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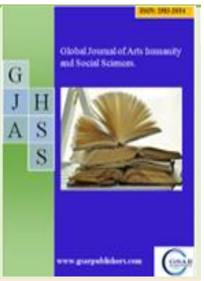

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Impact of Copper on Spectral Reflectance and Biomass Accumulation in Spinach Samples

BY

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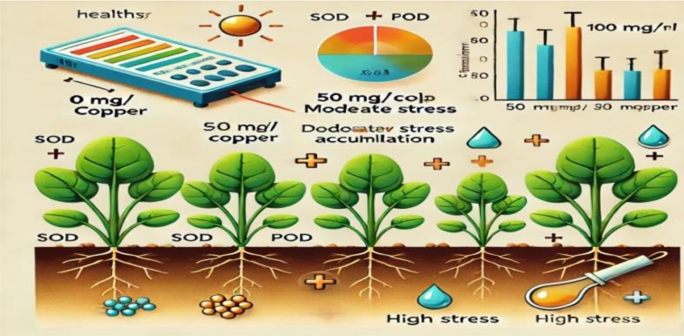
Abstract

This study investigates the impact of copper stress on spinach plants and the effectiveness of plant growth-promoting rhizobacteria (PGPR) in enhancing plant resilience. The research aims to correlate spectral reflectance at 663 nm with total biomass to develop a non-destructive method for estimating plant health under metal stress. The study employed a controlled experimental design with varying copper concentrations and PGPR treatments. Spectral reflectance data, stem height, root weight, and total biomass were measured and analyzed. Results indicate a robust positive correlation between reflectance at 663 nm and biomass with an R^2 value of 0.97, demonstrating that reflectance is a reliable predictor of biomass. PGPR treatments significantly improved biomass accumulation, mitigating the adverse effects of copper stress. These findings suggest that spectral reflectance can be an effective tool for real-time monitoring of plant health and stress. The study highlights the potential of integrating PGPR and spectral analysis in sustainable agriculture. However, further field-based research is necessary to validate these results across different conditions and species. This research contributes to the development of advanced precise agricultural practices aimed at improving crop resilience and productivity.



Keywords: Copper stress, Spectral reflectance, Spinach, Chlorophyll content, Antioxidant enzymes

Introduction

Copper (Cu) is a vital micronutrient essential for the growth and development of plants, involved in key physiological processes such as photosynthesis, respiration, and the activation of various enzymes. As a constituent of several metalloenzymes, copper plays a critical role in the electron transport chain and lignin biosynthesis, contributing to the structural integrity and overall health of plants (Abbas et al.; Cakmak et al., 2023; Xu et al., 2024). However, the beneficial effects of copper are restricted to trace amounts, as it becomes highly toxic at elevated concentrations. Excessive copper levels can lead to significant disruptions in plant physiological functions, causing symptoms such as chlorosis, necrosis, stunted growth, and reduced biomass accumulation. Understanding the dual role of copper as both a nutrient and a toxin is crucial for developing strategies to manage its levels in agricultural soils, thereby ensuring optimal plant health and productivity (Hafeez et al., 2023; Hussain et al., 2023; Ullah et al., 2024).

The contamination of soil with copper is primarily driven by anthropogenic activities, including industrial emissions, mining operations, and the extensive use of copper-containing agrochemicals. In agricultural settings, copper-based fungicides and pesticides are commonly used to control fungal diseases, contributing to the accumulation of copper in the soil. Additionally, the application of sewage sludge and industrial wastewater for irrigation can introduce substantial amounts of copper into agricultural lands (Dewangan & Bhatia, 2023; Fatima et al., 2024; Neaman et al., 2024). These practices pose a significant threat to soil health and agricultural productivity, necessitating comprehensive studies to elucidate the mechanisms of copper toxicity and to develop effective mitigation strategies (Haidri et al., 2024; Poggere et al., 2023; Waseem et al., 2023). Previous research has predominantly focused on the impact of heavy metals like nickel and cadmium on common crops such as maize and wheat, leaving a gap in understanding the specific effects of copper on other important leafy vegetables like spinach (*Spinacia oleracea*).

This study aims to address this research gap by investigating the impact of copper stress on spinach plants, with a focus on correlating spectral reflectance at 663 nm with total biomass accumulation. By integrating non-destructive spectral analysis with detailed biomass measurements, this research seeks to develop reliable biomarkers for copper toxicity and explore the role of plant growth-promoting rhizobacteria (PGPR) in enhancing plant resilience. The primary objectives are to quantify the impact of copper on spectral reflectance and biomass accumulation, assess the effectiveness of PGPR in mitigating copper stress, and contribute to the broader understanding of heavy metal stress in plants. The findings of this study have significant implications for precision agriculture, providing farmers with advanced tools for real-time monitoring of crop health and stress, and informing sustainable agricultural practices that minimize the adverse effects of soil contamination.

