Gorgan University of Agricultural Sciences and Natural Resources

Environmental Resources Research (ERR)

Print ISSN: 2783-4832
Online ISSN: 2783-4670

Effect of heavy metals Fe, Cu, and Zn on soil free-living N-fixing bacteria

Nasrin Sabourmoghaddam[1*], Paniz Abdollahi Saeed[2]

[1] Department of Environmental and Natural Resources, Payame Noor University, Tehran, Iran
[2] Plant Protection Department, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Article Info

Article type:
Research Article

Article history:
Received: February 2025
Accepted: October 2025

Corresponding author:
sabourmoghaddam@pnu.ac.ir

Keywords:
Nitrogen-fixing bacteria
Heavy metal pollution
Soil fertility
Azospirillum irakense
Azotobacter chroococcum
Sustainable agriculture

Abstract

Nitrogen-fixing bacteria play a crucial role in sustainable agriculture by enhancing soil fertility and reducing the need for chemical fertilizers. However, heavy metal pollution, particularly from iron (Fe), copper (Cu), and zinc (Zn), poses a significant threat to these beneficial microorganisms. This study investigates the effects of three heavy metals - iron (Fe), copper (Cu), and zinc (Zn) - on the populations of two free-living nitrogen-fixing bacterial strains, *Azospirillum irakense* (AR) and *Azotobacter chroococcum* (AC), under controlled laboratory conditions. The experimental treatments included sulfate ion (SO_4^-) solutions of Fe, Cu, and Zn at four concentrations (0.1, 1, 10, and 100 mg/L), alongside a control group. The results revealed that Zn had the most pronounced inhibitory effect, with population reductions of up to 76% for AR and 80% for AC at the highest concentration of 100 mg/L. Cu also exhibited significant inhibitory effects, particularly at higher concentrations, reducing AR and AC populations by 28% and 15%, respectively, at 100 mg/L. Lower concentrations of Cu (0.1 and 1 mg/L) showed no significant impact on the bacterial populations. In contrast, Fe did not demonstrate any significant inhibitory effects across all tested concentrations. Additionally, we investigated the accumulation of heavy metals within bacterial cells and the production of reactive oxygen species (ROS) under metal stress. Both experiments revealed significant intracellular metal accumulation and elevated ROS levels at higher metal concentrations, further elucidating the mechanisms of metal toxicity. These findings highlight the potential risks associated with the accumulation of heavy metals, particularly Zn and Cu, in agricultural soils. The study underscores the importance of managing heavy metal levels in fertilizers to safeguard soil microbial health and ensure sustainable agricultural practices. This research provides valuable insights for developing strategies to mitigate the adverse effects of heavy metals on soil ecosystems.

Cite this article: Sabourmoghaddam, Nasrin; Abdollahi Saeed; Paniz. 2025. Effect of Heavy metals Fe, Cu, and Zn on Soil Free-Living N-Fixing Bacteria. *Environmental Resources Research*, 13(2), 251-260.

DOI: 10.22069/IJERR.2025.23287.1479
Publisher: Gorgan University of Agricultural Sciences and Natural Resources

Introduction

Soil nitrogen-fixing bacteria are fundamental components of the agricultural ecosystem, significantly contributing to sustainable crop production. By converting atmospheric nitrogen into bioavailable forms, these bacteria reduce the reliance on chemical nitrogen fertilizers, which are associated with environmental degradation, including water eutrophication and greenhouse gas emissions (Tyagi et al., 2022). Nitrogen-fixing bacteria are broadly classified into two groups: symbiotic nitrogen-fixing bacteria and free-living nitrogen-fixing bacteria. The former, such as *Rhizobium* in legumes, form highly specific relationships with plant hosts, creating root nodules for nitrogen fixation. While efficient, their host specificity limits their application across diverse cropping systems (Khosravi et al., 2024). In contrast, free-living nitrogen-fixing bacteria, including *Azospirillum* and *Azotobacter* species, exist independently in the soil and are not constrained by host specificity (Sharma et al., 2021). These bacteria are versatile, contributing to nitrogen fixation across various crops, making them particularly valuable for sustainable agricultural practices (Coniglio et al., 2019). Moreover, free-living nitrogen fixers often play additional roles, such as producing plant growth-promoting substances and improving soil structure (Hindersah et al., 2020).

