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Impact of Plant Growth-Promoting Rhizobacteria on Spinach Growth Concerned with Industrial Effluents Contaminated with Chromium

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ABSTRACT: *Heavy metal pollution in soil poses a significant threat to the environment and human health as it accumulates throughout the food chain. Remediation of soil contamination involves biological, chemical, and physical methods. Plant growth-promoting rhizobacteria inoculation helps some plants stabilize heavy metals in polluted soil by forming chelates with the metals. An experiment was conducted to examine the impact of Plant growth-promoting rhizobacteria on spinach growth and heavy metal stability in polluted soil with industrial effluents. The study found that Plant growth-promoting rhizobacteria (S1 and S2) enhanced growth and yield parameters, with fresh weights reaching 18% and 14%, highest dry weights at 10% and 40%, and longest roots at 11%. The treatment with Plant growth-promoting rhizobacteria S1 recorded the highest SPAD values (27%). Chemical parameters revealed that Cr levels in roots peaked in treatments without PGPR. The results concluded that PGPR can effectively remediate heavy metal-contaminated soils and industrial effluent-irrigated soils used for food crop growth.*
Keywords: Spinach, Soil, Heavy metal, PGPR, Plant growth

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is considered an important nutritional vegetable. It is considered a vital component of a diet because it provides many micronutrients such as Mn (manganese), Mg (magnesium), Fe (iron), K (potassium), and folic acid, with small amounts of vitamins A, B2, K, and C (Sarwar et al., 2023). Furthermore, spinach exhibits strong antioxidant activity, primarily because it contains flavonoids, a key component of water-soluble polyphenols. Spinach also has anti-carcinogenic, antioxidant, and antimicrobial activities (Shilev et al., 2020). Moreover, adding spinach to the diet can reduce the post-ischaemic stroke in the brains of rats. Potentially due to its anti-apoptosis and antioxidant effects as well as its anti-inflammatory properties (Zaheer et al., 2020). Research has demonstrated that individuals consuming spinach or carrots more than twice a week have a lower risk of breast cancer recurrence and prostate cancer propagation compared to those who did not (Tirry et al., 2021). Moreover, early research documented that the people who consumed spinach had a lower risk of developing cancer than those who didn't consume any (Ali et al., 2023). Metal pollution in soil has become a significant hazard to agriculture, not only in Pakistan but also globally. It has risen dramatically in recent years, and it is now one of the aspects affecting agricultural production (Alengebawy et al., 2021). Furthermore, high levels of heavy metal pollution severely damage the ecosystem; the term HMs (heavy metal) refers to a metallic substance that has a greater density than others and is dangerous even in small quantities (Shahid et al., 2020). According to Renu et al. (2021), heavy metal substances are defined as those that have a density greater than 5 g/cm3, a specific gravity greater than four, and an atomic weight ranging from 63.54 to 200.5 g/mol. Both natural and anthropogenic activities can introduce heavy metals into the environment. Human activities that contribute to heavy metal pollution include mining activities, the use of pesticides containing high quantities of metals, the use of heavy metal-contaminated sludge in agro-ecosystems, and metallurgical and electronic processing (Tchounwou et al., 2012). The natural sources of heavy metal pollution include the movement of dust from the mainland, the identification of metal-bearing rocks, and volcanic activity (Ahmad et al., 2021). The addition of microbes to the soil can aid in HM remediation by reducing their availability. Plant growth-promoting rhizobacteria can significantly decrease HM stress in plants by producing siderophores, indole-3-acetic acid (IAA), organic acids, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and phytohormones (De Andrade et al., 2023). Other mechanisms may involve complexation, chelation, immobilization, and enzymatic transformation of HMs. Thus, plant growth-promoting rhizobacteria is the best alternative to chemical fertilizers and can contribute to sustainable agricultural production (Jamil et al., 2024). Various chemical and physiological methods, such as the reverse process of osmosis, reduction process, and ion-exchange process, are available to mitigate the harmful impact of heavy metals (HMs) (Batool et al., 2015). However, these remedial methods are costly and decrease the fertility of soil. Plant growth-promoting rhizobacteria is another method to reduce heavy metal pollution. The bacteria in the rhizosphere can enhance the developmental process of plants in various ways, including by fixing nitrogen, producing siderophores, solubilizing phosphorous, and enhancing the symbiotic process of plants. This plant growth-promoting rhizobium can perform three different roles, like production of specific substances, plant protection from diseases, and improved nutrient absorption (Sami et al., 2023).

MATERIALS AND METHODS

The Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, set up a pot experiment in the wire house to study the effect of industrial effluent and PGPR on spinach growth, using a completely randomized design (CRD). Six different treatments were used, including industrial effluent and PGPR, along with a control, and three replications were done. All pots were irrigated with water as and when needed. Every week, weeds were removed to promote better germination and growth. The recommended doses of nitrogen (N), potassium (K), and phosphorus (P) were provided by using urea, DAP, and sulphate of potash (SOP). All the recommended agronomic practices were carried out till crop maturity.

NPK Recommended for Spanish

A fertilizer with a 20-10-10 ratio may be more appropriate for Spanish, as they require a higher nitrogen concentration for lush development.

Making Samples

A dose of samples for the experiment were prepared by growing Cr-tolerant strains in LB broth media. To achieve this, we autoclaved the LB broth medium at 121 °C and 15 psi pressure for 20 minutes, followed by the inoculation of the plant growth-promoting rhizobacteria (U17) strain. Before being used for seed inoculation, it was shifted in a shaking incubator at 28 ˚C for 2 to 3 days.