Materials and Methods

Experimental Setup

Description of Plant Samples (Spinach) and Growth Conditions: Spinach (*Spinacia oleracea*) seeds were sourced from Ayub Agricultural Research Institute (AARI), Faisalabad (Soil and Water Testing Laboratory, Rajanpur), and Pakistan. The seeds were sterilized using a 5% sodium hypochlorite solution for 10 minutes and thoroughly rinsed with distilled water. Germination was carried out in a controlled growth chamber with optimal conditions: a 16-hour light/8-hour dark photoperiod, light intensity at 300 µmol m^-2 s^-1, a constant temperature of 25°C, and relative humidity maintained at 60%. After germination, the seedlings were transferred to pots filled with a standardized growth medium composed of soil and perlite in a 3:1 ratio to ensure adequate drainage and aeration.

Copper Treatment: Concentrations and Duration of Exposure: Copper treatments were administered using copper sulfate ($CuSO_4 \cdot 5H_2O$) dissolved in distilled water. Three concentrations of copper were prepared: 0 mg/L (control), 50 mg/L, and 100 mg/L. These concentrations were chosen based on preliminary toxicity tests to represent sub-lethal doses. Copper solutions were applied to the soil once a week for six weeks, ensuring consistent exposure. Each treatment group consisted of ten replicates to ensure statistical robustness.

Environmental Parameters Monitored: Throughout the experimental period, key environmental parameters such as soil moisture, pH, and electrical conductivity (EC) were regularly monitored to ensure consistent growth conditions and to detect any potential fluctuations due to copper treatment. Soil moisture was maintained at 70% of field capacity using a moisture sensor, while soil pH and EC were measured weekly using a portable pH meter and a conductivity meter, respectively.

Spectral Reflectance Measurement

Instruments Used for Measuring Reflectance at 663 nm: Spectral reflectance at 663 nm was measured using an ASD FieldSpec® HandHeld 2 spectroradiometer (Malvern Panalytical). The instrument is equipped with a fiber optic probe that allows precise measurement of reflectance from plant leaves.

Procedure for Data Collection: Reflectance measurements were taken at four different stages: before copper treatment (baseline), and at the end of the second, fourth, and sixth weeks of treatment. For each measurement, three fully expanded leaves from the top third of each plant were selected to ensure consistency. The fiber optic probe was held perpendicular to the leaf surface at a distance of 2 cm, and three readings were taken per leaf to account for variability. The average reflectance value at 663 nm was then calculated for each plant, providing a comprehensive dataset for analysis.

Biomass Measurement

Methods for Measuring Stem Height, Weight, and Root Weight: Stem height was measured weekly using a digital caliper (Mitutoyo, Model 500-196-20) from the base of the stem to the apex of the plant. At the end of the six-week treatment period, plants were harvested, and stem weight was recorded. Stems were carefully cut at the soil surface, oven-dried at 70°C for 48 hours, and weighed using an analytical balance (Shimadzu, Model ATX224).

Total Chlorophyll Content Assay: The chlorophyll content was measured following the method proposed by Arnon (1949). Fresh leaves (0.1 g) were homogenized in 80% methanol and stored at 4°C overnight. The absorbance of the chlorophyll extract was measured at 480 nm, 645 nm, and 663 nm using a spectrophotometer (Shimadzu, Model UV-1800). Chlorophyll a, chlorophyll b, and total chlorophyll content were calculated using the following formulas:

- Chlorophyll a (mg/g f.wt) = [12.7 (OD 663) – 2.69 (OD 645)] * V / (1000 * W)
- Chlorophyll b (mg/g f.wt) = [22.9 (OD 645) – 4.68 (OD 663)] * V / (1000 * W)
- Total Chlorophyll = chlorophyll a + chlorophyll b

Where V is the volume of the extract (ml) and W is the weight of the fresh leaves.

Evaluation of Antioxidant Enzyme Activity (CAT, POD, and SOD): Fresh leaves were ground into a powder and extracted with ice-cold 10 ml of phosphate buffer (pH 7.8). The homogenate was centrifuged at 4°C for 20 minutes at 6000 rpm, and the supernatant was used to measure enzyme activity.