Given their importance, understanding the impact of environmental factors, including heavy metals from fertilizers, on these bacteria is critical (Alengebawy et al., 2021; Sabourmoghaddam, 2017). Public attention often focuses on toxic metals like mercury (Hg), cadmium (Cd), chromium (Cr), or lead (Pb) (Sabourmoghaddam, 2024a). However, fertilizers also introduce substantial amounts of iron (Fe), copper (Cu), and zinc (Zn) into agricultural soils. Fe, Cu, and Zn are the only heavy metals naturally found in biosystems, such as plant cells, so their pollutant behaviors are often neglected by many experts (Mehri Yari et al., 2024; Naghshafkan et al., 2023). These metals intentionally are added to soil as essential micronutrients fertilizers without much environmental pollution concern, yet their accumulation can cause significant damage to soil's physical and biological structure and can become highly toxic at elevated concentrations (Alengebawy et al., 2021Sabourmoghaddam, 2024b; Sabourmoghaddam and Shakery, 2020).

This study aims to evaluate the effects of Fe, Cu, and Zn on the growth of *Azospirillum irakense* and *Azotobacter chroococcum*, key free-living nitrogen fixers, under controlled conditions. Additionally, we investigate the intracellular accumulation of these metals and the production of reactive oxygen species (ROS) under metal stress. The findings will provide insights into the potential risks of heavy metal pollution in agricultural soils and inform strategies for sustainable soil management.

Materials and Methods

Bacterial Strains and Media

The bacterial strains *Azospirillum irakense* and *Azotobacter chroococcum* were provided by the Soil Science Department of Tabriz Agriculture Faculty. These strains are previously isolated, purified, and molecularly confirmed. Both strains were cultured in Nitrogen-Free Malate (NFM) (malic acid, 5.0g; KOH, 4.0g; K_2HPO_4, 0.5g; $MgSO_4 \cdot 7H_2O$, 0.2g; NaCl, 0.1g; $CaCl_2$, 0.02g; $FeSO_4 \cdot 7H_2O$, 0.01g; $Na_2MoO_4 \cdot 2H_2O$, 0.002g; Water 1000ml; pH 6.8) (Khakvar et al., 2008), which provides an optimal environment for nitrogen-fixing bacteria without introducing external nitrogen sources.

Experimental Setup

Analytical grade $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $ZnSO_4 \cdot 7H_2O$ (Sigma-Aldrich) were used to prepare stock solutions. The sulfate forms were chosen to represent their most common ionic forms found in fertilizers. Metal concentrations were verified using atomic absorption spectroscopy (ChemTech, Model CTA 3000). Five treatments were designed for each metal: Control: 0 mg/L (no heavy metal added), Low; 0.1 mg/L, Moderate; 1 mg/L, High; 10 mg/L and Very High; 100 mg/L.

Each treatment was inoculated with *Azospirillum irakense* and *Azotobacter chroococcum* at an initial concentration of 10^6 CFU/mL at pH 6.8±0.1. Cultures were incubated at 25°C with constant shaking (90 rpm) for five days (120hour). The experiments were performed in triplicates and repeated twice to ensure consistency. Bacterial growth was monitored daily by measuring the optical density (OD) at 650 nm using a spectrophotometer (UV-3600 Plus). Growth rates were calculated relative to the control, and inhibitory effects were expressed as percentage reductions.

Heavy Metal Accumulation Assay

To assess the intracellular accumulation of Fe, Cu, and Zn, bacterial cells were harvested after 120 hours of incubation by centrifugation at 10,000 rpm for 10 minutes. The cell pellets were washed twice with phosphate-buffered saline (PBS) to remove extracellular metal ions. The pellets were digested with concentrated nitric acid (HNO_3) at 80°C for 2 hours. The metal content in the digested samples was quantified using inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer NexION 350D). To ensure accuracy and precision, standard calibration curves were generated using certified reference materials covering the anticipated concentration range. The calibration curves demonstrated excellent linearity ($R^2 > 0.995$), confirming the reliability of the measurements. Quality control samples were included at regular intervals to verify instrument performance, achieving a relative standard deviation (RSD) of less than 5%. The results were expressed as micrograms of metal per milligram of dry cell weight (µg/mg), with all measurements conducted in triplicate to ensure reproducibility.

