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Isolation and Characterization of Plant Growth-Promoting Microorganisms from Heavy Metal Contaminated Soil

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ABSTRACT:
The study aimed to isolate and characterize plant growth-promoting microorganisms (PGPMs) from heavy metal-contaminated soils to explore their potential in bioremediation and sustainable agriculture. Soil samples were collected from industrial, mining, and agricultural sites with heavy metal exposure, and microorganisms were isolated using selective media. Morphological, biochemical, and molecular analyses identified diverse bacterial and fungal genera, including Bacillus, Pseudomonas, and Trichoderma. These isolates exhibited significant plant growth-promoting traits, such as indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore production. Notably, Bacillus subtilis strains produced up to 40 µg/mL of IAA, and Pseudomonas fluorescens showed efficient phosphate solubilization. Heavy metal tolerance was assessed through minimum inhibitory concentration (MIC) tests, revealing several isolates with high tolerance to lead (Pb), cadmium (Cd), and zinc (Zn). For instance, Bacillus megaterium and Pseudomonas aeruginosa maintained growth at metal concentrations exceeding 500 mg/L. Mechanisms of tolerance included exopolysaccharide (EPS) production and enhanced antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase), which were particularly notable in Bacillus subtilis and Aspergillus niger. These traits enable the microorganisms to bind heavy metals, reduce their bioavailability, and neutralize reactive oxygen species (ROS), thereby enhancing their survival and function in contaminated environments. The findings suggest that these PGPMs can be utilized for bioremediation by detoxifying and stabilizing heavy metals in soils, reducing their ecological risk. Additionally, their application in agriculture as biofertilizers can promote plant growth and soil health, reducing reliance on chemical fertilizers. Future research should focus on field trials to validate laboratory findings, exploring microbial

consortia for synergistic effects, and developing formulations for large-scale application. Overall, the successful isolation and characterization of PGPMs from heavy metal-contaminated soils provide a promising approach to addressing soil contamination and enhancing agricultural productivity. These microorganisms offer a sustainable solution to improve plant growth in challenging environments, contributing to environmental conservation and food security.

Keywords:
Plant growth-promoting microorganisms, heavy metal contamination, bioremediation, soil microbiology, sustainable agriculture

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Introduction

Heavy metal contamination in soil has emerged as a significant environmental concern over the past few decades, primarily due to rapid industrialization [1], urbanization, and agricultural practices. Heavy metals, such as lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), and zinc (Zn), are non-biodegradable and persist in the environment for extended periods, causing detrimental effects on soil health, plant growth, and overall ecosystem balance. The presence of these metals at elevated concentrations in the soil not only impairs soil microbial activity but also affects plant growth and development through phytotoxicity [2], leading to reduced agricultural productivity and potential entry into the food chain, posing serious health risks to humans and animals. The urgent need to address heavy metal contamination has led to the exploration of various remediation techniques. Conventional methods such as soil excavation, landfilling, and chemical treatments are often expensive, labor-intensive, and can lead to secondary pollution.

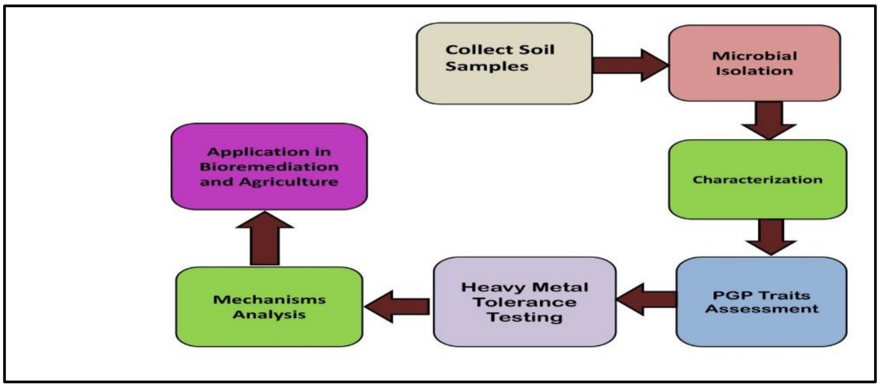

Figure 1: Overall Workflow of Isolating and Characterizing Plant Growth-Promoting Microorganisms from Heavy Metal-Contaminated Soil