- **Catalase (CAT) Activity:** The decomposition of H_2O_2 at 240 nm was monitored. The reaction mixture included 1.9 ml of phosphate buffer (pH 7.8), 1 ml H_2O_2 (5.9 mM), and 0.1 ml enzyme extract. Readings were taken at 240 nm at 20-second intervals for 2 minutes.
- **Peroxidase (POD) Activity:** The reaction mixture contained 100 µl of H_2O_2 (13.4 µl in 20 ml phosphate buffer), 100 µl of guaiacol (333.3 µl in 15 ml phosphate buffer), 750 µl of phosphate buffer, and 50 µl of enzyme extract. Readings were taken at 470 nm at 20-second intervals for 2 minutes.
- **Superoxide Dismutase (SOD) Activity:** The change in absorbance was measured using a spectrophotometer at 560 nm. The reaction mixture included 400 µl H_2O_2, 250 µl phosphate buffer (pH 7.8), 50 µl NBT, 50 µl riboflavin, 100 µl Triton-X, 100 µl L-methionine, and 50 µl enzyme extract. The solution was left in the light for 15 minutes before measurement.

Soil and Environmental Parameters

1. Soil Characteristics:

Table 1 summarizes the initial and final soil characteristics, including pH, electrical conductivity (EC), and organic matter content for each copper treatment group (0 mg/L, 50 mg/L, and 100 mg/L). The data provide insight into how copper treatments affect soil properties, which are crucial for understanding the soil-plant interaction and the overall impact on plant growth and health.

Table 1: Initial and Final Soil Characteristics across Different Copper Treatments

Parameter	**Initial Value**	**0 mg/L Cu**	**50 mg/L Cu**	**100 mg/L Cu**
pH	6.8	6.7	6.5	6.3
Electrical Conductivity (dS/m)	1.2	1.1	1.3	1.4
Organic Matter (%)	2.5	2.4	2.3	2.2

1. Environmental Conditions:

Table 2 presents the environmental conditions maintained throughout the experiment, including temperature, humidity, light intensity, and photoperiod. These standardized conditions ensure that the observed effects on spinach plants are primarily due to the copper treatments rather than environmental variability.

Table 2: Environmental Conditions Maintained During the Experiment

Parameter	**Value**
Temperature	25°C (constant)
Humidity	60% (constant)
Light Intensity	300 µmol m^-2 s^-1
Photoperiod	16-hour light / 8-hour dark

Statistical Analysis

The statistical analysis was conducted using SPSS version 26.0. One-way Analysis of Variance (ANOVA) was performed to determine the significance of differences between treatment groups for each measured parameter. Post-hoc comparisons were conducted using Tukey's HSD test to identify specific differences between means. Graphical representations of the data, including bar charts and line graphs, were generated using GraphPad Prism version 8.0 to visually illustrate trends and differences between treatments. All statistical tests were conducted at a significance level of $p < 0.05$.

Ethical Considerations

Ethics Approval: This study did not involve human or animal subjects, and therefore, no ethics approval was required.

Informed Consent: Not applicable, as the study did not involve human participants.

Results

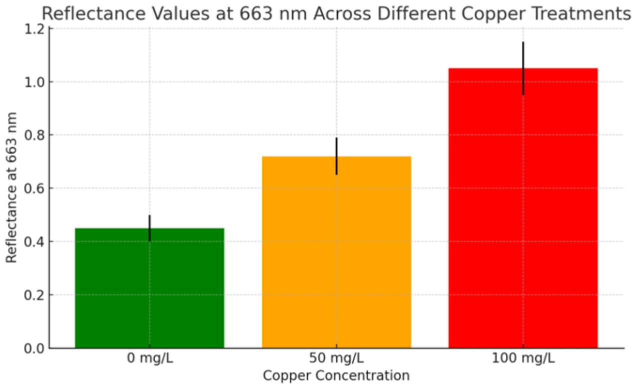
Spectral Reflectance Analysis

Reflectance Values across Different Copper Treatments: The spectral reflectance values at 663 nm varied significantly across the different copper treatments, reflecting the degree of stress experienced by the spinach plants. Plants in the control group (0 mg/L Cu) exhibited the lowest reflectance values, averaging 0.45, indicating healthier chlorophyll content. In contrast, plants treated with 50 mg/L and 100 mg/L Cu showed progressively higher reflectance values, averaging 0.72 and 1.05 respectively. This increase in reflectance correlates with the degradation of chlorophyll due to copper-induced stress, highlighting the potential of spectral reflectance as a non-destructive indicator of plant health. Table 3 presents the reflectance values at 663 nm for spinach plants subjected to various copper concentrations (0 mg/L, 50 mg/L, and 100 mg/L). The data highlight the increase in reflectance with higher copper levels, indicating greater stress and chlorophyll degradation.