Reactive Oxygen Species (ROS) Assay

The production of ROS in bacterial cells under heavy metal stress was measured using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Gholami and Etemadifar, 2013). Bacterial cells were exposed to Fe, Cu, and Zn at concentrations of 0.1, 1, 10, and 100 mg/L for 120 hours. After incubation, cells were washed with PBS and incubated with 10 µM DCFH-DA for 30 minutes at 37°C in the dark. The fluorescence intensity was measured using a microplate reader (ELISA-reader BioTek Synergy H1) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. ROS levels were expressed as relative fluorescence units (RFU) normalized to the control.

Data Analysis

Statistical significance of growth inhibition, metal accumulation, and ROS production was determined using ANOVA followed by Tukey's test. A p-value < 0.05 was considered statistically significant. Error bars in growth curves represent standard deviations from the mean. SPSS22 and Excel 2022 were utilized for data analysis and graph design.

Results

Effect of Zinc on Bacterial Growth

Zinc exhibited the most severe inhibitory effects on both bacterial strains. *A. irakense* showed concentration-dependent growth reduction, with significant inhibition observed at all tested concentrations ($p<0.05$). After 120 hours, population reductions of 12.0±1.2%, 22.0±2.1%, 35.0±2.8%, and 76.0±3.5% were recorded at 0.1, 1, 10, and 100 mg/L Zn^{2+}, respectively. *A. chroococcum* displayed similar sensitivity patterns but with more pronounced effects at higher concentrations, showing reductions of 8.0±0.9%, 20.0±1.8%, 28.0±2.5%, and 80.0±3.8% at corresponding concentrations (Figure 1).

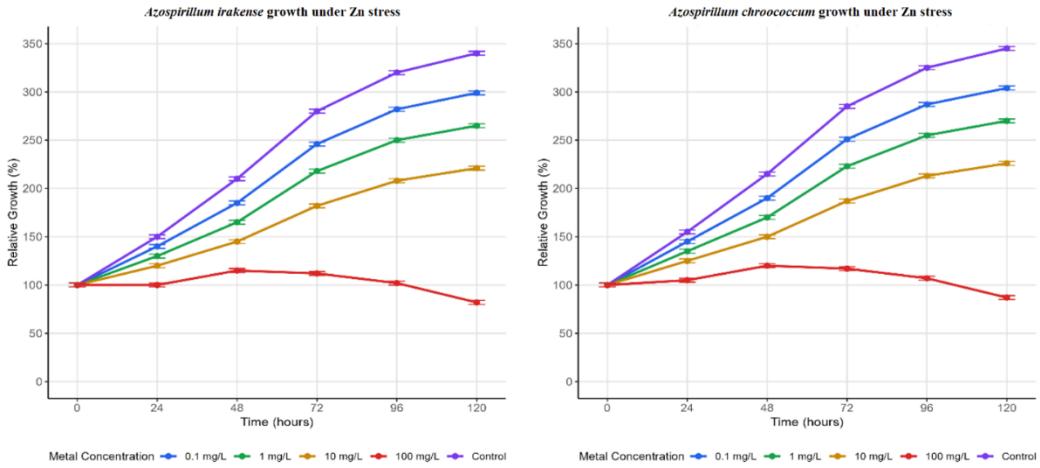

Figure 1. Growth response of *Azospirillum irakense* and *Azotobacter chroococcum* to different Zn treatments

Copper-Induced Growth Inhibition

Copper demonstrated moderate toxicity, with significant effects observed only at higher concentrations. *A. irakense* growth was unaffected at 0.1 and 1 mg/L Cu^{2+}, while 10 and 100 mg/L resulted in 8.0±0.7% and 22.0±2.0% growth reduction, respectively. *A. chroococcum* showed slightly higher tolerance, with reductions of 5.0±0.5% and 29.0±2.4% at Cu2+ concentrations of 10 and 100 mg/L, respectively. (Figure 2).