T1 represents the control group (no industrial effluent and no PGPR), T2 represents the industrial effluent (50 mL/pot), T3 represents S1

(PGPR alone), T4 represents S2 (PGPR combined with industrial effluent), T5 represents S1 + industrial effluent, and T6 represents S2 + industrial effluent. The spinach crop was used as a test crop.

STATISTICAL ANALYSIS

The collected data had been submitted to an ANOVA (analysis of variance) using the computer program Statistix-8.1 (Statistics, 2006). LSD was employed to examine treatment variations at ($P<0.05$).

RESULTS

Shoot fresh weight

The plant's fresh weight revealed that the application of industrial effluent resulted in a minimum value of 40.33 g (Fig. 1). Compared to the control, applying industrial effluent suppressed spinach growth by 8%. The application of metal-resistive strains was observed better under normal and contaminated conditions.

However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. While the application of plant growth-promoting rhizobacteria (S1) was observed to be more effective under contamination conditions, strain (S1) increased the fresh weight of spinach by 23% under contaminated conditions with industrial effluents, while the strain (S2) increased the fresh weight of spinach by 25% compared to the respective control. Overall, the findings revealed that plant growth-promoting rhizobacteria strains were improving crop growth in contaminated soil with industrial effluents.

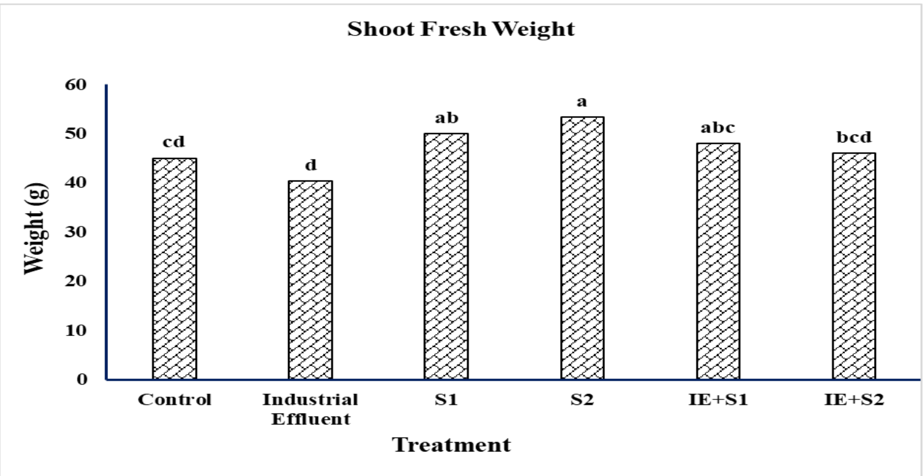

Fig. 1. Effect of industrial effluents and Plant growth-promoting rhizobacteria on shoot fresh weight

Shoot dry weight

According to the shoot dry weight industrial effluent application had a minimum value of 7 g (Fig. 2). The application of industrial effluent reduced spinach growth by 14% compared to the control. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. Under contamination conditions, the application of S1 yielded superior results, enhancing the shoot dry weight of spinach by 83% when combined with industrial effluents. Strain S2 significantly enhanced the dry weight of spinach by 50% compared to its reference control. The overall findings showed that plant growth-promoting rhizobacteria strains were effective in improving the growth of spinach under contaminated soil.

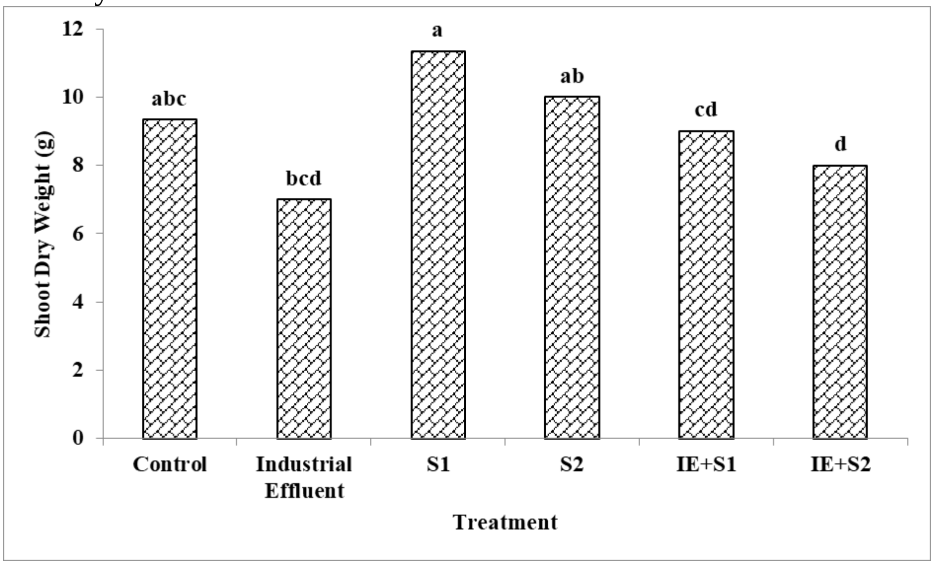

Fig. 2. Effect of industrial effluents and plant growth-promoting rhizobacteria on shoot dry weight

Root Fresh Weight (g)

When applying industrial effluent without the use of plant growth-promoting Rhizobacteria strains or other industrial effluents, we measured a minimum mean value of 9.66 g based on the root fresh weight data in Figure 3. Applying industrial effluent results in 21% slower spinach growth compared to the control group. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. Conversely, the control group did not use any plant growth-promoting rhizobacteria strain. However, the application of plant growth-promoting rhizobacteria strains (S1 and S2) increased plant growth by 17% and 20%, respectively, under polluted conditions containing industrial effluents. Thus, we deduced that rhizobacteria strains that promote plant growth improved crop growth in polluted soil.

