

Comparative Effects of NiSO_4 , ZnSO_4 , and CuSO_4 on Chlorophyll a and b in Hydroponically Cultivated *Spinacia oleracea*

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Introduction

This investigation was chosen to see the effects of heavy metal salts on the Chlorophyll concentrations in plants because of the increasing levels of toxicity caused due to heavy metals in waterbodies, thereby affecting marine plants (Tchounwou et al. 2014). The issue of soil contamination by heavy metals is widespread and poses significant risks to plant health and productivity – something which was observed in an article on Farmer Strikes in North India (Sharma 2024). Therefore, it was decided to see the impact of Heavy metal contamination negatively on crop yield and quality, and high levels in the soil can lead to reduced plant productivity, stunted growth, and lower yields which can be measured by the chlorophyll concentrations in a plant (Vasilachi, Stoleru, and Gavrilescu, 2023).

Abstract

Chlorophyll a and b

Chlorophyll molecules are fundamental pigments involved in the photosynthetic process, predominantly located within the chloroplasts of plant cells. These pigments are crucial for the absorption of light energy, which is subsequently converted into chemical energy during photosynthesis. Plant leaves contain several pigments, which can be broadly classified into Chlorophylls and carotenoids. Both carotenoids and Chlorophyll play a role in light harvesting, photon absorption, and excitation energy transmission in the reaction center of photosystems (Polívka and Frank 2010). Chlorophylls a and b help in capturing non-green light during photosynthesis. This study will examine the impact of photo-oxidative damage on Chlorophyll a and b, each of which has distinct absorption maxima at specific wavelengths. Chlorophylls consist of a porphyrin ring with a central magnesium ion (Mg^{2+}) (PubChem, n.d.). This basic structure can cause chlorophyll a to be absorb blue/green pigment with maximum absorbance at 664 nm and Chlorophyll b at 648 nm. The chemical structure of chlorophyll may degrade due to exposure to light and oxygen known as photooxidative damage caused due to interference of a heavy metal (Hong et al. 2020). The heavy metal's ions interfere with the central magnesium ion thereby leading to color loss. Photodegradation of chlorophyll pigments due to heavy metals can result in irreversible breakdown of chlorophyll a and b through damage of the chlorophyll porphyrin ring structure, causing a slight modification which no longer allows it to absorb light

at their respective wavelength range. This leads to a lack of any photosynthesizing agent for the plant (Hong et al. 2020)

The molecular formula given to the structure of Chlorophyll a is $C_{55}H_{72}O_5N_4Mg$ as seen in Figure 1. The molecular formula of Chlorophyll b is $C_{55}H_{70}MgN_4O_6$, as seen in figure 2 and it absorbs light predominantly in the violet-blue region of the spectrum.

Figure 1 – Chemical structure of Chlorophyll-a, one of the main unsaturated molecules used in photosynthesis.²

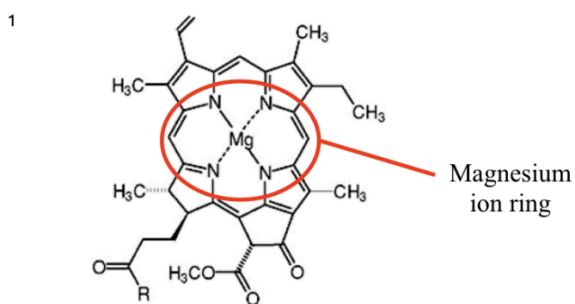
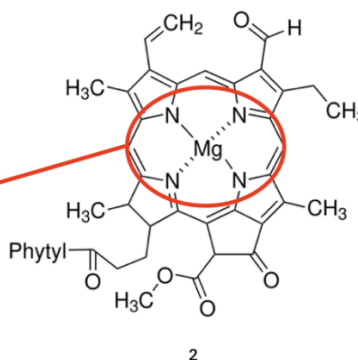


Figure 2 – Chemical structure of Chlorophyll-b, which includes a formyl group.



Figures 1 and 2 taken from (Sigma-Aldrich, 2020)

Chemical Properties of Spinacia oleracea

Spinach (*Spinacia oleracea*) leaves contain Chlorophyll a, Chlorophyll b and b-carotene as major pigments as well as smaller amounts of other pigments such as xanthophylls. Spinach is a leafy green plant well-suited for this experiment because it has a very high Chlorophyll content (Phuong Dung and Ngoc Thang 2022). This allows for precise measurement of changes in Chlorophyll a and b under different conditions as seen in table 1, the study Bahceci et al, 2005 checked the chlorophyll a and b concentrations on spinach leaves and purée, where the leaves average chlorophyll content for a and b was significantly greater than as a purée, which is why I chose to experiment on leaves. The selection of spinach leaves as a subject for studying the extraction of Chlorophyll-based natural dyes, specifically Chlorophylls a and b, stems from the fact that this vegetable is an abundant source of these pigments (Nugmanov et al. 2021). In this essay, spinach is used to investigate how exposure to various concentrations of heavy metal salts in a controlled hydroponic system affects Chlorophyll levels (Bahceci et al, 2005).