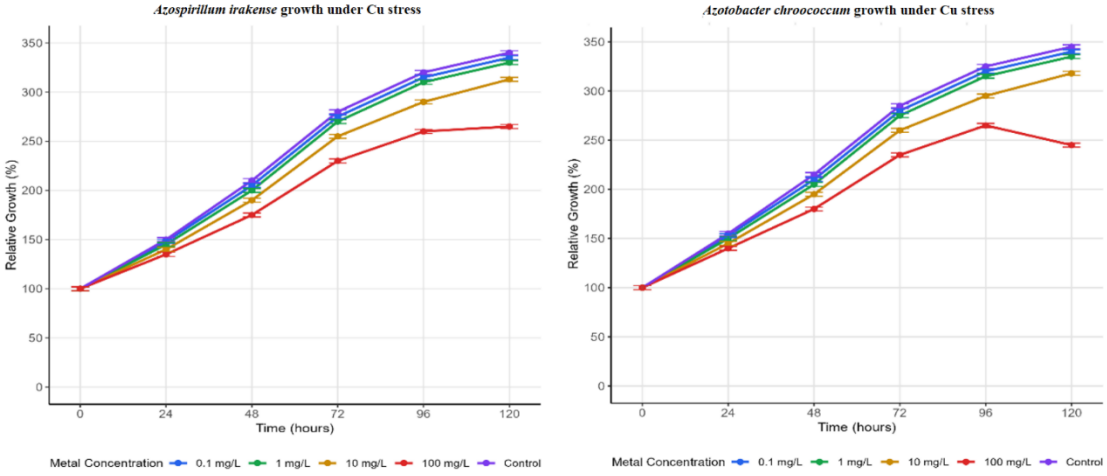

Figure 2. Growth response of *Azospirillum irakense* and *Azotobacter chroococcum* to different Cu treatments

Iron Impact on Bacterial Growth

Iron treatments showed minimal inhibitory effects. *A. irakense* exhibited slight growth reduction (4.0±0.4%) only at 100 mg/L Fe^{2+}, while *A. chroococcum* showed no statistically significant growth inhibition across all concentrations ($p>0.05$).

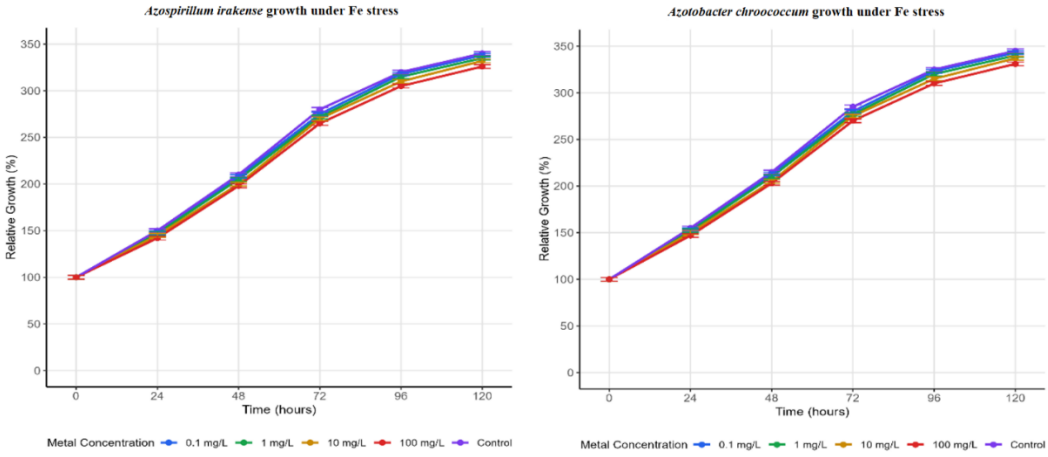

Figure 3. Growth response of *Azospirillum irakense* and *Azotobacter chroococcum* to different Fe treatments

Heavy Metal Accumulation in Bacterial Cells

The intracellular accumulation of Fe, Cu, and Zn was significantly higher at elevated metal concentrations. For *A. irakense*, Zn accumulation increased from 0.5±0.1 µg/mg at 0.1 mg/L to 18.2±1.5 µg/mg at 100 mg/L. Similarly, Cu accumulation increased from 0.3±0.05 µg/mg at 0.1 mg/L to 7.8±1.2 µg/mg at 100 mg/L. Unlike inhibitory effect, Fe accumulation was relatively higher than both previous metals, ranging from 0.2±0.03 µg/mg at 0.1 mg/L to 25.4±0.8 µg/mg at 100 mg/L. *A. chroococcum* showed a similar trend, with Zn accumulation reaching 16.5±1.8 µg/mg at 100 mg/L, Cu accumulation at 11.2±1.3 µg/mg, but Fe accumulation at 29.8±0.9 µg/mg which were even more than *A. irakense* (Figure 4).

