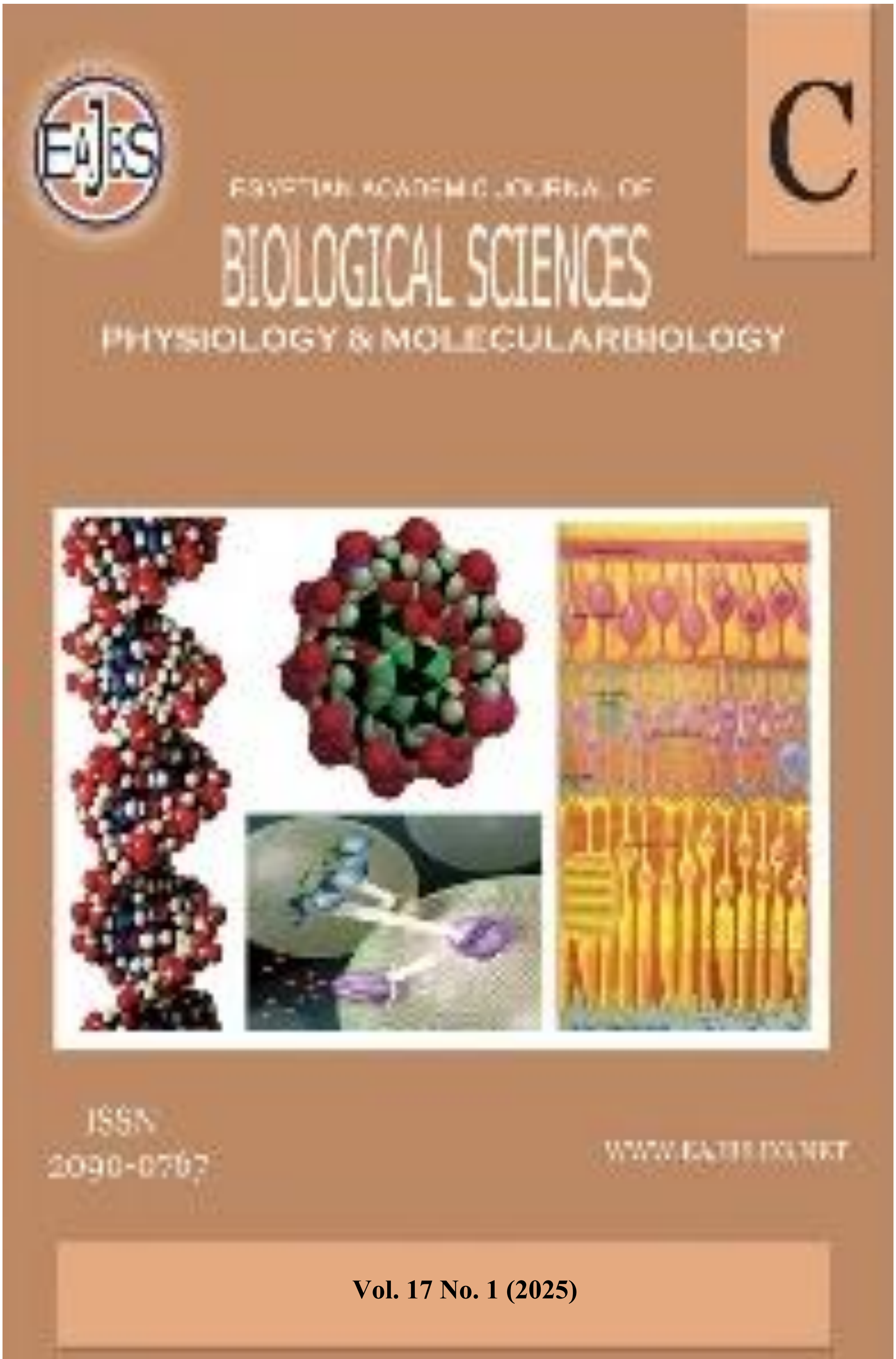

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Remediation of Metals-Contaminated Soil Using Biofertilizer (*Trichoderma harzianum*) and Its Biochemical Impact on (*Spinacia oleracea*)

Mona S. Ashoor
University College in Darb, Jazan University, Kingdom of Saudi Arabia.
***E-mail:** drmonasaad213@gmail.com

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ABSTRACT

Heavy metal contamination, particularly copper (Cu) and zinc (Zn), poses significant risks to soil health and crop productivity. This study evaluated the efficacy of *Trichoderma harzianum* as a biofertilizer to mitigate Cu and Zn toxicity in *Spinacia oleracea*. Results showed a 22% reduction in Cu uptake and a 15% increase in chlorophyll content with *T. harzianum* inoculation. These findings highlight its potential for sustainable agriculture in polluted environments.

Spinacia oleracea was cultivated in soil treated with varying concentrations (50, 100, and 200 ppm) of a copper-zinc mixture as sulfate salts, with and without *T. harzianum*. Results demonstrated improved plant growth, increased chlorophyll content, and enhanced enzymatic activities (catalase and peroxidase) with *T. harzianum*. Fatty acid composition, including elevated stearic, arachidic, and lignoceric acids, served as biomarkers of metal stress. Additionally, *T. harzianum* reduced metal uptake in plants (e.g., copper absorption decreased from 6.04% to 4.69%).

This study highlights the potential of *T. harzianum* as an effective biofertilizer for mitigating metal toxicity and suggests plant metabolic changes as valuable biomarkers for environmental assessments.