Table 1 showing concentration of chlorophyll a and b of Spinach (Spinacia oleracea) leaves vs puree (taken from Bahceci et al, 2005)

<i>Form</i>	<i>Chlorophyll a (mg.g⁻¹)</i>	<i>Chlorophyll b (mg.g⁻¹)</i>	<i>Carotenoids (mg.g⁻¹)</i>
<i>Leaves</i>	<i>0.47</i>	<i>0.84</i>	<i>27.21</i>
<i>Purée</i>	<i>0.38</i>	<i>0.70</i>	<i>24.29</i>

Heavy Metals and Plants

Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, electroplating, energy and fuel production, power transmission, and intensive agriculture (Radmila Filipović-Trajković et al. 2012). In scientific research conducted by Frontiers in Science (2012) it was found that the Mithi river in BKC, Mumbai contained a toxic level of heavy metal sulfates including Al, Zn, Cd, Cr, Hg, Ni, Pb, Cu and Ni (Singare, Mishra, and Trivedi 2024). Zinc, Nickel and Copper sulfates were specifically chosen given their high prevalence in Indian local soils of Maharashtra. (Bhagure, 2012). Therefore, these metal sulfates were chosen because they are not only classified as toxic but also mimic the heavy metal make-up of soil in the immediate local environment. Their excessive accumulation can inhibit growth and productivity, and in some cases can even lead to plant death. Hydroponics was chosen because it is the practice of growing plants in a water-based nutrient solution instead of soil, and spinach thrives when grown submerged in water (USDA, 1965).

Research Question

To what extent will the concentration of Chlorophyll a and b in the leaves of spinach (*Spinacia oleracea*) change when grown hydroponically in heavy metal sulfate solutions of Nickel sulfate, Zinc sulfate and Copper sulfate, of concentration 0.2M, 0.4M, 0.6M, 0.8M, 1.0M kept at 25°C for 5 days, measured by differences in absorbance at wavelengths 664nm and 648nm?

Hypothesis

Null Hypothesis (H0): The concentration of heavy metals(*Spinacia oleracea*) has no effect on the concentration of Chlorophyll a and b in spinach leaves (*Spinacia oleracea*). This hypothesis supports the fact that Any observed variations in chlorophyll content would be attributed to random factors or natural variability, rather than the influence of heavy metals.

Alternative Hypothesis (H1): The concentration of Chlorophyll a and b in spinach leaves changes significantly with varying concentrations varying from 0.2M, 0.4M, 0.6M, 0.8M, and 1.0M of heavy metal salt solutions.

The concentration of Chlorophyll a and b in the leaves of spinach (*Spinacia oleracea*) grown hydroponically is dependent on the concentration of heavy metal salt solutions. This hypothesis posits that heavy metals inhibit metabolic processes primarily by affecting the structural chemical porphyrin ring by interfering with the Mg^{2+} ion in the centre. 25°C was chosen because it is close to the optimal temperature for spinach growth and most plant metabolic processes, ensuring that temperature-related stress is minimized and any observed effects on chlorophyll content can be attributed primarily to the heavy metal exposure rather than temperature fluctuations.

Methodology

The initial preliminary experiment involved assessing the impact of sulfate metal salts on plant health through a seed germination test. 10 seeds each were germinated in cotton beds soaked in the metal sulfate solutions of different concentrations at room temperature. The preliminary included the quantitative result of the percentage of successfully germinated seeds served as an indicator of the effects of the metal salts. This approach was chosen for its simplicity and direct measure of plant health. However, it exhibited limitations due to its time-consuming nature and the irregular variability of the length of seed germination, which necessitated the exploration of more precise and rapid methods. This would change my Research Question by steering it in the direction of investigating the effects of sulfate metal salts on early plant development using rapid and quantifiable measures of physiological stress, measured by metal sulfate salt solution concentration.

