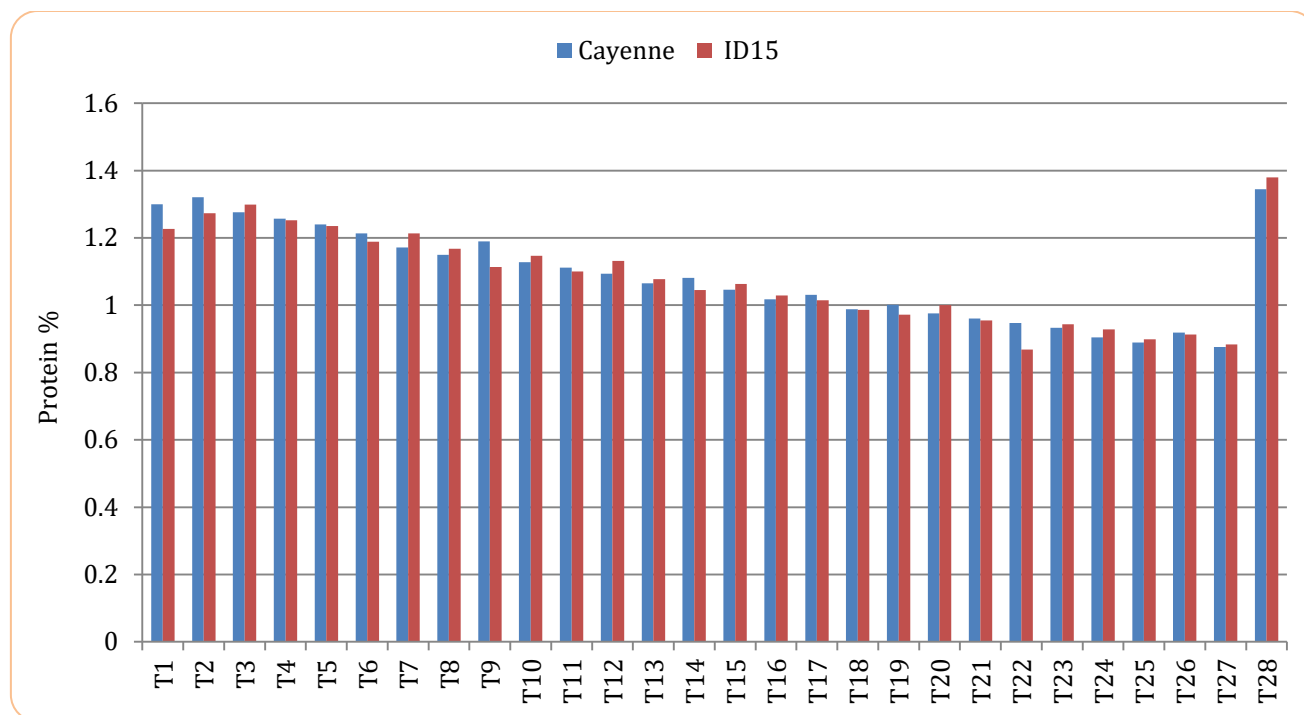


Figure 9. Effect of genotype $\times$ heavy metal interaction on fruit protein content (%) of chili pepper under different Ni, Pb, and Cd combinations.

Conclusion

Heavy metal stress significantly affected the physiological and biochemical characteristics of chili pepper genotypes. The Cayenne genotype maintained significantly higher leaf chlorophyll content than ID15, indicating superior tolerance to heavy metal stress through better preservation of chloroplast integrity and photosynthetic capacity. Conversely, ID15 accumulated higher levels of total soluble sugars, suggesting a more effective osmotic adjustment mechanism and greater metabolic adaptation under stress conditions. Fruit protein content was not significantly influenced by genotype but declined markedly with increasing concentrations of nickel, lead, and cadmium, demonstrating that heavy metal toxicity disrupts nitrogen metabolism and protein synthesis. Overall, the results indicate that Cayenne exhibits greater tolerance in maintaining photosynthetic pigments, whereas ID15 displays stronger biochemical adaptation through soluble sugar accumulation, highlighting distinct genotype-specific strategies for coping with heavy metal stress.

Author Contributions

AHM conceptualized and designed the study. AHM conducted the experimental work, performed field

investigations, collected and analyzed the data, and prepared the initial manuscript draft. SKY supervised the research, contributed to the experimental design, data interpretation, and critical revision of the manuscript. AMSH co-supervised the study, provided scientific guidance on data analysis and interpretation, and critically reviewed and revised the manuscript. All authors contributed to the intellectual content of the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

Policy Addressed

This study supports policies promoting heavy metal

monitoring in agricultural soils, the development of stress-tolerant *Capsicum annuum* cultivars, and sustainable soil management practices. Integrating physiological and biochemical traits into breeding programs can enhance crop resilience, improve food safety, and strengthen agricultural sustainability in metal-contaminated environments.

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