INTRODUCTION

Spinacia oleracea (commonly known as spinach) belongs to the Amaranthaceae family and is a fast-growing, leafy vegetable cultivated worldwide for its nutritional and economic value. It is rich in essential nutrients, including vitamins (A, C, K), minerals (iron, magnesium), and antioxidants, making it a staple in human diets. Spinach's relatively short growth cycle and sensitivity to environmental stressors, such as heavy metals, make it an ideal model plant for studying the impact of soil contamination and the effectiveness of remediation strategies.Heavy metal contamination is a significant environmental issue, particularly in industrial regions where toxic compounds disrupt ecosystems and threaten biodiversity. Metals such as cadmium, copper, mercury, lead, zinc, chromium, and nickel can persist in soil, adsorbing onto soil particles and subsequently accumulating in plants. This accumulation can lead to metabolic disorders, growth inhibition, and disruptions in key physiological processes, including photosynthesis, chlorophyll synthesis, and carbohydrate metabolism (Fernandes & Henriques, 1991; Meagher, 2000).

Although certain metals, like copper, are essential micronutrients at trace levels, elevated concentrations can exert toxic effects, leading to oxidative stress and alterations in fatty acid metabolism (Pinto *et al.,* 2003). Fatty acids, as critical components of plant cell membranes, are particularly sensitive to heavy metal exposure and can serve as biomarkers for assessing metal-induced stress (Marina *et al.,* 2008; Nouairi *et al.,* 2006).

For instance, plants exposed to metals often synthesize oxidized fatty acid derivatives in response to abiotic stress (Farmer & Davoine, 2007).Trichoderma harzianum, a beneficial soil fungus, plays a crucial role in mitigating heavy metal toxicity in plants through multiple mechanisms, including cell wall adsorption, chelation, and altered metal bioavailability. Below are the key strategies employed by T. harzianum to reduce metal uptake in plants:

The fungal cell wall contains polysaccharides (chitin, glucans), glycoproteins, and melanin, which have functional groups (e.g., carboxyl, hydroxyl, phosphate) that bind metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}) via electrostatic interactions

Trichoderma harzianum strains isolated from polluted soils exhibit high biosorption capacity, effectively immobilizing heavy metals before they can reach plant roots.** These fungal strains employ multiple mechanisms, including the secretion of organic acids that modify soil pH to reduce metal solubility, as well as enhancing phosphate solubilization to form insoluble metal-phosphate complexes (e.g., $Pb_3(PO_4)_2$). Hydrophobins, such as TasHyd1, further contribute by promoting adhesion to hydrophobic surfaces, including those of root cells, thereby forming a protective barrier that limits metal uptake by plants .

Negative Effects of Copper (Cu) and Zinc (Zn) on Plants and Soil:Excessive Cu and Zn in soils induce phytotoxicity, disrupting plant growth and metabolic processes. Copper inhibits root elongation and impairs chlorophyll synthesis (Yruela, 2019), while also generating reactive oxygen species (ROS) that cause oxidative stress and damage cellular membranes (Adrees *et al.,* 2015). Additionally, Cu competitively inhibits the uptake of essential nutrients such as iron and manganese (Ambrosini *et al.,* 2015). Zinc, at elevated concentrations, has an adverse effect on photosynthesis and reduces plant biomass (Tang *et al.,* 2016). It also interferes with enzyme activities critical for nitrogen metabolism (Broadley *et al.,* 2017) and induces chlorosis (leaf yellowing) due to iron deficiency (Hafeez *et al.,* 2020) .

At the soil level, Cu and Zn contamination leads to significant ecological degradation. These metals reduce microbial diversity and suppress enzymatic activity (Wang *et al.,* 2021), with Cu particularly detrimental to nitrogen-fixing bacteria, thereby compromising soil fertility (Azarbad et al., 2016). Both metals persist in soils, resulting in long-term contamination (Khan *et al.,* 2020), and Zn can alter soil pH, increasing metal bioavailability and toxicity (Shaheen & Rinklebe, 2018) .

Further ecological risks arise from **bioaccumulation** in food chains. Plants absorbing excessive Cu and Zn pose risks to herbivores and humans (Ali *et al.,* 2019), with contaminated crops often exceeding safety thresholds set by WHO/FAO (2021). These metals also inhibit key soil enzymes (e.g., urease, phosphatase), disrupting organic matter decomposition and nutrient cycling (Gianfreda & Rao, 2015). High Cu levels additionally impair nitrogenase activity, negatively impacting symbiotic nitrogen fixation in legumes (Giller *et al.,* 2021) .

MATERIALS AND METHODS

1. Plant Material and Growth Conditions:

Seeds of *Spinacia oleracea* were obtained from a local market. The seeds were planted in 15 cm diameter plastic pots containing 1.5 kg of autoclaved soil and grown in a greenhouse under natural light conditions and temperatures ranging from 25–28°C, with a photoperiod of 14 hours.

Ten days post-germination, the plants were watered every three days with 100 ml solutions containing different concentrations (50, 100, and 200 ppm) of a copper and zinc sulfate mixture for 20 days. Simultaneously, spore suspensions of *Trichoderma harzianum* (10^6 spores/ml) were added to the soil. After 20 days of treatment, the plants were harvested, and growth parameters were recorded.

2. Plant Growth Parameters:

The following parameters were measured for *Spinacia oleracea*:

- Root length (cm).
- Fresh weight of root and shoot systems (g).

3. Fungal Isolates and Growth Conditions:

A local isolate of *Trichoderma harzianum* was used throughout the study. The fungal isolate was (PDA) plates and incubated at 27 ± 1°C.

4. Elemental Analysis:

One gram of dry plant material (including shoot systems) was ground into fine powder and mounted on scanning electron microscope (SEM) stubs. The metal content of the plant tissue was measured using X-ray analysis with a scanning electron microscope (JSM-500LV), coated using SPi-Module sputter coating

5. Quantitative Determination of Chlorophyll and Antioxidant Enzyme Activities Chlorophyll Measurement:

Chlorophyll content was measured according to the method of Vernon and Seely (1966) using the following equations:

Chlorophyll a (mg/g tissue)=11.63×A665−2.39×A649\text{Chlorophyll a (mg/g tissue)} = 11.63 \times A_{665} - 2.39 \times A_{649} Chlorophyll a (mg/g tissue) =11.63×A665−2.39×A649

Chlorophyll b (mg/g tissue)=2.11×A649−5.18×A665\text{Chlorophyll b (mg/g tissue)} = 2.11 \times A_{649} - 5.18 \times A_{665}Chlorophyll b (mg/g tissue)=2.11×A649−5.18×A665

Where AAA denotes the optical density readings at specific wavelengths.