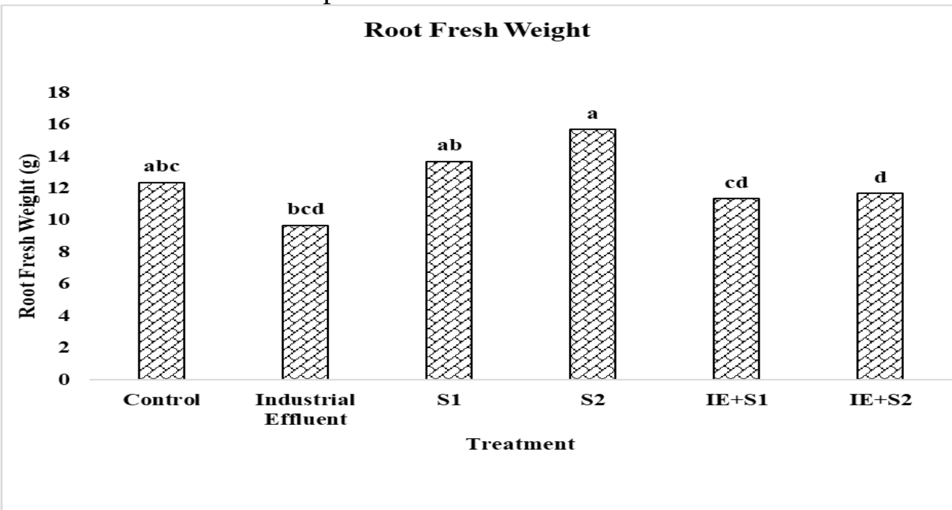

Fig. 3. Effect of industrial effluents and plant growth-promoting rhizobacteria on Root Fresh Weight

Root Dry weight (g)

The data on plant root dry weight indicated that the application of industrial effluent yielded a minimum value of 1 g (Fig. 4). Compared to the control, applying industrial effluent suppressed spinach growth by 39%. We observed better results with the application of metal-resistive strains under both normal and contaminated conditions. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. While the application of plant growth-promoting rhizobacteria (S1) was observed to be better under contamination conditions, S1 increased the root dry weight of spinach by 33% under contaminated conditions with industrial effluents, and strain (S2) increased the dry weight of spinach by 40% as compared to its respective control. Overall, the findings revealed that plant growth-promoting rhizobacteria strains were improving crop growth in contaminated soil with industrial effluents.

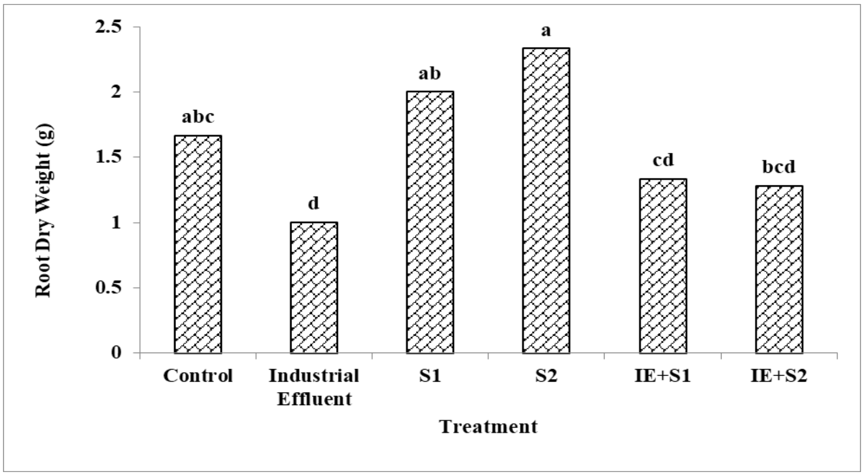

Fig. 4. Effect of industrial effluents and plant growth-promoting rhizobacteria on root dry weight

Shoot Length (cm)

The data presented in Fig. 5 regarding shoot length cm indicated a minimum value of 5.4 cm for the application of industrial effluents. The application of industrial effluent reduced spinach growth by 22% compared to the control. However, the application of S1 resulted in a 50% increase in the plant's growth compared to the control. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. Under contamination conditions, the application of S1 showed superior results, enhancing spinach shoot length by 48% when combined with industrial effluents. In contrast to its perspective control, strain S2 enhanced the shoot length cm of spinach by 60%. Overall, the findings showed that plant growth-promoting rhizobacteria strains were effective in improving spinach growth in contaminated soil.