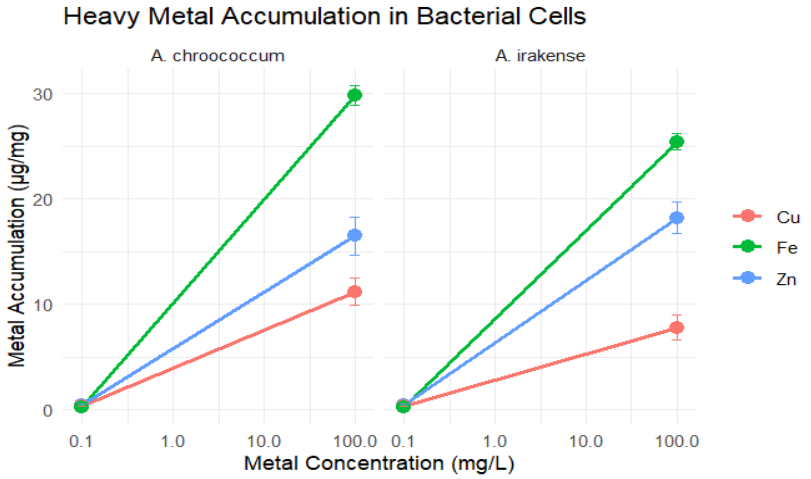

Figure 4. Heavy metal accumulation in *Azospirillum irakense* and *Azotobacter chroococcum* following exposure to Zinc, Copper, and Iron

Reactive Oxygen Species (ROS) Production

ROS levels increased with higher metal concentrations. For *A. irakense*, ROS production increased from 120±10 RFU at 0.1 mg/L Zn^{2+} to 850±75 RFU at 100 mg/L Zn^{2+}. Similarly, Cu^{2+} induced ROS levels increased from 110±8 RFU at 0.1 mg/L to 780±70 RFU at 100 mg/L. Fe^{2+} showed a less pronounced effect, with ROS levels increasing from 100±5 RFU at 0.1 mg/L to 450±50 RFU at 100 mg/L. *A. chroococcum* exhibited a similar pattern, with ROS levels reaching 900±80 RFU for Zn^{2+}, 800±75 RFU for Cu^{2+}, and 500±55 RFU for Fe^{2+} at 100 mg/L (Figure 5).

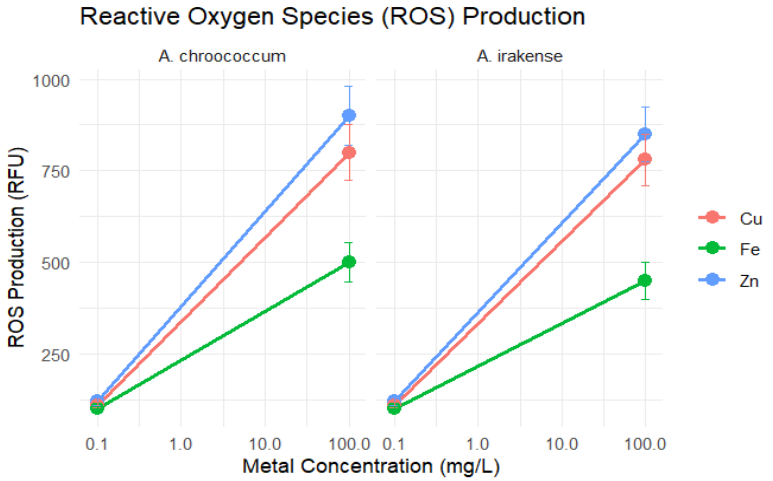

Figure 5. Reactive Oxygen Species (ROS) production in *Azospirillum irakense* and *Azotobacter chroococcum* following exposure to Zinc, Copper, and Iron

Discussion

The differential toxicity patterns observed among Fe, Cu, and Zn demonstrate complex metal-microbe interactions that significantly impact nitrogen-fixing bacterial populations. Our findings align with and expand upon previous research in this field, while providing new insights into metal tolerance mechanisms. In particular, we observed that Zn^{2+} exerts a pronounced inhibitory effect on bacterial growth, with reductions in activity ranging between 76% - 80% at the highest concentration tested (100 mg/L). These findings are in concert with the work of Roy (2021), who reported similar toxicity patterns in soil bacteria, underscoring the vulnerability of these microbes to zinc stress.