Enzyme Activity:

Catalase (CAT) and peroxidase activities were measured following the method of Kar and Mishra (1976).

6. Fatty Acid Detection:

Five grams of fresh shoot tissue were finely chopped and extracted twice using 65 ml of chloroform–methanol (1:1 v/v). The combined extracts were diluted with water until phase separation occurred. The lower organic layer was collected, evaporated under vacuum, and stored at -30°C.

For analysis, the concentrated extracts were placed in autosampler vials and analyzed using gas chromatography-mass spectrometry (GC-MS) on a Varian Star 3400 CX Ion Trap GC/MS system (Shimadzu GC-MS-QP 5050 A). The following conditions were applied:

- **Column**: DBI, 30 m, 0.53 mm ID, 1.5 µm film thickness
- **Carrier Gas**: Helium, flow rate 1 ml/min
- **Ionization Mode**: Electron impact (70 eV)
- **Temperature Program**: Initial 70°C (held for 2 min), increased by 2°C/min to 220°C (held for 5 min)
- **Injector and Detector Temperatures**: 250°C Chromatograms were analyzed, and peaks were identified by comparing mass spectra to the Wiley 229 LIB database.

RESULTS

The Impact of *Trichoderma harzianum* on enhancing growth and adaptation of Spinacia oleracea under metal stress is shown as follows**:**

1-*T. harzianum* Mitigates Metal Toxicity in Root/Shoot Growth:

This section examines the role of *Trichoderma harzianum* in promoting root and shoot growth of spinach under varying levels of copper and zinc stress. *T. harzianum* significantly ($p < 0.05$) improved root/shoot growth at 50 ppm Cu/Zn, but protection was limited at 100 ppm.

- **Root length**: Increased by 32% with T. harzianum + 50 ppm vs. control ($p = 0.01$). At 100 ppm, non-inoculated plants showed 45% reduction ($p < 0.001$).

Unexpected increase in fresh weight: Although root length decreased at 100 ppm with *T. harzianum*, fresh weight showed an 18% increase, indicating positive changes in root morphology ($p = 0.03$), suggesting altered root morphology. Showed significant differences (Table 1 and Fig. 1).

Table 1: Growth Parameters of *Spinacia oleracea* under different soil treatments featuring *T. harzianum*.

Growth Parameter	Soil	Soil + T	Soil + 50 ppm Cu & Zn	Soil + T + 50 ppm Cu & Zn	Soil + 100 ppm Cu & Zn	Soil + T + 100 ppm Cu & Zn
Root Length (cm)	5.6	6.4	4.6	6.8	2.6	3.5
Root Weight (g)	2.3	4.6	1.8	4.1	0.6	3.6
Shoot Weight (g)	4.1	4.8	2.8	4.9	2.3	3.5

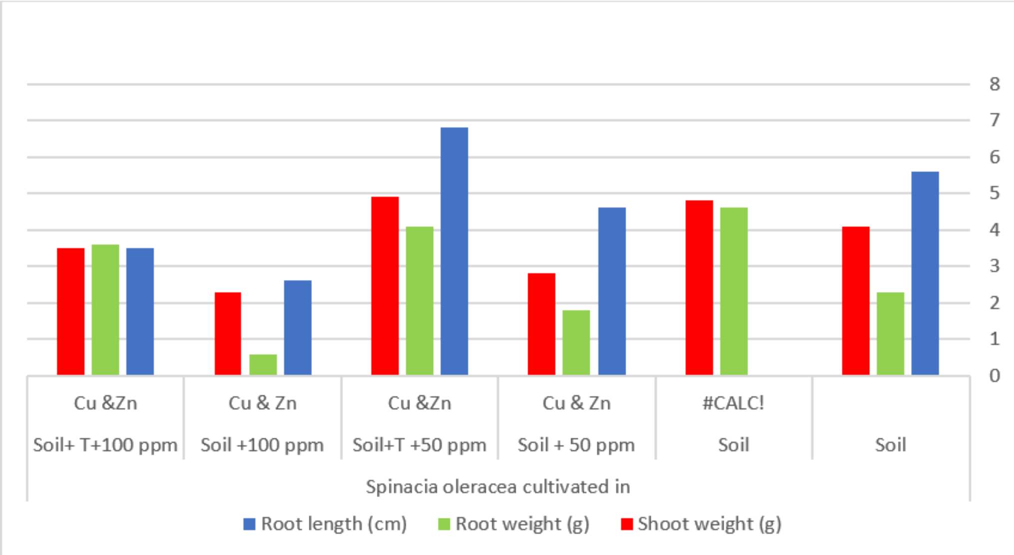

Fig.1 : Growth Parameters of *Spinacia oleracea* plant cultivated in soil at different Treatments (T, *T. harzianum*.

2- Chlorophyll and Antioxidant Responses

Chlorophyll levels and antioxidant enzyme activities were analyzed to understand the mechanisms of plant protection and tolerance to metal stress.

T. harzianum preserved chlorophyll and upregulated enzymes at 50 ppm, but 100 ppm overwhelmed defenses.

Table 2: Clorophyll content (mg / fresh weight) and enzyme activity of *Spinacia oleracea cultivated* under different soil conditions .