Given the challenges encountered with the previous methods, the decision was made to adopt colorimetry for the final methodology. This was selected because it was precise and provided accurate and reproducible quantitative data on the extent of damage to plant tissues by measuring Chlorophyll a and b levels. It is also efficient since it allows for rapid assessment of multiple samples, significantly reducing the time required for analysis. Colorimetry is also a relatively simple and easy to use given that it is based on computerized reading, as carried out by Hiscox et al during their chlorophyll extraction experiment in 1979 (Hiscox and Israelstam, 1979). This research paper was chosen because the experiment's method is precise, has high reproducibility, and is efficient for measuring chlorophyll a and b levels. The Colorimeter measures the absorbance of specific wavelengths of light by the plant extracts, allowing for the quantification of Chlorophyll a and b, as well as other pigments directly affected by sulfate metal salts.

1. Make the solutions of different concentrations:

Pour 10 cm³ of distilled water into a beaker. Gradually add the weighed amount of the respective heavy metal sulfate to the beaker while stirring continuously to ensure it dissolves completely. Repeat this process for each concentration and for each type of heavy metal. Clearly label each beaker with the concentration and type of heavy metal. Store the solutions in a cool, dry place until use.

Molar Concentration (mol/dm³)	Nickel Sulphate per 10cm³		Copper Sulphate per 10cm³		Zinc Sulphate per 10cm³	
	Moles	Grams	Moles	Grams	Moles	Grams
0.2	0.2	30.95	0.2	31.92	0.2	32.29
0.4	0.4	61.90	0.4	63.84	0.4	64.59
0.6	0.6	92.85	0.6	95.76	0.6	96.88
0.8	0.8	123.80	0.8	127.68	0.8	129.18
1.0	1.0	154.75	1.0	159.60	1.0	161.47

Table 3: masses of different sulfates required, and volume of water needed to make concentrated solutions.

2. Hydroponically grow the Spinach:

Cut fresh spinach stalks to a uniform length to ensure consistency across samples. Place each spinach stalk into a separate beaker, ensuring that the base of each stalk is fully submerged in the heavy metal solution. Ensure that there is one control beaker with only water. Arrange the beakers in a test tube stand to keep them upright and stable. Allow the spinach to grow hydroponically for 3 days. Monitor the spinach daily for any visible signs of growth or distress (wilting and color changes) Environmental conditions remain the same: set up was placed near the windowsill with adequate sunlight, temperature of 25 °C and standard atm. After 3 days, carefully remove a leaf from each spinach stalk and place it carefully on a white flat tile. Observe and note down any qualitative changes. Using a ruler, cut a 4 cm by 4 cm section from each leaf.

3. Chlorophyll Extraction:

Place each leaf section into a test tube containing warm water. Place the test tubes in a beaker containing boiling ethanol. This step ensures that the Chlorophyll and any absorbed heavy metals are extracted into the water in the test tube. Allow the leaves to soak in the boiling ethanol for 15 minutes (monitor time using stopwatch).

4. Spectrophotometric Analysis:

Using a pipette, transfer the green solution from the test tubes into cuvettes using a pipette. Take 30 readings using the same method described above. Set the photo spectrometer to the appropriate wavelength to measure the absorbance of Chlorophyll a and Chlorophyll b: For Chlorophyll a, set the wavelength to around 664 nm. (Dunne 1999) and for Chlorophyll b: Set

the wavelength to around 648 nm. (Dunne 1999). Measure and record the absorbance of each solution at these wavelengths using LoggerPro software.

Environmental, Safety, and Ethical Concerns

During the experimental procedures essential protective gear, including gloves and safety goggles, was utilized to prevent direct contact of metal sulphate solutions with skin and eyes, mitigating the risk of exposure to toxic substances. Additionally, ethanol, known for its highly flammable properties, was heated indirectly using a water bath rather than an open flame. This precaution, coupled with conducting the process in a well-ventilated area, effectively reduced the risk of fire hazards. Careful handling of hot glassware was also imperative to prevent burns and accidental breakage. Moreover, the spectrophotometer, a critical piece of electrical equipment, was operated with adherence to safety protocols to avoid electrical hazards and ensure the accuracy of measurements.

The heavy metal solutions were managed as hazardous waste to mitigate their environmental impact. Additionally, water conservation was a priority during the experiment, with conscientious efforts made to minimize water usage and reduce wastage. The spinach plants used in the experiment were obtained from a local farmer's market and were kept soaked in fresh water 5 hours before the experiment. It was treated with care to minimize unnecessary stress or damage, reflecting a commitment to ethical research practices. Furthermore, environmental responsibility was upheld throughout the experiment by using materials efficiently and minimizing waste, thereby reducing the overall ecological footprint of the study.