The heightened sensitivity of *A. chroococcum* compared to *A. irakense* under high Zn^{2+} exposure (80% vs. 76% reduction) suggests that species-specific differences are at play, likely reflecting distinct genetic and biochemical pathways involved in metal homeostasis. Zn^{2+} toxicity may be attributed to several mechanisms. For instance, zinc ions have the capacity to disrupt membrane integrity by displacing essential cations from their binding sites, jeopardizing the structure and function of the cell membrane (Patil et al., 2024). Moreover, Zn^{2+} may interfere with the assembly or function of critical nitrogenase enzymes involved in biological nitrogen fixation, thereby impairing energy metabolism and growth (Kushwaha et al., 2017; Ramazani and Kargar, 2023). Additionally, excessive Zn^{2+} accumulation is known to trigger the generation of reactive oxygen species (ROS), which inflict oxidative damage on vital cell components such as lipids, proteins, and nucleic acids (Johnson and Hug, 2019). This multifaceted toxicity highlights the necessity for careful monitoring of zinc-containing fertilizers and underscores the importance of maintaining soil Zn^{2+} levels within safe limits, especially in regions where zinc-enriched fertilizers are routinely applied.

In contrast, Cu^{2+} demonstrates moderate toxicity that becomes significant primarily at concentrations exceeding 10 mg/L. Copper plays a dual role in microbial physiology, acting as an essential micronutrient at low concentrations while becoming a harmful toxicant when present in excess. Our study's findings parallel those of Wyszkowska et al. (2008), who noted that soil bacteria exhibit threshold responses to Cu^{2+} exposure. The contrasting responses of *A. irakense* and *A. chroococcum*—evidenced by the 22% versus 29% reduction in growth at 100 mg/L—suggest that distinct metal homeostasis mechanisms may be responsible for this variation. For instance, species-specific metal efflux systems, as reported by Kushwaha et al. (2017), could enable certain bacteria to more effectively export excess copper ions. Additionally, intrinsic differences in membrane permeability (Patil et al., 2024) may influence the rate and extent of Cu^{2+} uptake, while variations in cellular

sequestration mechanisms, such as the binding of copper to specialized proteins or storage molecules (Gupta et al., 2023), further modulate toxicity. The cumulative effects of copper in agricultural soils, particularly under conditions of continual heavy metal input, suggest that even moderate levels of Cu^{2+} stress may have long-term implications for microbial community structure and function.

In stark contrast to Zn and Cu, Fe^{2+} exhibited minimal inhibitory effects on bacterial growth even at high concentrations. This underscores iron's essential role as a nutrient critical for numerous metabolic pathways, especially those central to nitrogenase function in nitrogen fixation. Our observations are consistent with the work of Sahrawat (2005), who documented similar resilience among soil diazotrophs when exposed to varying levels of iron. The high tolerance to Fe^{2+} is likely due to the evolved regulatory mechanisms that bacteria possess to uptake and utilize iron efficiently, thereby maintaining the functionality of essential enzymes without incurring toxic side effects. The significant intracellular accumulation of Zn and Cu, as demonstrated by our heavy metal accumulation assay, reinforces the critical need for regulating metal concentrations in agricultural soils. In beneficial soil bacteria, such as nitrogen-fixing species, the internalization of Zn disrupts ion homeostasis, destabilizing cell membranes and impairing enzymatic activities that are crucial for nitrogen fixation (Nnaji et al., 2024). Similarly, when Cu accumulates within bacterial cells, it can interfere with critical metabolic pathways by altering protein conformations and inhibiting enzymatic reactions, markedly affecting energy production and nitrogenase activity (Fasnacht & Polacek, 2021). These disruptions compromise the bacteria's ability to support soil fertility and plant growth, ultimately impacting crop productivity.

The elevated levels of reactive oxygen species (ROS) detected under Zn and Cu stress provide further insight into the dual toxicity mechanism of these metals. ROS generation is a well-documented consequence of metal-induced oxidative stress, leading to damage across a range of biomolecules. For example, ROS can oxidize nucleic acids, proteins, and lipids, thereby impairing vital cellular functions (Van Acker et al., 2016). This oxidative damage is a critical factor in the observed reduction of bacterial viability and performance. In contrast, the lower ROS levels observed under Fe^{2+} treatment corroborate its minimal impact on bacterial growth, supporting its role as a carefully regulated micronutrient rather than a toxin under the conditions examined.

The mechanisms underlying metal toxicity, as elucidated by the heavy metal accumulation and ROS production assays, indicate that metal uptake and subsequent intracellular damage are key drivers of toxicity. The significant intracellular accumulation of Zn and Cu correlates strongly with the pronounced growth inhibition we observed. Moreover, the elevated ROS levels under Zn and Cu stress confirm that oxidative stress is a principal mediator of the toxic effects of these metals. These insights contribute to our broader understanding of how heavy metal exposure affects soil microbial communities and the mechanisms by which bacteria attempt to adapt or counteract these stresses.