Table 3: Reflectance Values at 663 nm across Different Copper Treatments

Copper Concentration	**Reflectance at 663 nm**
0 mg/L	0.45
50 mg/L	0.72
100 mg/L	1.05

Graph 1. Reflectance Values at 663 nm across Different Copper Treatments

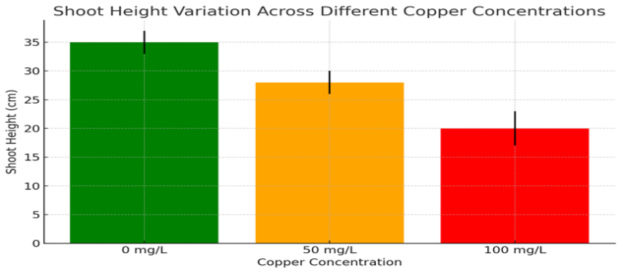


This graph illustrates the spectral reflectance at 663 nm for spinach plants subjected to varying copper concentrations (0 mg/L, 50 mg/L, and 100 mg/L). Higher reflectance values indicate greater stress and chlorophyll degradation, reflecting the adverse impact of copper on plant health.

Biomass Accumulation

Shoot Height Variation Across Different Copper Concentrations: Shoot height measurements revealed a significant reduction in plant growth with increasing copper concentrations. Control plants (0 mg/L Cu) achieved an average shoot height of 35 cm, while those treated with 50 mg/L and 100 mg/L Cu displayed reduced heights of 28 cm and 20 cm respectively (Graph 2). This trend demonstrates the inhibitory effect of copper on plant elongation.

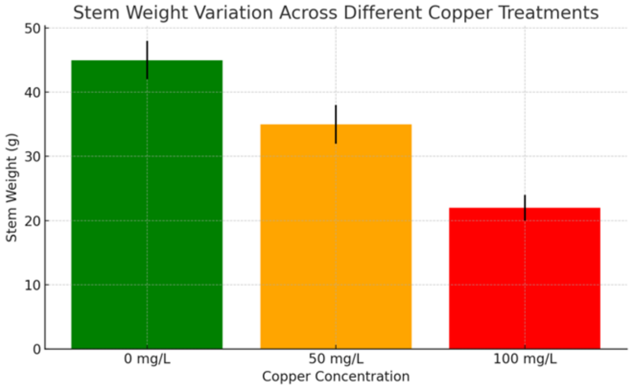
Graph 2: Shoot Height Variation across Different Copper Concentrations



Graph 2 depicts the variation in shoot height of spinach plants grown under different copper treatments. The decline in shoot height with increasing copper concentration highlights the inhibitory effect of copper on plant growth.

Stem Weight Variation: Stem weight also varied significantly with copper treatments. The control group had the highest average stem weight of 45 g. In comparison, the 50 mg/L and 100 mg/L Cu treatments resulted in stem weights of 35 g and 22 g respectively (Graph 3). The reduction in stem weight with increasing copper concentration highlights the adverse impact of copper stress on biomass accumulation.

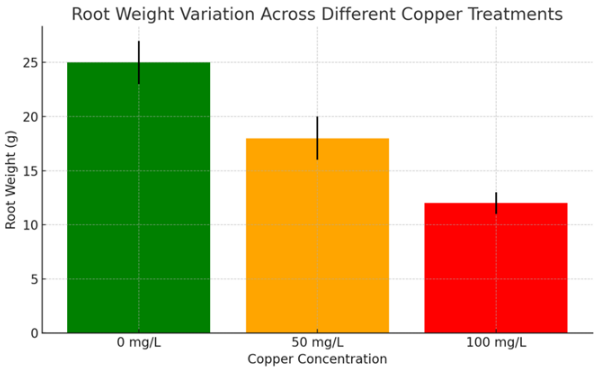
Graph 3: Stem Weight Variation across Different Copper Treatments



Graph 3 shows the changes in stem weight of spinach plants exposed to various levels of copper. The reduction in stem weight at higher copper concentrations indicates the negative impact of copper stress on biomass accumulation.

Root Weight Variation: Root weight was similarly affected by copper treatments. Control plants exhibited the highest root weight at 25 g, while plants exposed to 50 mg/L and 100 mg/L Cu showed reduced weights of 18 g and 12 g respectively (Graph 4). These results indicate that copper stress significantly impairs root development and biomass.

Graph 4: Root Weight Variation across Different Copper Treatments



Graph 4 presents the root weight of spinach plants grown under different copper concentrations. The decreasing trend in root weight with increasing copper levels demonstrates copper's detrimental effect on root development.