In contrast, bioremediation, which utilizes microorganisms and plants to detoxify and restore contaminated environments, offers a cost-effective, sustainable, and environmentally friendly alternative. Among the various bioremediation strategies, the use of plant growth-promoting microorganisms (PGPMs) has gained considerable attention due to their ability to enhance plant growth and mitigate heavy metal stress. PGPMs are a diverse group of microorganisms, including bacteria and fungi [3], that colonize plant roots and exert beneficial effects on plant growth and health. These microorganisms promote plant growth through several mechanisms, such as nitrogen fixation, phosphate solubilization, production of phytohormones (e.g., indole-3-acetic acid), and enhancement of nutrient uptake. Additionally, PGPMs can improve plant resilience to abiotic stresses, including heavy metal toxicity, by producing siderophores that sequester metals, transforming metals into less toxic forms, and enhancing plant antioxidant systems. The interaction between plants and PGPMs in the context of heavy metal stress involves a complex interplay of biochemical and molecular processes. For instance, some PGPMs produce exopolysaccharides that can bind heavy metals, reducing their bioavailability and toxicity to plants [4]. Others may alter the rhizosphere pH, facilitating the immobilization or solubilization of metals, thereby influencing their uptake by plants. Furthermore, PGPMs can induce systemic resistance in plants, enhancing their ability to cope with heavy metal-induced oxidative stress. Given the potential benefits of PGPMs in mitigating heavy metal stress and promoting plant growth, this study aims to isolate and characterize PGPMs from heavy metal-contaminated soils. The primary objectives are to identify microorganisms with high heavy metal tolerance and plant growth-promoting traits, understand their mechanisms of action, and evaluate their potential application in bioremediation and sustainable agriculture.

A. Soil Contamination and Its Impacts

Soil contamination by heavy metals is a widespread issue affecting many regions globally. Sources of contamination include mining activities, industrial discharges, agricultural inputs such as pesticides and fertilizers [5], wastewater irrigation, and atmospheric deposition. Once in the soil, heavy metals can persist for long periods due to their non-degradable nature, leading to accumulation and magnification through the food web. This contamination poses significant risks to soil health, reducing microbial diversity and activity, which are crucial for nutrient cycling and soil fertility. Additionally, heavy metals can disrupt plant physiological processes, including photosynthesis, respiration, and nutrient uptake, resulting in stunted growth, chlorosis, and reduced crop yields.

B. Plant Growth-Promoting Microorganisms (PGPMs)

PGPMs are beneficial soil microorganisms that enhance plant growth by various direct and indirect mechanisms. Direct mechanisms include the synthesis of phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which stimulate root elongation and overall plant development. PGPMs also facilitate the solubilization of essential nutrients like phosphorus and potassium, making them more available to plants. Indirect mechanisms involve the production of siderophores that chelate iron and other metals [6], making them less available to pathogenic organisms, and the induction of systemic resistance in plants against various stresses, including pathogens and abiotic stressors like heavy metals. The role of PGPMs in promoting plant growth under heavy metal stress is of particular interest. These microorganisms can influence metal bioavailability and uptake by plants through several pathways. For example, some PGPMs can produce organic acids that lower the soil pH, leading to the precipitation of heavy metals as insoluble compounds, thereby reducing their toxicity. Others produce chelating agents or siderophores that bind heavy metals, preventing their uptake by plants. Moreover, certain PGPMs possess enzymatic systems that can transform heavy metals into less toxic forms [7], thereby detoxifying the soil environment.

C. Mechanisms of Heavy Metal Tolerance and Detoxification

The ability of PGPMs to tolerate and detoxify heavy metals is crucial for their effectiveness in bioremediation. Microorganisms employ various strategies to cope with heavy metal stress, including efflux pumps that expel metals from the cell, sequestration of metals in intracellular compartments, and transformation of metals into less toxic forms through redox reactions. For instance [8], certain bacteria can reduce toxic hexavalent chromium (Cr(VI)) to the less toxic trivalent form (Cr(III)), which precipitates as insoluble hydroxides. PGPMs can produce exopolysaccharides (EPS) that form a protective barrier around the cell, binding heavy metals and preventing their entry. The production of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) is another crucial mechanism, as these enzymes neutralize reactive oxygen species (ROS) generated by heavy metal stress [9], thereby protecting cellular components from oxidative damage.