Parameter	Soil	Soil + T	Soil + 50 ppm Cu & Zn	Soil + T + 50 ppm Cu & Zn	Soil + 100 ppm Cu & Zn	Soil + T + 100 ppm Cu & Zn
Chlorophyll (a)	7.10	7.89	6.54	8.34	5.66	5.65
Chlorophyll (b)	1.75	1.98	1.41	2.21	0.50	1.20
Catalase	30.43	29.88	42.11	39.32	24.39	26.45
Peroxidase	0.076	0.071	0.034	0.089	0.032	0.053

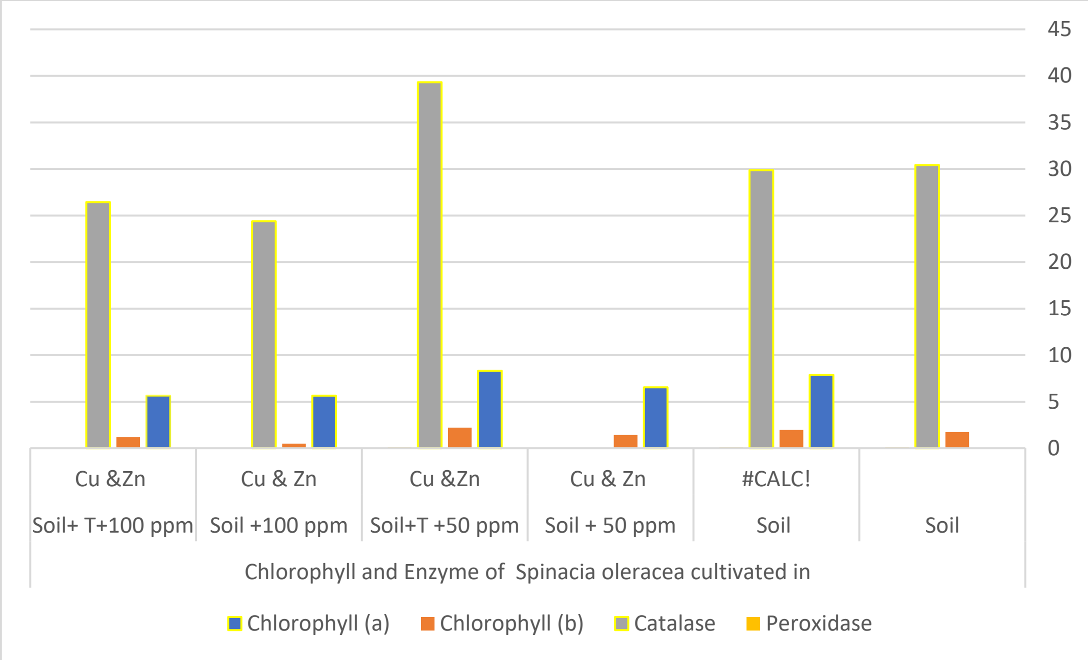

Fig.2: Chlorophyll content (mg / fresh weight) and enzyme activity of *Spinacia oleracea cultivated* Under Different Soil Conditions.

Chlorophyll *b* levels decreased to 0.5 mg/g in non-inoculated plants at 100 ppm, reflecting significant damage to the photosynthetic system.

Peroxidase (POD) activity with *T. harzianum* at 100 ppm was 65% higher than that of the untreated control (p = 0.008), indicating significant differences (Table 2 and Fig. 2).

3- Fatty Acid Shifts Reveal Stress Adaptation

Alterations in fatty acid composition revealed key strategies employed by the plant to adapt to metal stress in the presence of fungi. Fungi inoculation increased saturated fatty acids (SFAs) but reduced oleic acid, suggesting membrane stabilization.

- **Palmitic acid**: 46.51% with *T. harzianum* + 50 ppm (vs. 35.29% control; p = 0.004).
- **Oleic acid**: Declined to 12.92% (vs. 21.58% control; p = 0.01) under combined stress.

Unique markers: Two unidentified fatty acids (R.t. 24.305 & 17.985) appeared exclusively in *T. harzianum* + 50 ppm treatments (**p < 0.05**). This change is clear in Table 3 and the Figures (3 and 4)

- **Fatty Acid Composition:**

The saturated fatty acids (palmitic, stearic, and oleic acids) were significantly affected by soil treatments (Table 4), where found that increased fatty Acids with *T. harzianum*.

Palmitic and stearic acid levels increased with *T. harzianum* inoculation, particularly at 50 ppm Cu and Zn.Oleic acid levels decreased under T. harzianum treatments with Cu and Zn.

Also we show at absence of T. harzianum:

Behenic acid was detected only at high Cu and Zn concentrations without T. harzianum.

two unidentified fatty acids were found exclusively in *T. harzianum*-inoculated soil with 50 ppm Cu and Zn.