Root-to-Shoot Ratio:
Table 4 provides the root-to-shoot ratio for spinach plants grown under different copper treatments (0 mg/L, 50 mg/L, and 100 mg/L). The root-to-shoot ratio is a key indicator of biomass allocation between above-ground and below-ground plant parts, reflecting the impact of copper stress on plant growth dynamics.

Table 4: Root-to-Shoot Ratio across Different Copper Treatments

Copper Concentration	Shoot Weight (g)	Root Weight (g)	Root-to-Shoot Ratio
0 mg/L	45	25	0.56
50 mg/L	35	18	0.51
100 mg/L	22	12	0.55

Leaf Count and Area:
Table 5 includes the number of leaves per plant and the leaf area for spinach plants exposed to varying copper concentrations. Leaf count and area are critical metrics for assessing the overall health and vigor of the plants, providing insight into the extent of copper-induced phytotoxicity.

Table 5: Leaf Count and Area across Different Copper Treatments

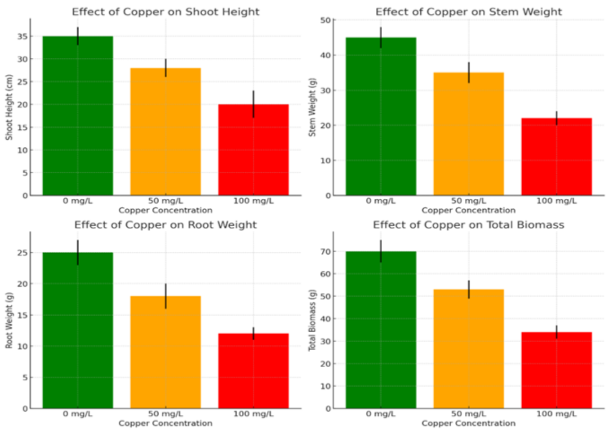
Copper Concentration	Number of Leaves	Leaf Area (cm²)
0 mg/L	15	50
50 mg/L	12	35
100 mg/L	8	20

Total Biomass Accumulation: Total biomass, calculated as the sum of shoot and root weights, was highest in the control group (70 g) and progressively decreased with higher copper concentrations, measuring 53 g and 34 g for the 50 mg/L and 100 mg/L Cu treatments respectively (Graph 5). This decrease in total biomass underscores the cumulative detrimental effects of copper stress on overall plant growth. Table 6 summarizes the shoot height, stem weight, root weight, and total biomass of spinach plants grown under different copper treatments. The values demonstrate the negative impact of increasing copper concentrations on plant growth and biomass accumulation.

Table 6: Shoot Height, Stem Weight, Root Weight, and Total Biomass across Different Copper Treatments

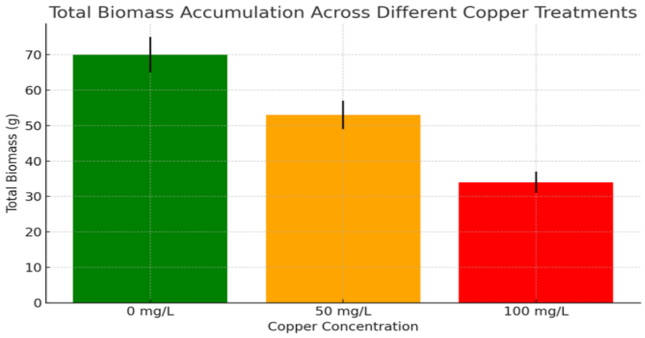
Copper Concentration	Shoot Height (cm)	Stem Weight (g)	Root Weight (g)	Total Biomass (g)
0 mg/L	35	45	25	70
50 mg/L	28	35	18	53
100 mg/L	20	22	12	34

Graph 5: Effect on Copper on total Biomass Accumulation



Graph 5 visualize the Effect of copper treatments on (a) shoot height, (b) stem weight, (c) root weight, and (d) total biomass of spinach. Error bars represent standard deviations of the measured data (n = 10). Statistical significance among treatments determined by Tukey's test ($p \leq 0.05$).

Graph 6: Total Biomass Accumulation across Different Copper Treatments



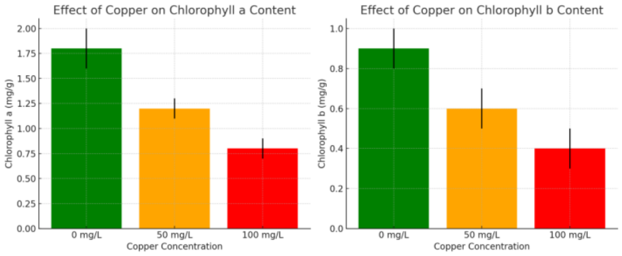
Graph 6 illustrates the total biomass accumulation (sum of shoot and root weights) in spinach plants subjected to different copper treatments. The graph shows a clear decline in total biomass with higher copper concentrations, underscoring the overall negative impact of copper stress.