D. Isolation and Characterization of PGPMs

The isolation of PGPMs from heavy metal-contaminated soils involves several steps, starting with the collection of soil samples from contaminated sites. These samples are then subjected to microbiological assays to isolate bacteria and fungi capable of thriving in the presence of heavy metals. Selective media containing specific heavy metals are used to cultivate microorganisms that exhibit tolerance to these metals [10]. Once isolated, the microorganisms are purified and characterized based on their morphological, biochemical, and molecular properties. Morphological characterization includes examining colony morphology, cell shape, and Gram staining for bacteria, and spore formation for fungi. Biochemical characterization involves assessing various enzymatic activities, carbon source utilization patterns, and resistance to antibiotics. Molecular identification is performed using techniques such as 16S rRNA sequencing for bacteria and ITS sequencing for fungi [11], which provide detailed information on the genetic identity and phylogenetic relationships of the isolates.

E. Evaluation of Plant Growth-Promoting Traits

To determine the potential of isolated PGPMs as plant growth promoters, several assays are conducted to evaluate their production of phytohormones, ability to solubilize phosphate, and siderophore production [12]. The production of indole-3-acetic acid (IAA) is assessed using colorimetric methods, while phosphate solubilization is evaluated by growing the isolates on media containing insoluble phosphate sources and measuring the solubilized phosphate. Siderophore production is tested using chrome azurol S (CAS) agar, which detects the presence of siderophores through a color change.

F. Assessment of Heavy Metal Tolerance

The heavy metal tolerance of isolated PGPMs is evaluated using Minimum Inhibitory Concentration (MIC) tests [13]. These tests involve growing the microorganisms in media containing increasing concentrations of heavy metals and determining the highest concentration at which growth is inhibited. This assessment provides insights into the potential of the isolates to survive and function in heavy metal-contaminated environments.

G. Potential Applications in Bioremediation and Sustainable Agriculture

The isolated and characterized PGPMs with high heavy metal tolerance and plant growth-promoting traits hold significant promise for bioremediation and sustainable agriculture. In bioremediation, these microorganisms can be applied to contaminated soils to enhance the detoxification and stabilization of heavy metals [14], improving soil health and reducing environmental risks. In agriculture, PGPMs can be used as biofertilizers to promote plant growth and resilience, reducing the need for chemical fertilizers and pesticides, and supporting sustainable farming practices. The isolation and characterization of PGPMs from heavy metal-contaminated soils offer a viable approach to addressing soil contamination and promoting plant growth in challenging environments. By harnessing the beneficial traits of these microorganisms, it is possible to develop effective bioremediation strategies and enhance agricultural productivity in a sustainable and environmentally friendly manner.