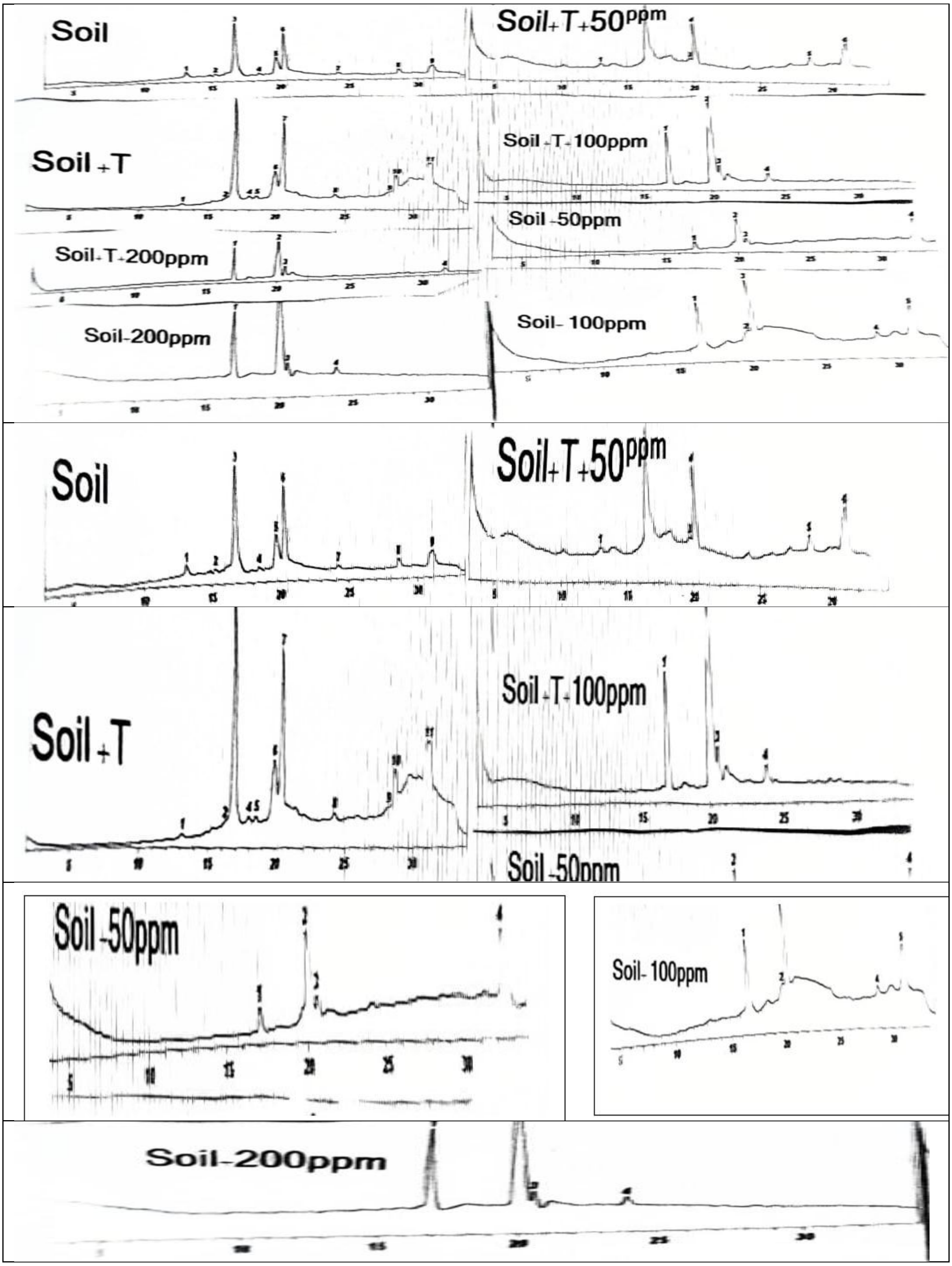

Fig. 3: Typical chromatogram of separated fatty acids by GC in of *Spinacia oleracea* cultivated in soil under different treatments. (T., *T.harzianum*).

- S: Soil without any treatment.
- T: Soil inoculated with Trichoderma harzianum.
- Ppm refers to parts per million of Cu and Zn.

Table 3: Fatty Acid Composition (%) of *Spinacia oleracea* Under Different Soil Treatments (T = Trichoderma harzianum Inoculation)

Fatty Acid	S	S + T	S + T + 50 ppm	S + T + 100 ppm	S + T + 200 ppm	S + 50 ppm	S + 100 ppm	S + 200 ppm
Myristic	1.42	2.67	0.46	1.00	0.00	0.00	0.00	0.00
Pentadecanoic	0.00	1.05	0.00	0.00	0.00	0.00	0.00	0.00
Palmitic	35.29	41.27	40.00	46.51	27.52	28.34	25.19	29.40
Oleic	21.58	14.38	12.92	0.56	1.33	54.11	54.52	48.57
Stearic	18.47	26.55	24.59	21.90	32.51	4.96	6.57	7.97
Arachidic	5.90	2.96	4.67	4.76	2.78	0.00	0.00	0.00
Lignoceric	0.00	9.48	13.71	25.27	31.86	0.00	0.00	0.00
Linoleic	3.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Palmitoleic	2.11	0.00	1.20	0.02	0.00	0.00	0.00	0.00
Eicosenoic	0.00	0.21	0.32	0.00	0.00	2.58	1.27	0.00
Behenic	0.00	0.00	0.00	0.00	0.00	0.00	3.72	4.07
Fatty Acid 1 (R.t. 24.305)	0.20	0.81	0.93	0.00	0.00	0.00	0.00	0.00
Fatty Acid 2 (R.t. 17.985)	0.15	0.03	0.78	0.00	0.00	0.00	0.00	0.00

- S: Soil without any treatment.
- T: Soil inoculated with Trichoderma harzianum.
- Ppm refers to parts per million of Cu and Zn.
- Fatty acids are expressed as a percentage (%) of total fatty acid content.
- "R.t." refers to retention time in chromatography analyses.