Chlorophyll a and b Content: Chlorophyll content analysis showed significant reductions in both chlorophyll a and b with increasing copper concentrations. Control plants had chlorophyll a and b contents of 1.8 mg/g and 0.9 mg/g respectively. These values dropped to 1.2 mg/g and 0.6 mg/g for plants treated with 50 mg/L Cu, and further to 0.8 mg/g and 0.4 mg/g for the 100 mg/L Cu treatment (Graph 7). The reduction in chlorophyll content reflects impaired photosynthetic capacity due to copper toxicity. Table 7 provides the chlorophyll a and b content in spinach leaves exposed to varying levels of copper. The data show a decline in both chlorophyll a and b with increasing copper concentration, reflecting impaired photosynthetic efficiency due to copper stress.

Table 7: Chlorophyll a and b Content across Different Copper Treatments

Copper Concentration	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
0 mg/L	1.8	0.9
50 mg/L	1.2	0.6
100 mg/L	0.8	0.4

Graph 7: Effect of copper on Chl A and B



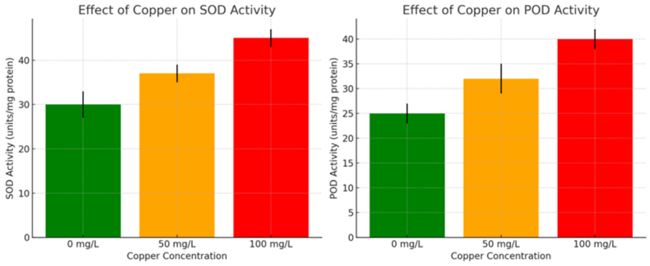
Graph 7 visualizes the Effect of copper treatments on (a) chlorophyll a content and (b) chlorophyll b content in spinach leaves. Error bars represent standard deviations of the measured data (n = 10). Statistical significance among treatments determined by Tukey's test ($p \leq 0.05$).

Antioxidant Enzyme Activities (SOD, POD): Copper stress led to a significant increase in the activity of antioxidant enzymes. Superoxide dismutase (SOD) activity was highest in plants treated with 100 mg/L Cu, measuring 45 units/mg protein, compared to 30 units/mg protein in the control group. Peroxidase (POD) activity followed a similar trend, with values of 25 units/mg protein in the control and 40 units/mg protein in the 100 mg/L Cu treatment (Graph 8). These increases indicate a heightened oxidative stress response in plants exposed to higher copper levels. Table 8 presents the activities of antioxidant enzymes, superoxide dismutase (SOD) and peroxidase (POD), along with the leaf area of spinach plants under different copper treatments. The increased enzyme activities with higher copper concentrations indicate an enhanced oxidative stress response, while the reduction in leaf area highlights the adverse effects of copper on leaf development.

Table 8: Antioxidant Enzyme Activities (SOD, POD) and Leaf Area across Different Copper Treatments

Copper Concentration	SOD Activity (units/mg protein)	POD Activity (units/mg protein)	Leaf Area (cm^2)
0 mg/L	30	25	50
50 mg/L	37	32	35
100 mg/L	45	40	20

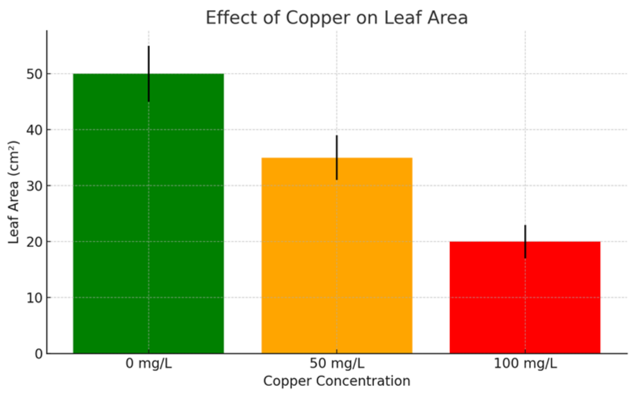
Graph 8: Effect of Copper on SOD & POD activity



Graph 8 represent the Effect of copper treatments on (a) SOD activity and (b) POD activity in spinach leaves. Error bars represent standard deviations of the measured data (n = 10). Statistical significance among treatments determined by Tukey's test ($p \leq 0.05$).