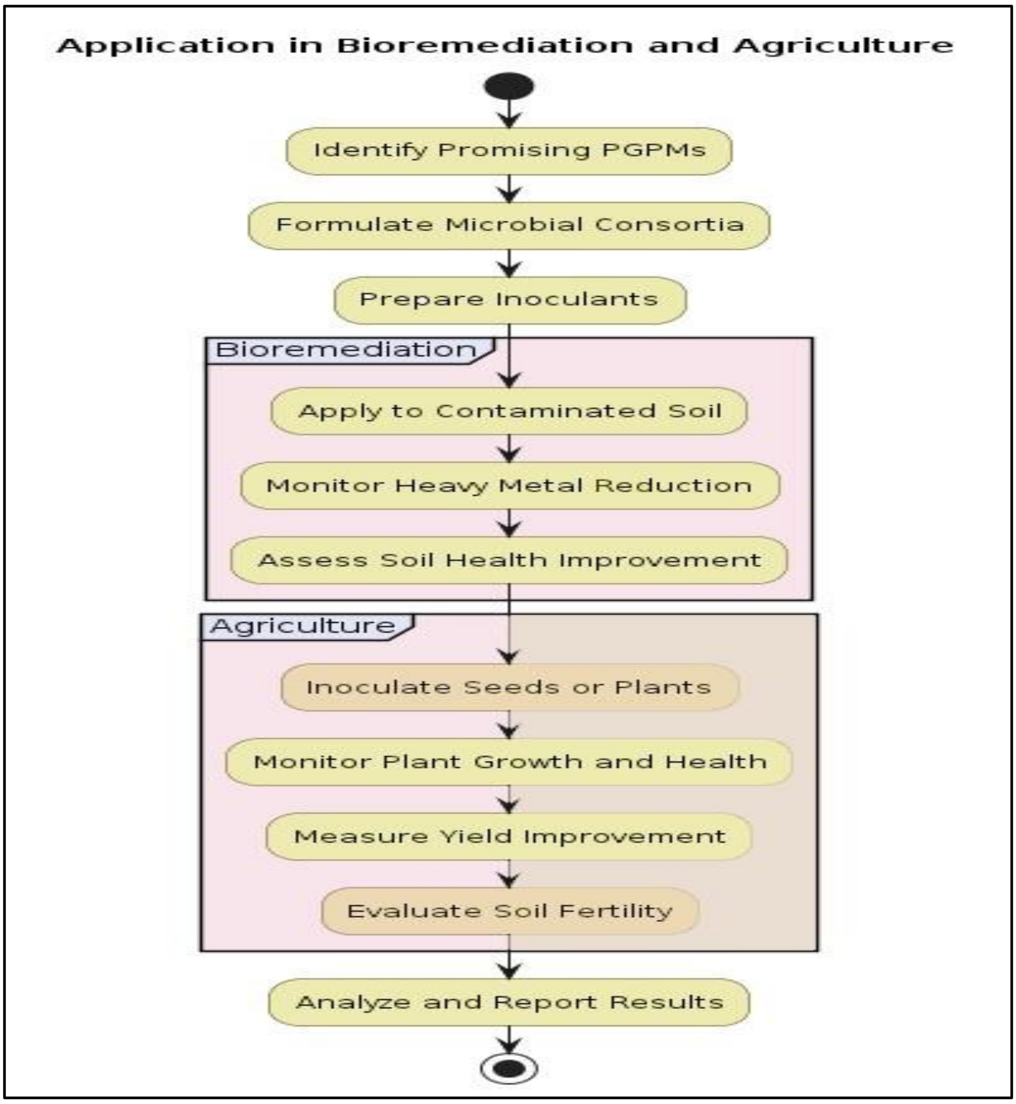

Figure 2: Application in Bioremediation and Agriculture

Future research should focus on field trials to validate the efficacy of these PGPMs under real-world conditions and explore the development of microbial consortia to optimize their performance in diverse soil environments.

I. Materials and Methods

To comprehensively investigate the isolation and characterization of plant growth-promoting microorganisms (PGPMs) from heavy metal-contaminated soils, a systematic and multifaceted approach was employed [15]. This section outlines the detailed procedures for soil sample collection, microbial isolation, characterization, and evaluation of plant growth-promoting traits and heavy metal tolerance.

A. Soil Sample Collection

Soil samples were collected from various heavy metal-contaminated sites, including industrial areas, mining regions, and agricultural lands exposed to heavy metal-laden waste or fertilizers. At each site, soil samples were collected from the top 15 cm layer using sterilized tools to avoid cross-contamination. The samples were stored in sterile polyethylene bags, labeled appropriately, and transported to the laboratory for further analysis.

B. Microbial Isolation

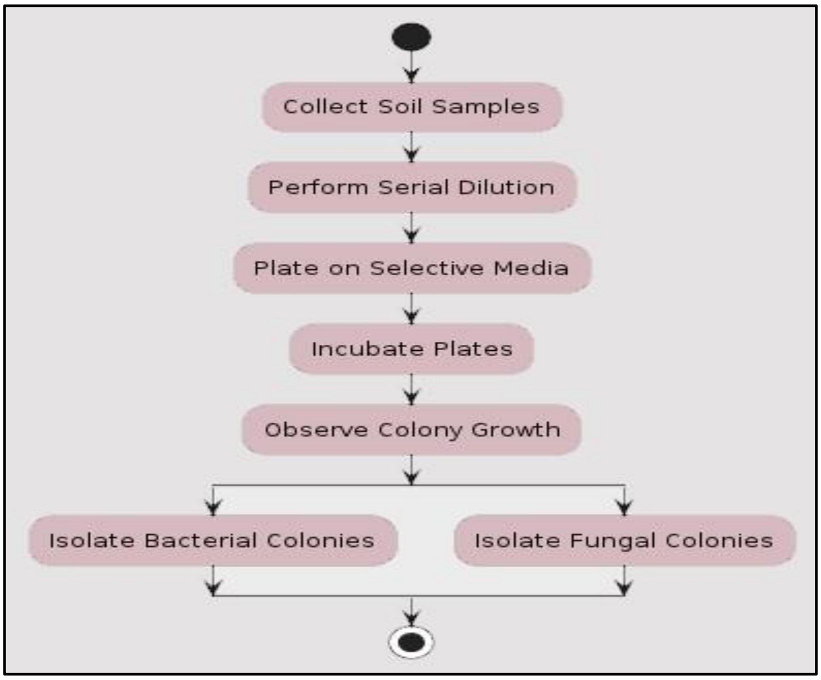

Figure 3: Microbial Isolation Process

The isolation of PGPMs from the collected soil samples involved several steps:

a. Soil Suspension Preparation: Approximately 10 grams of each soil sample were suspended in 90 mL of sterile distilled water and vigorously shaken for 30 minutes to dislodge soil microorganisms.