Data Processing

Table 4: Showing the Absorbance of Chlorophyll a decreasing with respect to the increased concentration of 3 different heavy metal sulfates (0.2,0.4, 0.6, 0.8, 1.0 in mol/dm³)

Metal	Average Absorbance of Chlorophyll a (nm) $\pm$ 0.001				
Sulphate	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	0.973	0.867	0.816	0.738	0.708
Copper	0.854	0.764	0.566	0.425	0.238
Nickel	0.879	0.770	0.648	0.531	0.449
Mean	0.727	0.703	0.658	0.624	0.599
Std. Dev	0.063	0.063	0.127	0.159	0.235

Table 5: Showing the Absorbance of Chlorophyll b decreasing with respect to the increased concentration of 3 different heavy metal sulfates (0.2,0.4, 0.6, 0.8, 1.0 in mol/dm³).

Metal	Average Absorbance of Chlorophyll b (nm) $\pm$ 0.001				
Sulphate	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	0.650	0.575	0.518	0.441	0.390
Copper	0.396	0.335	0.244	0.196	0.108
Nickel	0.575	0.470	0.441	0.358	0.265
Mean	0.455	0.445	0.451	0.449	0.441
Std. Dev	0.131	0.120	0.141	0.125	0.141

Experimental Data

$$\text{Mean} = \frac{\sum \text{Values}}{n}$$

$$\text{Standard Deviation} = \sqrt{\frac{\sum (\text{Value} - \text{Mean})^2}{n}}$$

$$\text{Percentage Error} = \left(\frac{\text{Standard Deviation}}{\text{Mean}} \right) \times 100$$

$$R^2 = 1 - \frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

Using sample data: For Zinc Sulfate for measuring Chlorophyll a at $0.2 \text{ mol/dm}^3 = 0.960, 0.990, 0.980, 0.970, 0.990, 0.960, 0.970, 0.970$.

1. Calculating the Mean:

Sample calculation for Zinc at 0.2 mol/dm^3 :

$$\text{Mean} = \frac{0.960 + 0.990 + 0.980 + 0.970 + 0.990 + 0.960 + 0.970 + 0.970}{8} = 0.973$$

2. Calculating the Standard Deviation:

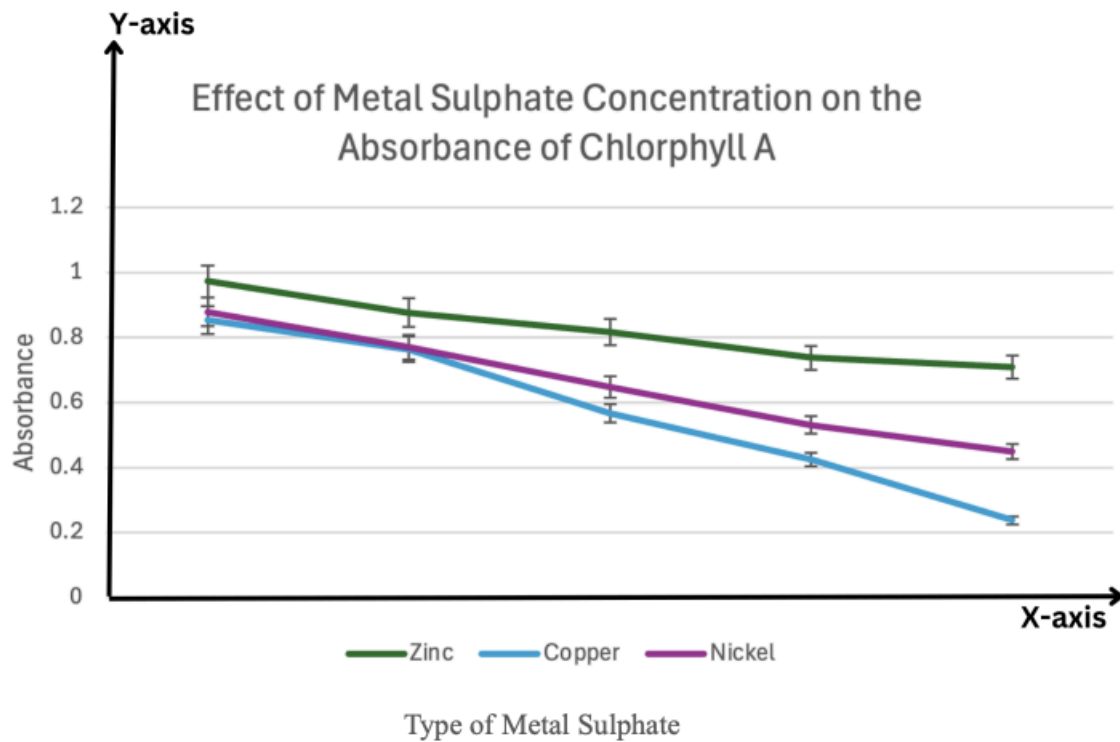
Sample calculation for Zinc at 0.2 mol/dm^3 :