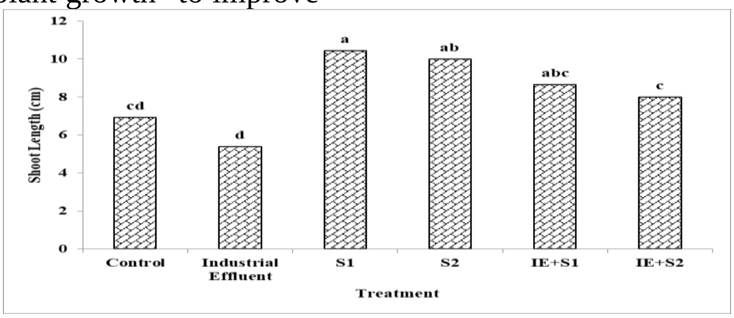

Fig. 5. Effect of industrial effluents and plant growth-promoting rhizobacteria on shoot length

Root length (cm)

The data on root length cm in Figure (6) revealed that the application of industrial effluent, without the use of plant growth-promoting rhizobacteria strains or

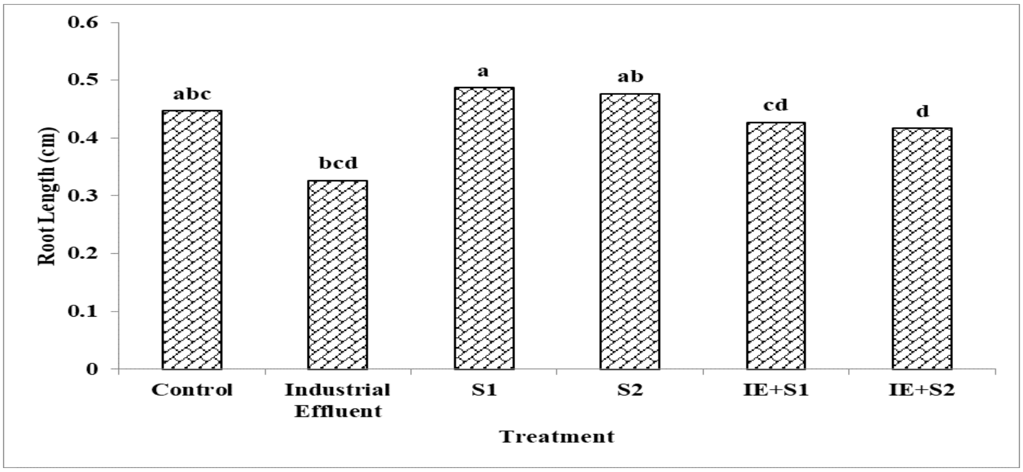

Fig. 6. Effect of industrial effluents and plant growth-promoting rhizobacteria on root length

industrial effluents, resulted in a minimum mean value of 0.32 cm. The application of industrial effluent retards the growth of spinach by 14% as compared to control. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. The use of plant growth-promoting rhizobacteria strains S1 and S2, on the other hand, increased plant growth by 14% and 8%, respectively, when industrial wastewater was present. Therefore, we concluded that the application of plant growth-promoting rhizobacteria strains enhanced crop growth in contaminated soil.

Total Nitrogen in Shoot

The plant's fresh weight indicated that an application of industrial effluent resulted in a minimum value of 0.32% (Fig. 7). Compared to the control, the application of industrial effluent suppressed spinach growth by 26%. The application of metal-resistive strains was observed better under normal and contaminated conditions. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. Under contaminated conditions with industrial effluents, the application of S1 performed better, increasing the fresh weight of spinach by 31%, while strain S2 increased the total nitrogen in the spinach shoot by 25% compared to its respective control. The overall findings revealed that plant growth-promoting rhizobacteria strains were improving crop growth in contaminated soil with industrial effluents.

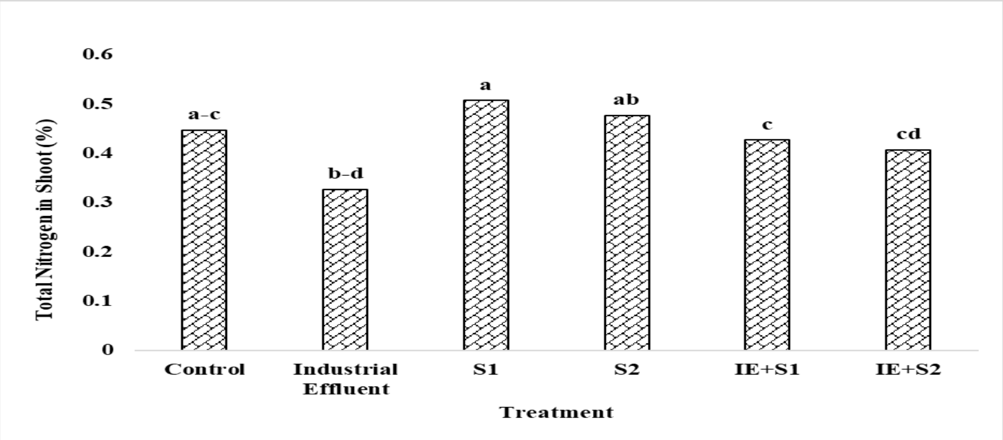

Fig. 7. Effect of industrial effluents and plant growth-promoting rhizobacteria on total Nitrogen in Shoot

Total Nitrogen in Root

The shoot dry weight data revealed that industrial effluent application had a minimum value (0.32%) (Fig. 8). The application of industrial effluent reduced spinach growth by 11% compared to the control. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. Application of S1 was observed better when used in contaminated conditions, strain S1 gave better results, increasing the total nitrogen in the spinach root by up to 15%. On the other hand, strain S2 made the spinach 19% heavier than the control group. Findings showed that plant growth-promoting rhizobacteria strains were effective at improving spinach growth in contaminated soil.