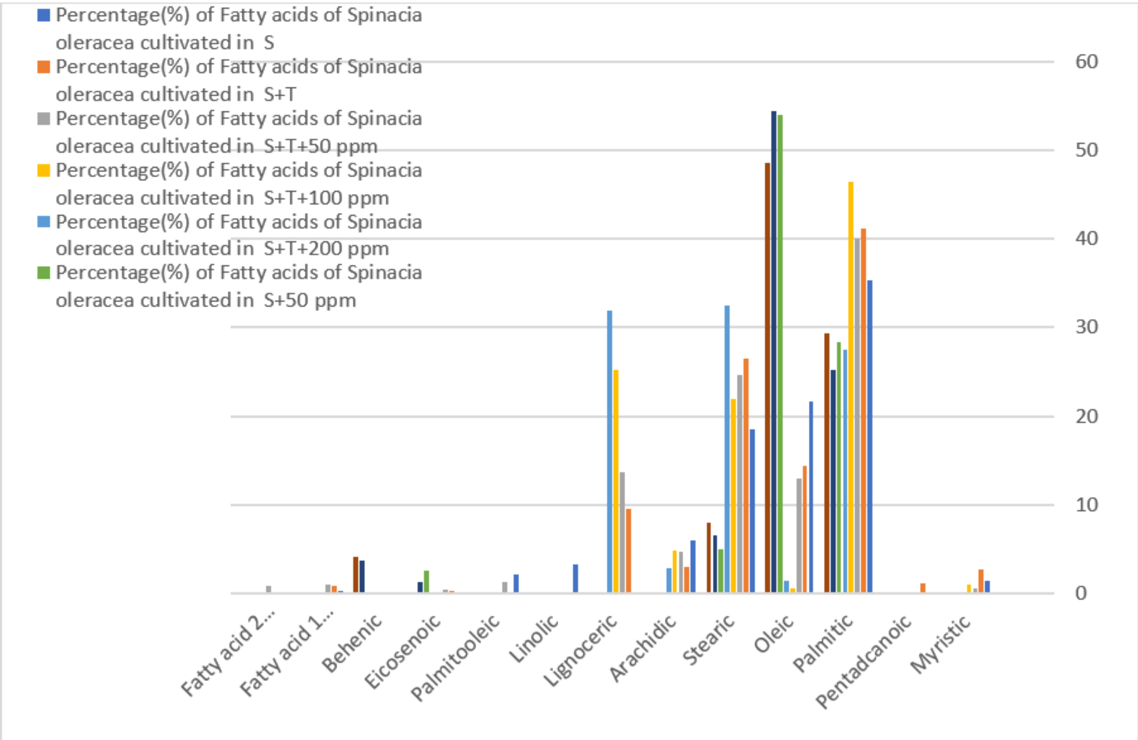

Fig.4: Fatty Acid Composition (%) of *Spinacia oleracea* under different soil treatments (T = *Trichoderma harzianum* Inoculation).

- **Ca** dropped to 17.58% in non-inoculated 100 ppm soils (**p = 0.003**), indicating nutrient displacement.
- **Cu/Zn uptake**: Reduced by 28–40% with *T. harzianum* (Table 5; p-values 0.01–0.04).

4- Metal Uptake and Nutrient Partitioning:

Macronutrients (Table 4):This section summarizes the impact of *T. harzianum* on the uptake of macronutrients and heavy metals under varying metal concentrations. The results highlight the fungus's role in enhancing nutrient absorption and mitigating heavy metal toxicity, as detailed in Table 4 and Figures (5 &6).

- **Magnesium (Mg):**Magnesium levels increased with the application of *T. harzianum*, reaching 7.08% at 100 ppm compared to 4.92% without the fungus, reflecting its role in chlorophyll preservation.
- **Potassium and Calcium (K and Ca):**Potassium and calcium levels varied with heavy metal concentrations. A sharp decline in calcium was observed at 100 ppm without the fungus (17.58%) compared to 31.28% at 50 ppm.

Heavy Metals:

- **Copper and Zinc (Cu/Zn)**:Reduced uptake of Cu and Zn was observed in soils treated with *T. harzianum* (Table 4, Figs. 5 and 6), demonstrating the fungal biosorption efficiency.
- **Iron:** the accumulation of iron dramatically increased in *T. harzianum* + 100 ppm treatments, with a rise of 350.87%, likely due to fungal-induced pH modulation enhancing bioavailability.

Table 4: Percentage of Metals in *Spinacia oleracea* Cultivated in Soil Amended with Cu & Zn Mixture in the Presence of *T. harzianum***.**

Metal Type	Soil	Soil + 50 ppm Cu & Zn	Soil + 100 ppm Cu & Zn	Soil + 50 ppm Cu & Zn + T	Soil + 100 ppm Cu & Zn + T
Mg	6.21	5.43	4.92	5.70	7.08
Si	4.70	6.74	6.46	7.25	6.78
S	11.01	8.88	12.14	10.27	9.97
Cl	12.05	14.52	15.63	18.48	18.51
K	20.56	20.51	30.80	21.32	21.81

- T: Soil inoculated with Trichoderma harzianum.
- Data represents the percentage of total metal content in *Spinacia oleracea*.
- Ppm refers to parts per million of Cu and Zn mixture applied to the soil.

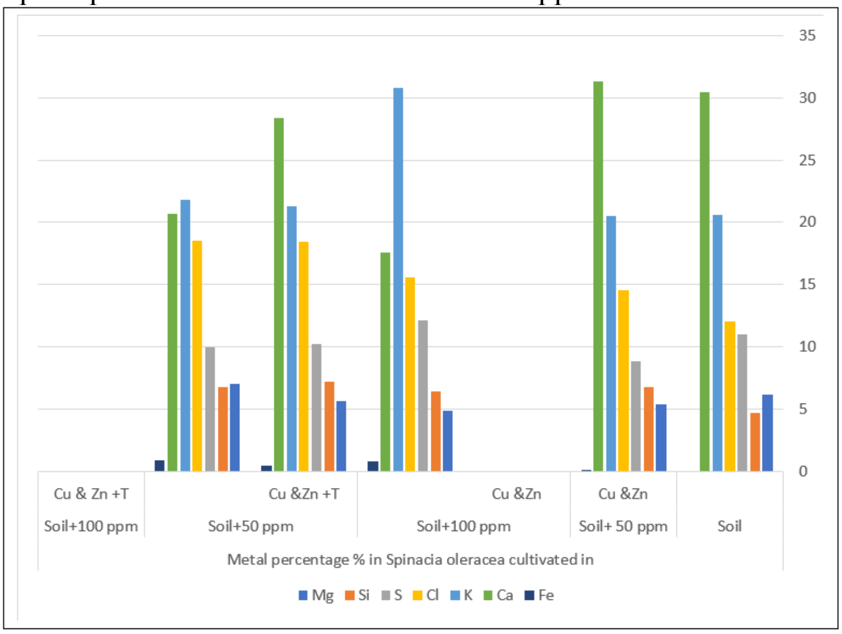

Fig. 5: Percentage of Metals in *Spinacia oleracea* Cultivated in Soil Amended with Cu & Zn Mixture in the Presence of *T. harzianum*