$$\text{Standard Deviation} = \sqrt{\frac{(0.960 - 0.973)^2 + (0.990 - 0.973)^2 + \dots + (0.970 - 0.973)^2}{8}} = 0.011$$

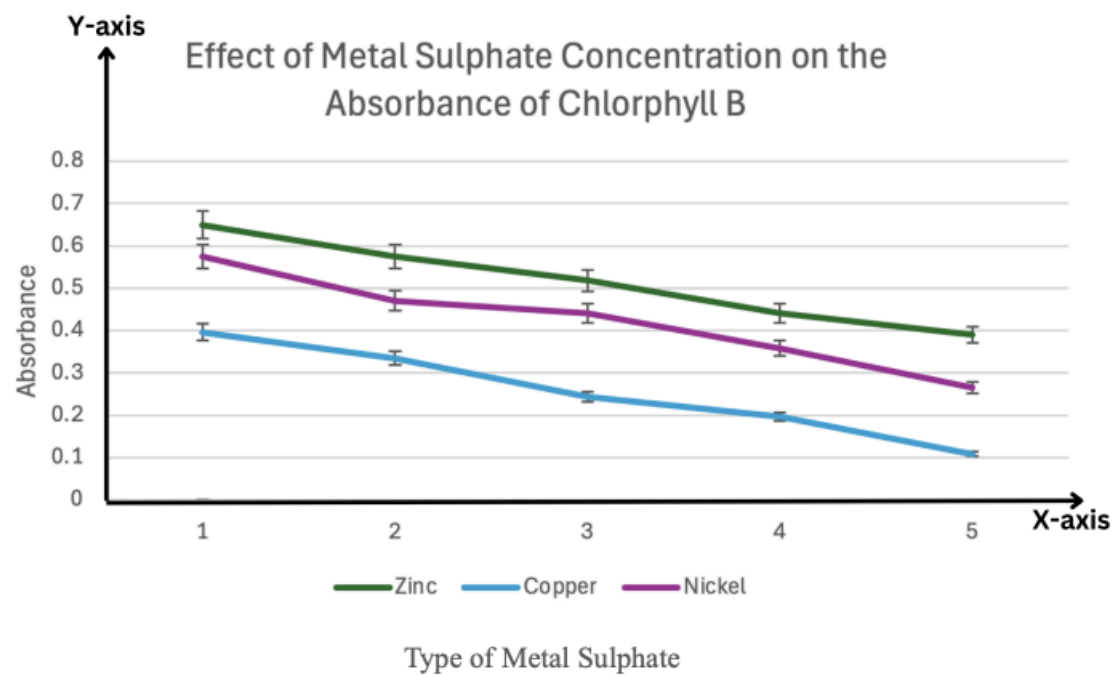
3. Calculating the % error:

$$\text{Percentage Error} = \left(\frac{0.011}{0.973} \right) \times 100 = 1.13\%$$

Graph 1- Type of Metal Sulfate Vs. Average Absorbance of Chlorophyll a at differing concentrations.



Graph 2- Type of Metal Sulfate Vs. Average Absorbance of Chlorophyll b at differing concentrations.



Discussion

The data from graph 1 and 2 indicate a clear trend where increasing concentrations of heavy metal sulfates (Zinc, Nickel, and Copper) reduce the concentration of both Chlorophyll a and Chlorophyll b, as measured by absorbance.

For Chlorophyll a (Graph 1), Zinc sulfate exhibits a gradual decline, followed by Nickel sulfate follows a similar pattern. Copper sulfate shows the steepest decline in absorbance. This steep gradient for Copper sulfate suggests it has the most detrimental impact on Chlorophyll a. The R^2 values for Zinc (0.974), Nickel (0.989), and Copper (0.996) indicate strong correlations between heavy metal concentration and decreased absorbance, particularly for Copper. Similarly, in Graph 2 (Chlorophyll b), the trend lines reflect a consistent decrease in absorbance as the concentration of metal sulfate increases. Copper sulfate again shows the most significant reduction. Again, the R^2 values—0.993 for Zinc, 0.977 for Nickel, and 0.996 for Copper—confirm strong correlations, with Copper sulfate having the strongest effect on Chlorophyll b. The R^2 value is a statistical measure that determines the proportion of variance in the dependent variable that can be explained by the independent variable. Given that the R^2 is 0.993, it shows a high correlation, stating that the decreasing concentration of chlorophyll a and b is highly likely due to the increasing metal sulfate concentration. A higher R^2 value indicates that more variability is explained by the model, and since this value is closer to 1, it indicates that the variability in the results is mostly explained by the experimental design.