Beyond the immediate physiological consequences, the species-specific responses observed in our study have profound implications for soil health assessments and the formulation of agricultural management practices. For instance, the differential responses between *A. irakense* and *A. chroococcum* highlight the importance of considering microbial diversity when evaluating soil health under metal stress conditions. Such diversity plays a critical role in the overall resilience of the soil ecosystem, with variations in metal tolerance potentially influencing the composition and functionality of microbial communities in contaminated or fertilized soils. From an agricultural practice's standpoint, our findings emphasize the need for careful management of heavy metals within fertilizers. Specific fertilizer management strategies should include the formulation of balanced nutrient packages that minimize the addition of potential toxicants like Zn and Cu, particularly in soils already known to harbor elevated background levels of these metals. For example, the

incorporation of organic amendments, such as compost or biochar, can help chelate heavy metals and reduce their bioavailability. Additionally, periodic monitoring using quantitative techniques like atomic absorption spectroscopy (AAS) ensures that fertilizer applications remain within safe limits, thereby preventing the accumulation of metals in the soil over time.

Furthermore, the use of metal-tolerant bacterial strains in bioaugmentation strategies could mitigate the adverse effects of heavy metals on soil microbial functions. By promoting the growth and activity of resistant diazotrophs, it may be possible to maintain nitrogen fixation processes even in environments where heavy metal stress is prevalent. Such biotechnological interventions, combined with optimized fertilizer formulations, represent promising avenues for safeguarding soil health and ensuring long-term agricultural productivity.

Conclusion

This study demonstrates that heavy metals—particularly Zn and Cu—profoundly inhibit the growth of free-living nitrogen-fixing bacteria through mechanisms that include membrane disruption, interference with essential enzyme functions (such as nitrogenase), and the induction of oxidative stress via reactive oxygen species. These multifaceted toxic effects not only compromise the physiological integrity of these microbes but also threaten the broader soil ecosystem, which is fundamental for sustainable agriculture. Our findings emphasize the critical need for careful management of heavy metal inputs in agricultural fertilizers. Regular monitoring of soil metal concentrations, using precise techniques such as atomic absorption spectroscopy, can guide the timely application of amendments to chelate and immobilize toxic metals. For instance, integrating organic amendments like compost or biochar can reduce the bioavailability of Zn and Cu, thereby mitigating their adverse impacts on microbial health. Moreover, the observed species-specific differences in tolerance—exemplified by the varying responses of *A. chroococcum* and *A. irakense*—highlight the importance of microbial diversity in soil resilience. Future research should explore the genetic and biochemical bases of metal tolerance in these bacteria, which may pave the way for developing metal-tolerant strains. Such strains could be employed in bioaugmentation strategies to sustain nitrogen fixation even under heavy metal stress, ensuring consistent soil fertility and improved crop productivity.

By optimizing fertilizer formulations and employing targeted microbial interventions, it is possible to mitigate heavy metal toxicity and foster long-term agricultural productivity. Future studies should focus on integrating these approaches into comprehensive soil management strategies that preserve microbial function and sustain ecosystem health.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., and Wang, M.Q. 2021. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics. 9(3),42.

Coniglio, A., Mora, V., Puente, M., and Cassán, F. 2019. *Azospirillum* as Biofertilizer for Sustainable Agriculture. Cham: Springer International Publishing. 45-70.

Fasnacht, M., and Polacek, N. 2021. Oxidative stress in bacteria and the central dogma of molecular biology. Frontiers in Molecular Biosciences. 8, 671037.

Gholami, M., and Etemadifar, Z. 2013. Isolation and molecular identification of a new strain of *Microbacterium resistens*, capable of tolerating the extreme conditions. Journal of Microbial Biology. 2(5),27-42.

Gupta, N., Singh, M., Gupta, P., Mishra, P., and Gupta, V. 2023. Heavy metal bioremediation and toxicity removal from industrial wastewater. Next-Generation Algae. 1,163-193.

Hindersah, R., Kamaluddin, N.N., Samanta, S., Banerjee, S., and Sarkar, S. 2020. Role and perspective of Azotobacter in crops production. SAINS TANAH - Journal of Soil Science and Agroclimatology. 17(2),170-179.