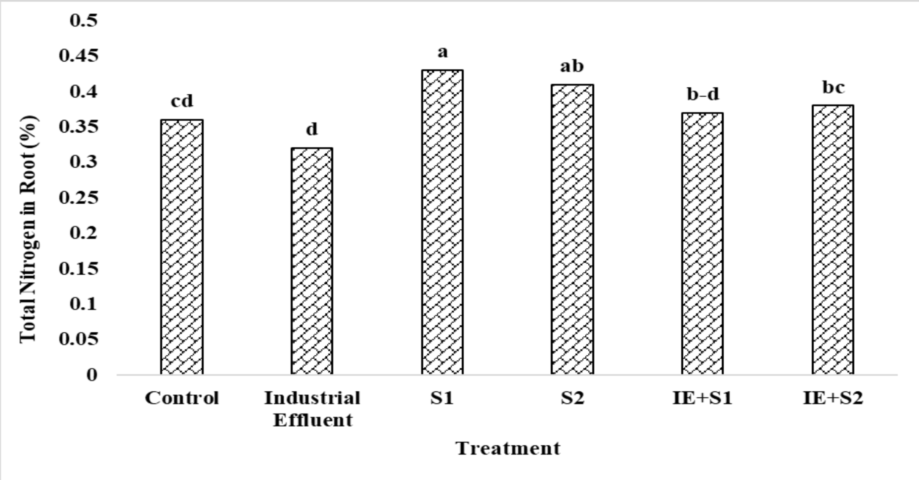

Fig. 8. Effect of industrial effluents and plant growth-promoting rhizobacteria on total Nitrogen in root

Phosphorus in Shoot

The data on root fresh weight, as presented in Fig. 9, revealed that the application of industrial effluent, without the use of plant growth-promoting Rhizobacteria strains and industrial effluents, yielded a minimum mean value of 0.11%. Compared to the control, applying industrial effluent retards spinach growth by 31%. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. This was different from the control, which did not use the plant growth-promoting rhizobacteria strain. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth by 27% and 45%, respectively. Therefore, we concluded that plant growth-promoting rhizobacteria strains enhanced crop growth in contaminated soil.

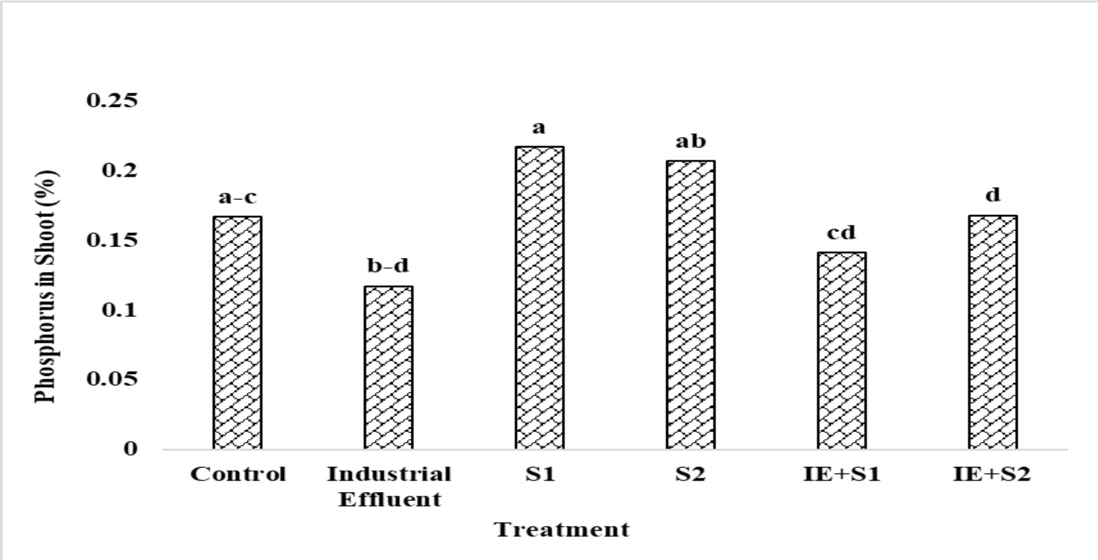

Fig. 9: Effect of industrial effluents and plant growth-promoting rhizobacteria on Phosphorus content in Shoot

Phosphorus in Root

Figure (10) data on plant fresh weight revealed that the application of industrial effluent resulted in a minimum value (0.1166%). Compared to the control, applying industrial effluent suppressed spinach growth by 35%. The application of metal-resistive strains was observed better under normal and contaminated conditions. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. In an experiment where industrial wastewater was present, strain S1 did better, increasing the fresh weight of spinach by 45%. On the other hand, strain S2 increased the amount of phosphorus in the spinach root by 63% compared to the control. It was revealed that strains of plant growth-promoting rhizobacteria were improving crop growth in contaminated soil with industrial effluents.