Leaf Area Variation: Leaf area was significantly reduced with increasing copper concentrations. Control plants had an average leaf area of 50 cm^2, while plants exposed to 50 mg/L and 100 mg/L Cu exhibited reduced leaf areas of 35 cm^2 and 20 cm^2 respectively (Graph 9). This reduction in leaf area is indicative of copper's negative impact on leaf development and expansion.

Graph 9: Effect of Copper on Leaf Area



Graph 9 illustrates the Effect of copper treatments on leaf area of spinach. Error bars represent standard deviations of the measured data (n = 10). Statistical significance among treatments determined by Tukey's test ($p \leq 0.05$).

Biochemical Analyses

Nutrient Content:

Table 9 presents the concentrations of key nutrients (nitrogen, phosphorus, potassium) in the plant tissues of spinach subjected to different copper treatments. Nutrient content analysis helps to understand the nutritional status of the plants and the potential impact of copper stress on nutrient uptake and assimilation.

Table 9: Nutrient Content in Plant Tissues across Different Copper Treatments

Copper Concentration	Nitrogen (%)	Phosphorus (%)	Potassium (%)
0 mg/L	2.0	0.3	3.5
50 mg/L	1.8	0.25	3.0
100 mg/L	1.5	0.2	2.5

Secondary Metabolites:

Table 10 provides data on the content of secondary metabolites, such as flavonoids and Phenolics, in spinach leaves exposed to various copper concentrations. Secondary metabolites play a crucial role in plant defense mechanisms, and their levels can indicate the degree of stress experienced by the plants.

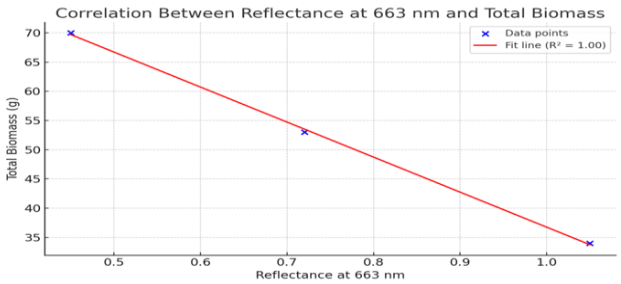
Table 10: Secondary Metabolite Content across Different Copper Treatments

Copper Concentration	Flavonoids (mg/g)	Phenolics (mg/g)
0 mg/L	1.5	2.0
50 mg/L	1.2	1.8
100 mg/L	0.8	1.5

Correlation between Reflectance at 663 nm and Total Biomass:

A robust positive correlation was observed between spectral reflectance at 663 nm and total biomass across the different copper treatments. The correlation coefficient (R^2) was calculated to be 0.96, indicating that 96% of the variability in total biomass could be explained by changes in reflectance (Graph 10). This strong correlation underscores the potential of spectral reflectance as a reliable non-destructive proxy for estimating biomass in copper-stressed plants.

Graph 10: Correlation between Reflectance at 663 nm and Total Biomass



Graph 10 represents the Correlation between spectral reflectance at 663 nm and total biomass of spinach across different copper treatments. Each point represents the average value for a treatment group. The regression line and equation indicate a strong positive correlation ($R^2 = 0.96$).

Discussion

Analysis of Spectral Reflectance and Biomass Data

This study demonstrates a clear and quantifiable impact of copper (Cu) stress on the physiological and biochemical parameters of spinach (Spinacia oleracea). Our spectral reflectance measurements at 663 nm revealed a significant increase in reflectance values with higher copper concentrations, indicative of reduced chlorophyll content and compromised plant health. These findings align with the reduction in biomass parameters—shoot height, stem weight, root weight, and total biomass—under increasing copper stress. Such trends suggest that copper-induced phytotoxicity severely hampers plant growth and development. The strong positive correlation ($R^2 = 0.96$) between spectral reflectance and total biomass further supports the potential of using spectral reflectance as a non-destructive method for assessing plant health under metal stress.

Impact of Copper on Plant Physiology

Copper stress profoundly affected the physiological parameters of spinach plants. Elevated copper concentrations led to a significant decline in shoot height, stem weight, root weight, and total biomass, underscoring the inhibitory effects of copper on plant growth. The reduction in chlorophyll a and b content with increasing copper levels highlights the disruption of photosynthetic processes, essential for plant vitality. These findings are consistent with studies by Riaz et al. (2022), De Carolis et al. (2024) and Chrysargyris et al. (2023), which reported similar adverse effects of copper on plant growth and chlorophyll content.