The trends observed in both graphs are largely similar, with increasing concentrations of heavy metals leading to a sharp reduction in the absorbance of both types of Chlorophyll. Copper consistently has the steepest negative gradient, followed by Nickel and then Zinc. This suggests that Copper sulfate has the most significant inhibitory effect on Chlorophyll, likely due to its strong interaction with Chlorophyll molecules, potentially forming stable complexes that disrupt their structure and function. A notable difference between Chlorophyll a and b is that Chlorophyll a consistently exhibits higher absorbance values across all concentrations of heavy metals compared to Chlorophyll b. The stronger effect of Copper suggests that this metal interferes with Chlorophyll structure or function more than Zinc or Nickel, possibly by forming stronger complexes that disrupt the ability of Chlorophyll molecules to absorb light.

The trends also highlight that higher concentrations of heavy metals exacerbate the toxicity, particularly with Copper, which begins to cause steep declines in absorbance from 0.4 M, 12 indicating its high toxicity even at moderate concentrations. The error bars in Graphs 1 and 2 indicate the variability in the data, showing smaller errors at lower concentrations, particularly for Zinc and Nickel. At higher concentrations, the error bars are larger but do not overlap, suggesting statistically significant differences in Chlorophyll concentration. This result being statistically significant means that it is unlikely to be explained solely by chance or random factors and solely by the changing of heavy metal type and concentration. In other words, a

statistically significant result has a very low chance of occurring if there were no true effects caused by the type and concentration of heavy metal sulfates on the concentration of chlorophyll a and b. The increased variability at higher concentrations could be due to random errors or the increased sensitivity of Chlorophyll to higher metal concentrations. In conclusion, the trend lines in the graphs clearly illustrate the detrimental effects of heavy metal sulfates on both Chlorophyll a and b, with Copper being the most harmful. This supports the hypothesis that higher concentrations of heavy metals severely reduce Chlorophyll concentration, potentially impairing plant photosynthesis and growth.

Statistical Testing

Figure 3: ANOVA Online Calculator Results

Summary of Data						
	<i>Treatments</i>					
	1	2	3	4	5	Total
N	6	6	6	6	6	30
ΣX	4.327	3.79	3.233	2.671	2.158	16.179
Mean	0.7212	0.6317	0.5388	0.4452	0.3597	0.539
ΣX^2	3.3586	2.6077	1.9285	1.3495	0.9935	10.2378
Std.Dev.	0.2182	0.2067	0.1931	0.1791	0.2085	0.2284

The ANOVA test was used because it helps determine if there are significant differences between the groups being studied. This statistical test can help determine whether or not there are significant differences between the means of the independent variable which is the 13 concentration and type of heavy metal salts. The statistical significance of the results were determined using an online calculator. The f-ratio value is 3.05361. The p-value is .035331. The result is significant at $p < 0.5$. The results are statistically significant if a large f-ratio value and a $p\text{-value} < 0.5$ are obtained. This means that whatever data has been used to plot the graphs are statistically significant. A positive result from this ANOVA test tells us that there is a significant difference between at least two of the groups. Additionally, the R^2 value is 0.990, indicating a

strong linear relationship between the concentration of metal sulfate and the mean absorbance of chlorophyll a and b.

Evaluation

Heavy metals can hinder plant metabolism, yet certain metals like copper (Cu), iron (Fe), and manganese (Mn) are crucial for photosynthesis. Heavy metals are vital in various plant physiological processes, with specific ions required in trace amounts for proper metabolism, growth, and development. These metals are involved in various proteins and enzymes essential for plant growth, development, and maintaining proper metabolic function. A deficiency in these metals directly impairs photosynthesis, but when their concentrations become too high, they turn toxic to plants and negatively affect the photosynthetic process. These essential ions are naturally found in soil or growth media and are crucial for several biochemical functions in plants. However, when their concentration exceeds certain levels, they can cause significant cellular damage in plants (Avery, 2001; Schutzendubel and Polle, 2002; Gaetke and Chow, 2003).