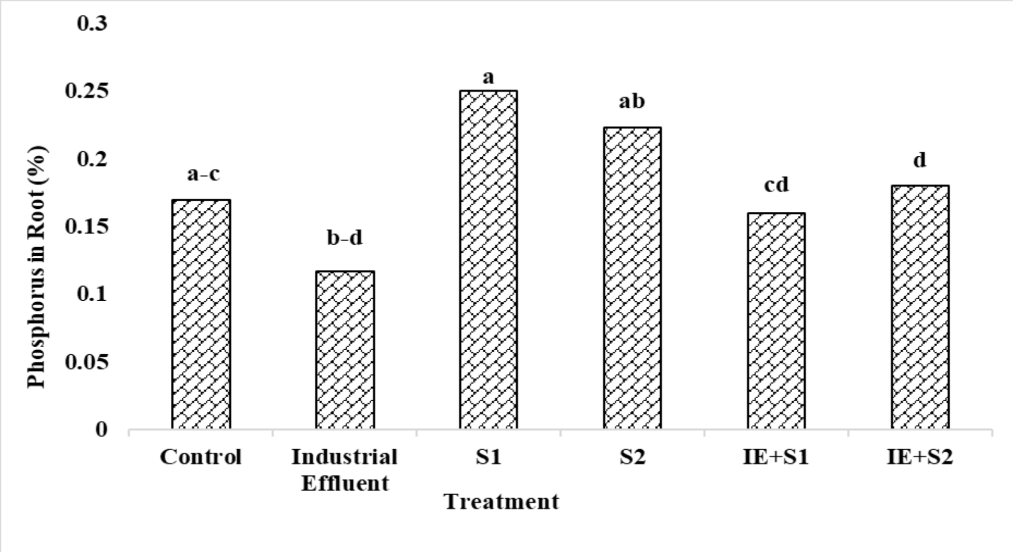

Fig. 10. Effect of industrial effluents and plant growth-promoting rhizobacteria on Phosphorus content in Root

Potassium in Shoot

The shoot dry weight data shown in Figure (11) revealed that the application of industrial effluents had a minimum value (0.4%). The application of industrial effluent reduced spinach growth by 24% compared to control. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth" to improve the structure and reduce redundancy. Under contamination conditions, the application of S1 yielded better results, and when combined with industrial effluents, S1 enhanced the dry weight of spinach by up to 21%. Strain S2 boosted potassium levels in the spinach root by 17% in comparison to the control. Overall, the findings showed that plant growth-promoting rhizobacteria strains were effective in improving spinach growth in contaminated soil.

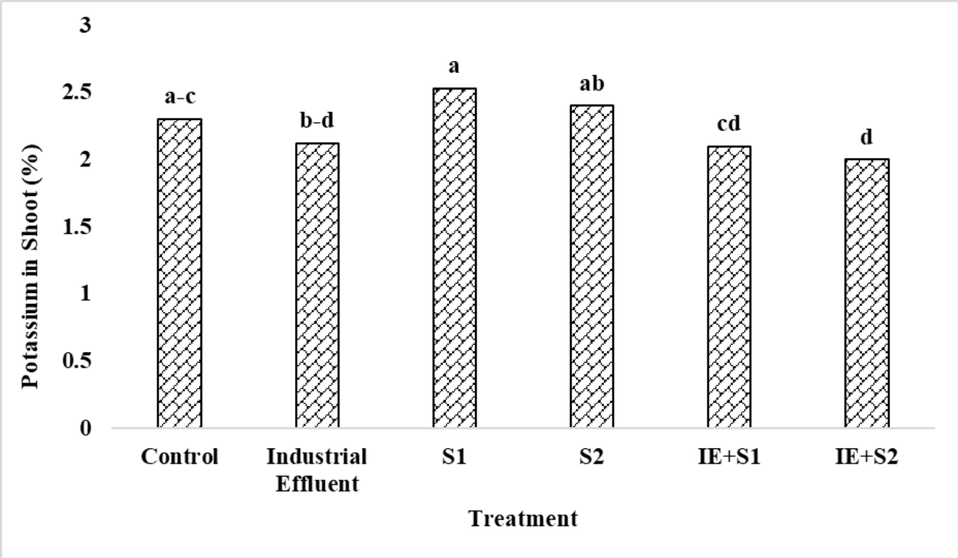

Fig. 11. Effect of industrial effluents and plant growth-promoting rhizobacteria on Potassium content in Shoot

Potassium in Root

Fig. 12 showed the application of industrial effluent, without the use of plant growth-promoting rhizobacteria strains and industrial effluents, results in a minimum mean value of potassium (0.2%). Compared to the control, applying industrial effluent retards spinach growth by 32%. However, the application of S1 boosted plant growth by 65% and S2 enhanced plant yield by 47%, respectively, compared to the control, which did not use any plant growth-promoting rhizobacteria strain. However, under contaminated conditions with industrial effluents, the applications of strains S1 and S2 promoted plant growth by 36% and 28%, respectively. Therefore, we concluded that rhizobacteria strains that promote plant growth enhanced crop growth in contaminated soil.

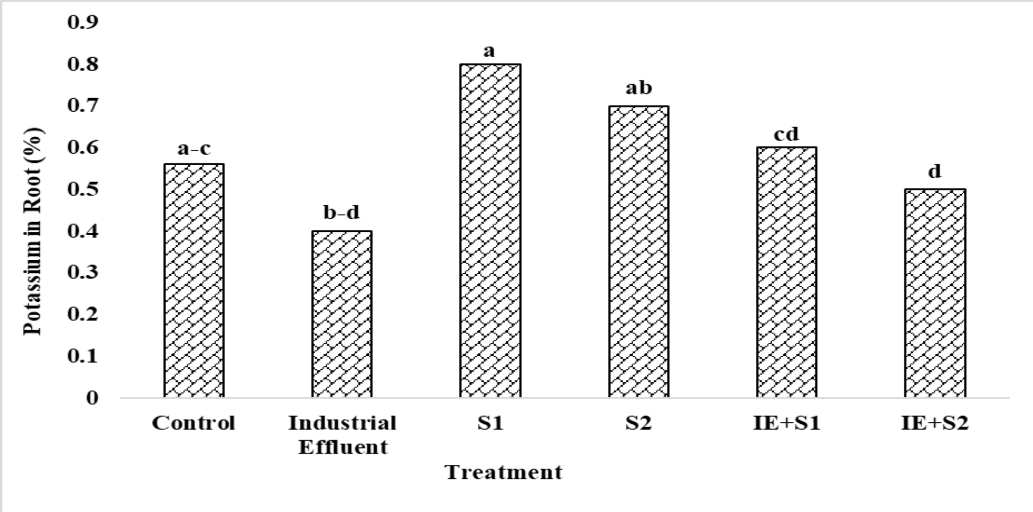

Fig. 12. Effect of industrial effluents and plant growth-promoting rhizobacteria on Potassium content in Root