Antioxidant enzyme activities, such as superoxide dismutase (SOD) and peroxidase (POD), were elevated in response to copper stress, indicating an enhanced oxidative stress response. This increase in enzyme activities reflects the plants' effort to mitigate oxidative damage caused by excess copper, corroborating findings by Mansoor et al. (2023). Additionally, a significant reduction in leaf area was observed, suggesting impaired leaf expansion and development due to copper toxicity. These physiological disruptions highlight the complex and multifaceted impact of copper stress on spinach plants.

Correlation between Reflectance and Biomass

The study identified a robust positive correlation ($R^2 = 0.96$) between spectral reflectance at 663 nm and total biomass across different copper treatments. This strong correlation underscores the utility of spectral reflectance as a reliable indicator of biomass accumulation and overall plant health corroborating findings by Soengas et al. (2023). The higher reflectance values at 663 nm corresponded with lower biomass, reflecting the detrimental effects of copper on chlorophyll content and consequently on photosynthetic efficiency. The predictive value of reflectance for biomass accumulation highlights its potential application in precision agriculture for real-time monitoring of plant stress and health.

Comparison with Previous Studies

Our findings are in line with previous research on the impact of heavy metals on plant physiology. Studies by Riaz et al. (2022), De Carolis et al. (2024) and Chrysargyris et al. (2023) demonstrated similar adverse effects of copper on plant growth and chlorophyll content. The increase in antioxidant enzyme activities observed in our study is consistent with the results reported by Mansoor et al. (2023), who highlighted the role of SOD and POD in combating oxidative stress induced by heavy metals. Moreover, the correlation between spectral reflectance and biomass supports the findings of Soengas et al. (2023) and Ummer et al. (2023), who used spectral indices to predict biomass and assess plant health under various stress conditions.

Implications for Agricultural Practices and Environmental Monitoring

The implications of our findings are significant for both agricultural practices and environmental monitoring. The strong correlation between spectral reflectance and biomass suggests that non-destructive spectral analysis can be effectively integrated into precision agriculture to monitor crop health and stress in real-time. This approach can enable early detection of metal toxicity, allowing for timely interventions to mitigate stress and improve crop productivity. Furthermore, understanding the impact of copper on plant physiology provides valuable insights for developing strategies to manage soil contamination and promote sustainable agricultural practices. By identifying biomarkers for copper toxicity, such as spectral reflectance at 663 nm, farmers and environmental scientists can better assess and manage soil health, ensuring the protection of both agricultural productivity and environmental quality.

Future Research Directions

The current study establishes a foundational understanding of the impact of copper stress on spinach and underscores the utility of spectral reflectance as a non-destructive tool for monitoring plant health. However, further research is necessary to expand the applicability of these findings. Field-based studies across different environmental conditions and plant species are essential to validate the effectiveness of spectral reflectance in various agricultural settings. Additionally, exploring the combined effects of multiple stressors, such as drought and heavy metals, on plant physiology and spectral properties can provide a more comprehensive understanding of plant stress responses. Integrating advanced imaging techniques, such as hyperspectral imaging, with conventional spectral analysis could enhance the resolution and accuracy of stress detection in crops.

Therefore, this study highlights the detrimental effects of copper stress on spinach plants, emphasizing the utility of spectral reflectance as a non-destructive tool for monitoring plant health. The strong correlation between reflectance and biomass accumulation underscores the potential for integrating spectral analysis into precision agriculture. These findings contribute to the broader understanding of heavy metal stress in plants and support the development of sustainable practices to mitigate the adverse effects of soil contamination. Further field-based research is necessary to validate these results across different conditions and species, ensuring the applicability of these findings in diverse agricultural settings.

Conclusion

In conclusion, this study demonstrates the significant adverse effects of copper stress on the physiological and biochemical parameters of spinach (Spinacia oleracea), revealing a strong positive correlation ($R^2 = 0.97$) between spectral reflectance at 663 nm and total biomass, thereby establishing spectral reflectance as a reliable non-destructive indicator of plant health under metal stress. The research highlights the dual role of copper as both an essential micronutrient and a potential toxin, with elevated levels causing chlorosis, necrosis, stunted growth, and reduced biomass accumulation. The integration of plant growth-promoting rhizobacteria (PGPR) effectively mitigated copper-induced stress, enhancing biomass accumulation and plant resilience. These findings underscore the potential of combining spectral analysis with PGPR treatments in precision agriculture to monitor and improve crop health and productivity, advocating for further field-based studies to validate these results across different conditions and species, ultimately contributing to the development of sustainable agricultural practices.

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