The experiment confirmed this, demonstrating that copper ions inhibited spinach growth more severely than zinc ions or nickel ions at all tested concentrations. The most significant reduction in chlorophyll concentration was observed with copper, confirming its pronounced toxicity in high amounts (Baszynski et al., 1988; Henriques, 1989). The results indicated that at a concentration of 1.0 mol/dm³, copper significantly reduced growth, supporting previous findings that high copper levels degrade photosynthetic enzyme activity, leading to chlorosis and reduced integration of chlorophyll into photosystems (Kupper et al., 2003).

Nickel ions also exhibited toxic effects, although less severe than copper. At lower concentrations, such as 0.2 mol/dm³, nickel's impact on growth was minimal, but at higher concentrations, it became toxic, shrinking and crumpling the leaves as evidenced in the 14 qualitative results (Kaamoush et al., 2022). Zinc ions, though crucial for gene regulation and metabolism, was found to be the least toxic of the metals tested. At lower concentrations, zinc maintained a darker chlorophyll solution compared to copper or nickel, indicating a less detrimental effect on spinach growth (Ishimaru et al., 2011).

To improve the accuracy and reliability of the experiment, a few adjustments could be made. One key improvement would be to include a broader range of heavy metals beyond copper, nickel, and zinc. Incorporating additional metals like lead or cadmium would provide a more complete picture of their varying impacts on spinach growth, helping to better understand the differences in toxicity levels between metals. While the experiment was largely well-executed, some potential sources of error could have influenced the results. For example, ensuring that the preparation process is free from contamination is crucial. Any cross-contamination between the heavy metal solutions could affect the outcome. To avoid this, stricter protocols during solution

preparation could be applied. Another improvement could be more frequent calibration of the instruments used, such as the devices for measuring chlorophyll content, to ensure that the data collected are as precise as possible. Additionally, repeating the experiment with a larger number of replicates or varying the environmental conditions, such as altering light exposure or soil pH, would provide a more robust data set and offer greater insight into how these external factors interact with heavy metal toxicity. Increasing the sample size would also allow for more statistically reliable results, making the findings more conclusive.

In future studies, extending the experiment beyond what was tested would be valuable. Since the results indicated that metal toxicity impairs plant growth, a logical next step could be to investigate how other environmental factors like pH or light intensity influence this relationship. This would deepen the understanding of how metal toxicity interacts with different variables, thereby taking the experiment further. As for the research question (RQ), the collected data provided a strong answer to the query, which sought to understand the extent to which varying concentrations of metal ions impact spinach growth and chlorophyll levels. The findings revealed that while copper was the most detrimental to spinach growth and chlorophyll content, other metals, such as nickel and zinc, also had a measurable impact. However, additional studies involving different plant species or extended exposure times could enrich the conclusions drawn from the current data. When evaluating the sources used, it is essential to consider their reliability and relevance. Many of the references, like Avery (2001), Schützendübel and Polle (2002), and Gaetke and Chow (2003), were published several years ago, potentially limiting their relevance to current scientific research. Recent developments in heavy metal toxicity research may not be reflected in these older studies. It would be beneficial to include newer references to offer a more up-to-date perspective. Additionally, a review of the authors' backgrounds and their affiliations with reputable institutions would further validate the reliability of these sources. Though the studies referenced were thorough, adding more contemporary research would help ensure that the results align with the latest findings.

Reflecting on the quality of the data collected, the moderate variability observed in the findings—particularly at higher concentrations of metal ions—suggests that further trials would be necessary to confirm the results. Conducting more trials and refining the data collection process would increase the reliability of the data. Variability observed was within the limits of experimental error.

Conclusion

In conclusion, the study demonstrated that the interaction between heavy metal sulfates (Zinc, Copper, and Nickel) and Chlorophyll in a model system results in a noticeable decline in Chlorophyll content, evidenced by decreasing absorbance values over increasing concentrations of metal ions. Chlorophyll a consistently exhibited higher absorbance levels compared to

Chlorophyll b across all tested concentrations, suggesting it may possess a greater resilience or a higher initial concentration that allows it to better maintain light absorption under metal stress. However, both Chlorophylls showed significant reductions in absorbance, particularly in response to Copper sulfate, indicating that metal pollutants can markedly impair Chlorophyll function, potentially disrupting photosynthesis.

The results underline the complex nature of Chlorophyll-metal interactions, where multiple factors may influence the extent to which Chlorophyll content is affected. Despite the observed decline, the persistence of absorbance signals suggests that the Chlorophylls, particularly Chlorophyll a, retain some functional capacity even under environmental stress.