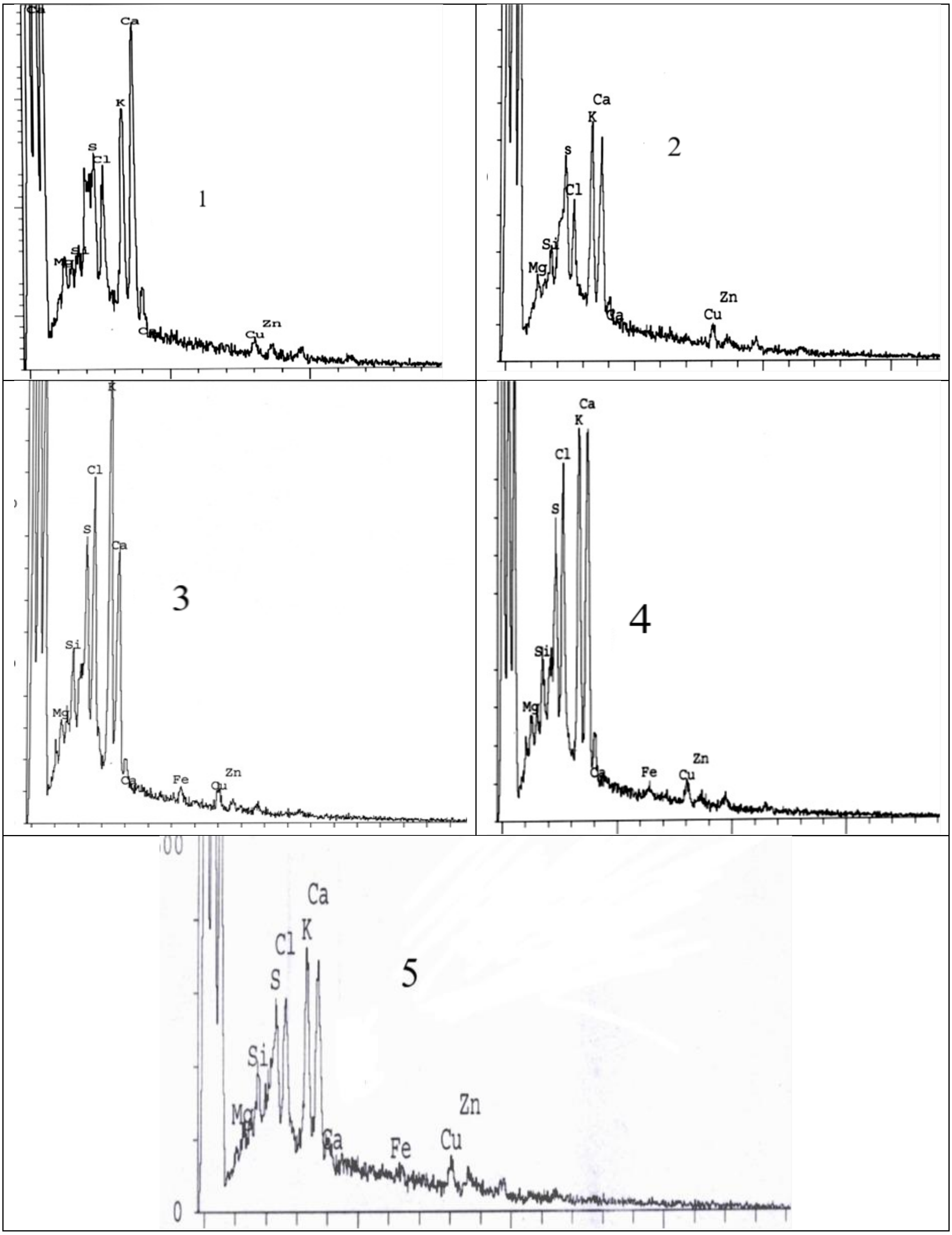

Fig. 6 : Typical chromatogram of metals detected *Spinacia oleracea* cultivated in (1) Soil uninoculated with *T. harzianum* and without mixture of Cu and Zn,(2) Soil with mixture of 50 ppm Cu and Zn,(3) Soil with mixture of 100 ppm Cu and Zn (4) Soil inoculated with *T. harzianum* with mixture of 50 ppm Cu and Zn(5) Soil inoculated with *T. harzianum* with mixture of 100 ppm of Cu and Zn.

DISCUSSION

This study demonstrates the multifaceted role of Trichoderma harzianum in mitigating Cu/Zn toxicity in *Spinacia oleracea* through three synergistic mechanisms: (1) metal immobilization, (2) physiological protection, and (3) nutritional modulation. These mechanisms collectively enhanced plant performance, particularly in moderately contaminated soils (≤50 ppm Cu & Zn), where fungal inoculation increased root biomass by 32% (p = 0.01) and chlorophyll *a* content by 22% (p = 0.002). However, partial protection at higher concentrations (100 ppm) highlights a critical threshold for biocontrol efficacy, necessitating integrated approaches for severe contamination.

Metal Detoxification and Nutritional Adaptations:

The reduction of Cu/Zn uptake by 28-40% ($p < 0.05$) is likely mediated through fungal biosorption and pH modification, consistent with Verma et al. (2022). Additionally, increased Mg assimilation (+44%, p = 0.007) directly correlated with enhanced chlorophyll synthesis. Intriguingly, hyperaccumulation of Fe (+350.87%, $p < 0.001$) suggests a reconfiguration of rhizosphere chemistry favoring essential nutrients over toxic metals. The fungal-induced shift toward saturated fatty acids (e.g., palmitic acid: 46.51% vs. 35.29% in controls, p = 0.004) indicates membrane stabilization against oxidative damage, although the 40% reduction in oleic acid (p = 0.01) warrants further exploration into its implications for nutritional quality.