Johnson, L.A., and Hug, L.A. 2019. Distribution of reactive oxygen species defense mechanisms across domain bacteria. Free Radical Biology and Medicine. 140, 93-102.

Khakvar, R., Sijam, K., Wong, M. Y., Radu, S., Jones, J., and Thong, K.L. 2008. Genomic diversity of *Ralstonia solanacearum* strains isolated from banana farms in west Malaysia. Plant Pathology Journal. 7(2),162-167.

Khosravi, H., Khoshru, B., Nosratabad, A.F., and Mitra, D., 2024. Exploring the landscape of biofertilizers containing plant growth-promoting rhizobacteria in Iran: Progress and research prospects. Current Research in Microbial Sciences.100268.

Kushwaha, A.N., Rani, R.A., and Kumar, S.A. 2017. Mechanism of soil-metal-microbe interactions and their implication on microbial bioremediation and phytoremediation. Environmental science and engineering. Biodegradation and Bioremediation. 28, 279-305.

Mehri Yari, F., Pirkharrati, H., Farhadi, K., and Soltanalinezhad, N. 2024. Investigation of heavy metals contamination in east Urmia agricultural soil based on contamination indicates. Environmental Resources Research. 12(2),319-332.

Naghshafkan, F., Pirkharrati, H., Farhadi, K., and Soltanalinejad, N. 2023. Evaluation of some heavy metals' contamination around Urmia Lake and city by soil pollution indicators. Environmental Resources Research. 11(2),271-282.

Nnaji, N.D., Anyanwu, C.U., Miri, T., and Onyeaka, H. 2024. Mechanisms of Heavy Metal Tolerance in Bacteria: A Review. Sustainability. 16(24),11124.

Patil, A., Chakraborty, S., Yadav, Y., Sharma, B., Singh, S., and Arya, M. 2024. Bioremediation strategies and mechanisms of bacteria for resistance against heavy metals: a review. Bioremediation Journal. 1-33.

Ramazani, S.H.R., and Kargar, M. 2023. Germination and seedling growth of two canola (*Brassica napus* L.) cultivars under heavy metals. Environmental Resources Research. 11(2), 283-290.

Roy, R. 2021. Effect of cadmium and zinc heavy metals on the soil bacteria isolated from coal mine region. Journal of Advanced Scientific Research. 12(04), 68-77.

Sabourmoghaddam, N. 2017. Comparative assessment of heavy metals changes in the ambient air of two different zones of Tabriz city, Iran. Global Nest Journal. 19(1), 69-73.

Sabourmoghaddam, N. 2024a. Investigating the Concentration of Heavy Metals in the Soil and Water of Sardroud Region; an Industrial and Agricultural Area in Suburb of Tabriz City. Journal of Water and Soil Resources Conservation (WSRCJ). 14(2),1-8. (in Persian)

Sabourmoghaddam, N. 2024b. Investigating the effect of soil contamination with heavy metals on the growth and development of wheat seedlings in greenhouse conditions. Journal of Genetic Engineering and Biosafety. 13 (1),42-50. (in Persian)

Sabourmoghaddam, N., and Shakery, M. 2020. Impact assessment of heavy metals pollution in the air on bacterial microflora of elm trees. Carpathian Journal of Earth and Environmental Sciences. 15(1), 87–92.

Sahrawat, K.Á. 2005. Iron toxicity in wetland rice and the role of other nutrients. Journal of Plant Nutrition. 27(8),1471-1504.

Sharma, S., Kumar, A., Nongthombam, J., and Nakum, K. 2021. A Review on the aspects of *Azospirillum*: Used as a tool in sustainable agriculture. Bulletin of Environment, Pharmacology and Life Sciences. 10(6),20-31.

Tyagi, J., Ahmad, S., and Malik, M. 2022. Nitrogenous fertilizers: impact on environment sustainability, mitigation strategies, and challenges. International Journal of Environmental Science and Technology. 19, 11649–11672.

Van Acker, H., Gielis, J., Acke, M., Cools, F., Cos, P., and Coenye, T. 2016. The role of reactive oxygen species in antibiotic-induced cell death in *Burkholderia cepacia* complex bacteria. PloS one. 11(7), e0159837.

Wyszkowska, J., Kucharski, J., Borowik, A., and Boros, E. 2008. Response of bacteria to soil contamination with heavy metals. Journal of Elementology. 13(3),443-453.