Further studies are recommended to explore the underlying mechanisms driving these interactions, such as examining the structural changes in Chlorophyll molecules or the impact of metal concentration on Chlorophyll fluorescence. Additionally, employing advanced techniques like measuring actual photochemical efficiency could provide deeper insights into the physiological state of Chlorophyll under metal stress. These approaches could help elucidate the full impact of heavy metals on plant photosynthesis and their broader ecological implications. Further research into the effects of heavy metals on plants like spinach can also raise the issue of the importance of limiting the negative effects of pollution.

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Appendix

Tables showing the 8 trials taken showing the absorbance of chlorophyll a and b values against the type of metal sulfate and the concentration in mol/dm³

Trial No.1						
Type of metal sulphate and chlorophyll		Absorbance (±0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.960	0.890	0.810	0.760	0.710
	B	0.660	0.590	0.510	0.460	0.410
Copper	A	0.860	0.770	0.590	0.420	0.220
	B	0.450	0.320	0.260	0.210	0.090
Nickel	A	0.890	0.790	0.650	0.520	0.460
	B	0.580	0.490	0.460	0.380	0.270

Trial No.2						
Type of metal sulphate and chlorophyll		Absorbance (±0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.990	0.860	0.790	0.730	0.690
	B	0.630	0.560	0.490	0.410	0.380
Copper	A	0.870	0.780	0.560	0.450	0.240
	B	0.480	0.340	0.230	0.190	0.140
Nickel	A	0.880	0.760	0.670	0.560	0.420
	B	0.560	0.450	0.410	0.330	0.290

Trial No.3						
Type of metal sulphate and chlorophyll		Absorbance (±0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.980	0.870	0.830	0.740	0.720
	B	0.650	0.570	0.530	0.440	0.400
Copper	A	0.850	0.750	0.570	0.400	0.230
	B	0.460	0.350	0.240	0.200	0.120
Nickel	A	0.870	0.780	0.660	0.540	0.440
	B	0.570	0.470	0.450	0.350	0.280

Trial No.4						
Type of metal sulphate and chlorophyll		Absorbance (±0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.970	0.880	0.820	0.750	0.730
	B	0.640	0.580	0.520	0.450	0.390
Copper	A	0.850	0.760	0.550	0.410	0.250
	B	0.430	0.310	0.240	0.190	0.070
Nickel	A	0.870	0.760	0.630	0.510	0.470
	B	0.590	0.470	0.450	0.360	0.250

Trial No.5						
Type of metal sulphate and chlorophyll		Absorbance (± 0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.990	0.890	0.840	0.710	0.670
	B	0.660	0.570	0.530	0.470	0.390
Copper	A	0.850	0.780	0.570	0.440	0.250
	B	0.440	0.330	0.270	0.200	0.150
Nickel	A	0.880	0.790	0.670	0.540	0.450
	B	0.570	0.480	0.430	0.370	0.250

Trial No.6						
Type of metal sulphate and chlorophyll		Absorbance (± 0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.960	0.880	0.790	0.710	0.670
	B	0.650	0.600	0.490	0.410	0.360
Copper	A	0.850	0.760	0.570	0.450	0.230
	B	0.470	0.360	0.230	0.190	0.080
Nickel	A	0.880	0.760	0.610	0.520	0.450
	B	0.570	0.480	0.450	0.360	0.280

Trial No.7						
Type of metal sulphate and chlorophyll		Absorbance (± 0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.970	0.860	0.810	0.760	0.740
	B	0.650	0.570	0.530	0.440	0.380
Copper	A	0.840	0.770	0.540	0.420	0.240
	B	0.440	0.320	0.250	0.180	0.080
Nickel	A	0.880	0.750	0.640	0.530	0.450
	B	0.580	0.460	0.440	0.350	0.210

Trial No.8						
Type of metal sulphate and chlorophyll		Absorbance (± 0.01)				
Metal Sulphate	Type of Chlorophyll	0.2 mol/dm ³	0.4 mol/dm ³	0.6 mol/dm ³	0.8 mol/dm ³	1.0 mol/dm ³
Zinc	A	0.970	0.880	0.840	0.750	0.730
	B	0.660	0.560	0.540	0.450	0.410
Copper	A	0.860	0.740	0.580	0.410	0.240
	B	0.470	0.350	0.230	0.210	0.130
Nickel	A	0.880	0.770	0.650	0.530	0.450
	B	0.580	0.460	0.440	0.360	0.290