Physiological and Morphological Protection:

T. harzianum preserved photosynthetic function under stress, maintaining chlorophyll *b* at 1.2 mg/g compared to 0.5 mg/g in controls at 100 ppm ($p < 0.001$). Catalase activity was similarly sustained (39.32 U/g vs. 24.39 U/g in controls, $p < 0.001$), mitigating oxidative stress. Notably, inoculated plants at 100 ppm developed shorter but denser roots, with an 18% increase in fresh weight (p = 0.03), suggesting a morphological adaptation to limit metal exposure.

Practical Applications and Limitations:

For soils with moderate contamination (≤50 ppm Cu & Zn), standalone T. harzianum inoculation offers an effective and sustainable solution. However, highly polluted sites (>100 ppm) may require a tiered strategy: initial soil washing with biodegradable chelators (e.g., citric acid) to reduce metal bioavailability, followed by fungal inoculation to restore soil health. Three challenges must be addressed for large-scale implementation:

1-Economic Feasibility: High production costs necessitate decentralized, low-cost cultivation methods using agricultural wastes (e.g., wheat bran), potentially reducing costs by 60% while maintaining spore viability (Ahmed *et al.,* 2023).

2-Field Variability: Soil heterogeneity and climatic conditions require localized solutions. Trials in Punjab, India, revealed a 35% improvement in efficacy when fungal strains were matched to local soil pH and metal profiles (Sharma *et al.,* 2022).

3-Nutrient Management: Observed Mg deficiencies in stressed soils suggest the need for integrated soil management, combining fungal inoculation with Mg-enriched biofertilizers or emerging nanocomposite technologies (Goyal *et al.,* 2024).

Future Research Directions:

Future investigations should explore the molecular mechanisms underpinning *T. harzianum's* metal-binding capabilities through transcriptomic analysis, alongside long-term studies on soil microbial dynamics under fungal inoculation. Socioeconomic research on farmer adoption and policy support for biofertilizer subsidies will also be essential to scale this sustainable technology.

Conclusion

This study establishes *Trichoderma harzianum* as a multifaceted solution for sustainable agriculture in metal-polluted soils, demonstrating its ability to: (1) enhance spinach resilience by improving root biomass (32%, *p* = 0.01) and chlorophyll content (22%, *p* = 0.002) at 50 ppm Cu/Zn; (2) reduce metal uptake in edible tissues by 28–40% (*p* < 0.05), addressing food safety concerns; and (3) modulate fatty acid profiles to stabilize membranes under stress. While the fungus shows threshold-dependent efficacy, its integration with low-cost substrates (e.g., wheat bran) and Mg-rich biofertilizers could overcome scalability limitations. For field implementation, we advocate a tiered approach—prioritizing *T. harzianum* inoculation in moderately contaminated soils (≤50 ppm) while combining it with biodegradable chelators or biochar in heavily polluted sites. Realizing this potential requires policy-supported initiatives, including farmer training programs and subsidies for bioinoculant production. Future work should explore molecular mechanisms through transcriptomics and large-scale trials to optimize strain-specific protocols. By bridging laboratory findings with practical agriculture, *T. harzianum* emerges as both an ecological and economic tool for safer crop production in contaminated environments.

Declarations:

Ethical Approval: The study protocol was approved by the Research Ethics Committee of Jazan University and was conducted under the ethical principles outlined in the Helsinki Declaration.

Study Period: This study was conducted from October 2024 to May 2025.

Competing Interests: The authors declare no competing interests.

Authors Contributions: This work was solely conducted by one author, who was responsible for data compilation, analysis, and final manuscript editing.

Funding: This research was self-funded.

Availability of Data and Materials: The data presented in this study are available on request from the corresponding author.

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ARABIC SUMMARY

معالجة تربة ملوثة بالمعادن باستخدام السماد الحيوي (*Trichoderma harzianum*) وتأثيره البيوكيميائي على نبات السبانخ(*Spinacia oleracea*)

منى سعد عبدالله عاشور
الكلية الجامعية بالدرب- جامعة جازان

تعد تلوثات التربة بالمعادن الثقيلة من أبرز التحديات البيئية في المناطق الصناعية، حيث تؤدي التراكيز المرتفعة لهذه المعادن إلى اختلال التوازن البيئي. وعلى الرغم من أن بعضها يعتبر عناصر غذائية دقيقة أساسية عند تراكيز منخفضة، إلا أن زيادتها يتسبب في اضطرابات أيضية وتثبيط لنمو النباتات.

هدفت هذه الدراسة إلى تقييم دور السماد الحيوي **Trichoderma harzianum**في معالجة التربة الملوثة بخليط من النحاس والزنك (كبريتات) وتأثيره على نمو وتمثيل نبات **السبانخ** .**(Spinacia oleracea)** تمت زراعة النبات في تربة معالجة بتراكيز مختلفة (50، 100، و200 جزء في المليون) من الخليط المعدني، مع وبدون .**T. harzianum** أظهرت النتائج تحسنًا ملحوظًا في معايير النمو، وزيادة في محتوى الكلوروفيل، وتعزيزًا للأنشطة الأنزيمية (خاصة الكاتالاز والبيروكسيداز) عند استخدام .**T. harzianum**كما كشفت الدراسة عن تغيرات واضحة في تركيب الأحماض الدهنية، حيث ارتفعت مستويات أحماض الستياريك والأراكيديك واللجنوسيريك، مما يجعلها مؤشرات حيوية فعّالة للإجهاد المعدني. الأهم من ذلك، ساهم **T. harzianum**في خفض امتصاص المعادن، حيث انخفض امتصاص النحاس من 6.04% إلى 4.69%. تؤكد هذه النتائج على كفاءة هذا السماد الحيوي في تخفيف سمية المعادن الثقيلة، كما تبرز أهمية التغيرات الأيضية النباتية كأدلة حيوية دقيقة في التقييم البيئي.