DISCUSSION

The first sign of heavy metal poisoning is thought to be a reduction in biomass output and plant height (Chen et al., 2019). Heavy metal toxicity may occur at any stage of plant development, including the vegetative and reproductive stages. When the level of heavy metal in the soil rises, it becomes directly accessible to plants (Khaliq et al., 2022). Plants absorb metals, which affect plant metabolism and prevent the absorption of other necessary elements. Compared to other methods, using different metal-tolerant plant growth-promoting rhizobacteria strains alone or together successfully makes the plant stronger, less likely to contract diseases, taller, less susceptible to biotic and abiotic stressors, and less likely to absorb and accumulate metals. In this study, applying plant growth-promoting rhizobacteria (Strain 1) without any other external factors resulted in a 50% improvement in plant height. A study by Dubey et al. (2018) validates my findings, demonstrating a 20% rise in plant height compared to soils irrigated with metal-contaminated industrial effluents. Researchers reported the same results: using plant growth-promoting rhizobacteria in spinach crops leads to increased plant heights. The application of plant growth-promoting rhizobacteria (Strain1 and Strain2) resulted in the highest fresh and dry weights of shoots compared to the normal control. The obtained results aligned with the findings of Cordero et al. (2018), who also observed an increase in root fresh and dry weight upon the application of plant growth-promoting rhizobacteria strains in industrial effluent soils. The application of industrial effluents blocked important nutrients within the plants, resulting in a decrease in the fresh and dry weight of the shoots. Abiotic stress can alter a plant's defence mechanisms. However, plant growth-promoting rhizobacteria and their combinations increased plant biomass production and enhanced plant defence mechanisms against abiotic stress. They also doubled the fresh and dry weight of shoots by lowering metal uptake and increasing mineral nutrient uptake. The quantity of roots and their penetration into the soil determine plant erection and stability. Roots in regular soil travel a long distance in quest of vital nutrients. The presence of metals in the soil negatively affects the structure of the roots, leading to a restricted root zone. Consequently, the industrial effluent roots exhibited reduced root length, root freshness, and dry weight compared to the control roots, which did not receive plant growth-promoting rhizobacteria or industrial effluent treatment. plant growth-promoting rhizobacteria -1, plant growth-promoting rhizobacteria -2, and their combinations significantly reduced metal absorption in plants. The plant growth-promoting rhizobacteria (Strain 1 and Strain 2) showed the maximum root length and root fresh and dry weights in comparison to all other treatments. This was the same outcome as Paredes et al. (2018). Where no plant growth-promoting rhizobacteria were used to reduce heavy metal buildup in plants, the concentration of heavy metals in industrial effluents rose in plant control treatment. Zafar-ul-Hye et al. (2018) applied plant growth-promoting rhizobacteria (Strain 1 and Strain 2) to reduce metal accumulation in wheat, confirming my findings that industrial effluents increased plant metal accumulation, but the application of plant growth-promoting rhizobacteria made plants more resistant to metals and reduced their accumulation. These findings are consistent with those of Gonzalez et al. (2021), who found that plant growth-promoting rhizobacteria may reduce metal toxicity in regions irrigated with industrial effluents.

CONFLICT OF INTEREST

There is no conflict of interest shown by the research manuscript authors.

ACKNOWLEDGEMENT

Authors are highly graceful to Department of ??????

CONCLUSION

The study demonstrated that Cr-contaminated industrial effluents negatively affect spinach growth by reducing plant height, shoot, and root fresh weights. However, the application of plant growth-promoting rhizobacteria significantly improved these growth parameters, including nutrient uptake of N, P, and K. Thus, rhizobacteria inoculation effectively mitigates heavy metal toxicity and enhances spinach growth under contaminated soil conditions.

REFERENCES

1. Ahmad M, Ahmad I, Nazli F, Mumtaz MZ, Latif M, Al-Mosallam MS, El-Shafei AA (2021). Lead-tolerant bacillus strains promote growth and antioxidant activities of spinach (*Spinacia oleracea*) treated with sewage water. Agron., 11(12): 2482.
2. Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang MQ (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxic., 9(3): 42.
3. Ali, A., Alghanem, S. M. S., Al-Haithloul, H. A. S., Muzammil, S., Adrees, M., Irfan, E., & Abeed, A. H. (2023). Co-application of copper nanoparticles and metal tolerant Bacillus sp. for improving growth of spinach plants in chromium contaminated soil. *Chemosphere*, *345*, 140495.
4. Batool, R., Yrjälä, K., & Hasnain, S. (2015). Alleviation of phyto-toxic effects of chromium by inoculation of chromium (VI) reducing Pseudomonas aeruginosa Rb-1 and Ochrobactrum intermedium Rb-2. *International Journal of*

Agriculture & Biology, *17*, 21-30.

5. Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. Frontiers in Plant Science, 9, 1945.
6. Cordero, I., Balaguer, L., Rincón, A., & Pueyo, J. J. (2018). Inoculation of tomato plants with selected PGPR represents a feasible alternative to chemical fertilization under salt stress. *Journal of Plant Nutrition and Soil Science*, *181*(5), 694-703.
7. De Andrade, L.A., Santos, C.H.B., Frezarin, E.T., Sales, L.R. and Rigobelo, E.C., 2023. Plant growth-promoting rhizobacteria for sustainable agricultural production. Microorganisms, 11(4), p.1088.
8. Dubey, S., Shri, M., Gupta, A., Rani, V., & Chakrabarty, D. (2018). Toxicity and detoxification of heavy metals during plant growth and metabolism. *Environmental Chemistry Letters*, *16*, 1169-1192.
9. Gonzalez, D., Blanco, C., Probanza, A., Jiménez, P. A., & Robas, M. (2021). Evaluation of the PGPR capacity of four bacterial strains and their mixtures, tested on Lupinus albus var. dorado seedlings, for the bioremediation of mercury-polluted soils. *Processes*, *9*(8), 1293.
10. Jamil, M., Malook, I., Rehman, S. U., Aslam, M. M., Fayyaz, M., Shah, G., & Abeed, A. H. (2024). Inoculation of heavy metal resistant bacteria alleviated heavy metal-induced oxidative stress biomarkers in spinach (Spinacia oleracea L.). *BMC Plant Biology*, *24*(1), 221.
11. Khaliq, M. A., Javed, M. T., Hussain, S., Imran, M., Mubeen, M., Nasim, W., & Abdo, H. G. (2022). Assessment of heavy metal accumulation and health risks in okra (Abelmoschus Esculentus L.) and spinach (Spinacia Oleracea L.) fertigated with wastwater. *International*

Journal of Food Contamination, *9*(1), 11.

12. Naili, F., Neifar, M., Elhidri, D., Cherif, H., Bejaoui, B., Aroua, M., & Cherif, A. (2018). Optimization of the effect of PGPR–based biofertlizer on wheat growth and yield. *Biometrics & Biostatistics International Journal*, 7, 226-232.
13. Paredes-Paliz, K., Rodríguez-Vázquez, R., Duarte, B., Caviedes, M. A., Mateos-Naranjo, E., Redondo-Gómez, S., ... & Pajuelo, E. (2018). Investigating the mechanisms underlying phytoprotection by plant growth-promoting rhizobacteria in Spartina densiflora under metal stress. *Plant Biology*, *20*(3), 497-506.
14. Renu, Sarim, K. M., Sahu, U., Bhoyar, M. S., Singh, D. P., Singh, U. B., & Manna, M. C. (2021). Augmentation of metal-tolerant bacteria elevates growth and reduces metal toxicity in spinach. *Bioremediation Journal*, *25*(2), 108-127.
15. Sami, H., Ashraf, K., Sultan, K., Alamri, S., Abbas, M., Javied, S., & uz Zaman, Q. (2023). Remediation potential of biochar and selenium for mitigating chromium-induced stress in spinach to minimize human health risk. *South African Journal of Botany*, *163*, 237-249.
16. Sarwar, M. J., Shabaan, M., Asghar, H. N., Ayyub, M., Ali, Q., Zulfiqar, U., & Elshikh, M. S. (2023). Interaction of chromium (Cr) resistant plant growth promoting rhizobacteria with compost to phytostabilize Cr in spinach rhizosphere. *Plant Stress*, *10*, 100261.
17. Shahid, M., Javed, M. T., Tanwir, K., Akram, M. S., Tazeen, S. K., Saleem, M. H., & Chaudhary, H. J. (2020). Plant growth-promoting Bacillus sp. strain SDA-4 confers Cd tolerance by physio-biochemical improvements, better nutrient acquisition and diminished Cd uptake in Spinacia oleracea L. *Physiology and molecular biology of plants*, *26*, 2417-2433.

18. Shilev, S., Babrikova, I., &Babrikov, T. (2020). Consortium of plant growth-promoting bacteria improves spinach (Spinacea oleracea L.) growth under heavy metal stress conditions. *Journal of Chemical Technology & Biotechnology*, *95*(4), 932-939.

19. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. Molecular, clinical and environmental toxicology: volume 3: environmental toxicology, 133-164.

20. Tirry, N., Kouchou, A., El Omari, B., Ferioun, M., & El Ghachtouli, N. (2021). Improved chromium tolerance of Medicago sativa by plant growth-promoting rhizobacteria (PGPR). *Journal of Genetic Engineering and Biotechnology*, *19*, 1-14.

21. Yanez-Yazlle, M. F., Romano-Armada, N., Rajal, V. B., &Irazusta, V. P. (2021). Amelioration of saline stress on chia (Salvia hispanica L.) seedlings inoculated with halotolerant plant growth-promoting bacteria isolated from hypersaline environments. *Frontiers in Agronomy*, *3*, 665798.

22. Zafar-Ul-Hye, M., A. Shahjahan, S. Danish, M. Abid and M.F. Qayyum. 2018. Mitigation of cadmium toxicity induced stress in wheat by ACC-deaminase containing PGPR isolated from cadmium polluted wheat rhizosphere. *Pakistan Journal of Botany*, 50: 1727-1734.

23. Zaheer, I. E., Ali, S., Saleem, M. H., Noor, I., El-Esawi, M. A., Hayat, K., & Wijaya, L. (2020). Iron–lysine mediated alleviation of chromium toxicity in spinach (Spinacia oleracea L.) plants in relation to morpho-physiological traits and iron uptake when irrigated with tannery wastewater. *Sustainability*, *12*(16), 6690.