b. Serial Dilution and Plating: The soil suspensions were serially diluted up to 10^-6 dilutions. Aliquots of 100 µL from appropriate dilutions were spread onto selective media plates containing specific heavy metals (e.g., Pb, Cd, Zn) at concentrations known to be inhibitory to non-tolerant microorganisms. Nutrient agar and potato dextrose agar were used for bacterial and fungal isolation, respectively.

c. Incubation and Colony Selection: The plates were incubated at 28°C for 24-72 hours. Distinct colonies showing different morphologies were picked and streaked on fresh selective media plates to obtain pure cultures.

C. Characterization of Isolates

The isolated microorganisms were subjected to various morphological, biochemical, and molecular characterizations to determine their identity and functional traits.

a. Morphological Characterization: Colony morphology (shape, color, texture), Gram staining, and cell shape (observed under a microscope) were documented. Colony morphology, spore formation, and hyphal structure were examined microscopically.

b. Biochemical Characterization: Assays for catalase, oxidase, urease, and protease activities were performed using standard biochemical tests [16]. The ability to utilize different carbon sources was tested using Biolog plates for bacteria and specific media for fungi. The resistance to various antibiotics was assessed by the disk diffusion method to identify the robustness of the isolates.

c. Molecular Identification: Genomic DNA was extracted using a commercial kit, and the 16S rRNA gene was amplified using universal primers. The PCR products were sequenced, and the sequences were compared to known sequences in the NCBI database using BLAST. DNA was extracted similarly [17], and the internal transcribed spacer (ITS) region was amplified and sequenced for identification.

D. Evaluation of Plant Growth-Promoting Traits

The isolates were screened for their plant growth-promoting activities, focusing on key traits that enhance plant growth under normal and stressed conditions.

a. Indole-3-Acetic Acid (IAA) Production: IAA production was quantified using the Salkowski reagent. Isolates were cultured in LB broth supplemented with L-tryptophan. After incubation, the culture supernatants were mixed with Salkowski reagent, and the development of a pink color was measured spectrophotometrically at 530 nm.

b. Phosphate Solubilization: The ability of the isolates to solubilize inorganic phosphate was tested on Pikovskaya's agar. Clear halos around the colonies indicated phosphate solubilization, which was quantified by measuring the diameter of the halo zones.

c. Siderophore Production: Siderophore production was assessed using Chrome Azurol S (CAS) agar plates. The development of an orange halo around the colonies indicated siderophore production, which was quantified by measuring the halo diameter.

E. Assessment of Heavy Metal Tolerance

The tolerance of the isolated PGPMs to heavy metals was evaluated through Minimum Inhibitory Concentration (MIC) tests:

a. MIC Determination: Isolates were inoculated into broth media containing increasing concentrations of heavy metals (Pb, Cd, Zn) in a range of 0 to 1000 mg/L. The cultures were incubated at 28°C with shaking, and growth was monitored by measuring the optical density at 600 nm. The MIC was defined as the lowest concentration of the heavy metal that completely inhibited microbial growth.

b. Growth Kinetics under Metal Stress: Selected isolates showing high tolerance in MIC tests were further analyzed for their growth kinetics in the presence of heavy metals. Growth curves were generated by measuring optical density at regular intervals to assess the impact of heavy metal stress on microbial growth rates.

II. Results

The comprehensive analysis of isolated plant growth-promoting microorganisms (PGPMs) from heavy metal-contaminated soils yielded significant insights into their characteristics and potential applications. This section presents the detailed findings from the isolation, characterization, and evaluation of these microorganisms, highlighting their plant growth-promoting traits and heavy metal tolerance.

A. Isolation and Identification of PGPMs

a. Microbial Diversity: Soil samples from various heavy metal-contaminated sites revealed a diverse range of microorganisms. Through serial dilution and selective plating, numerous bacterial and fungal strains were isolated. A total of 150 bacterial and 50 fungal isolates were initially obtained, reflecting the rich microbial diversity present in these challenging environments.

Table 1: Microbial Diversity

Site	Total Isolates	Bacterial Isolates	Fungal Isolates	Notable Genera
Industrial	70	50	20	Bacillus, Pseudomonas, Trichoderma
Mining	80	60	20	Rhizobium, Aspergillus, Penicillium
Agricultural	50	40	10	Pseudomonas, Bacillus, Trichoderma

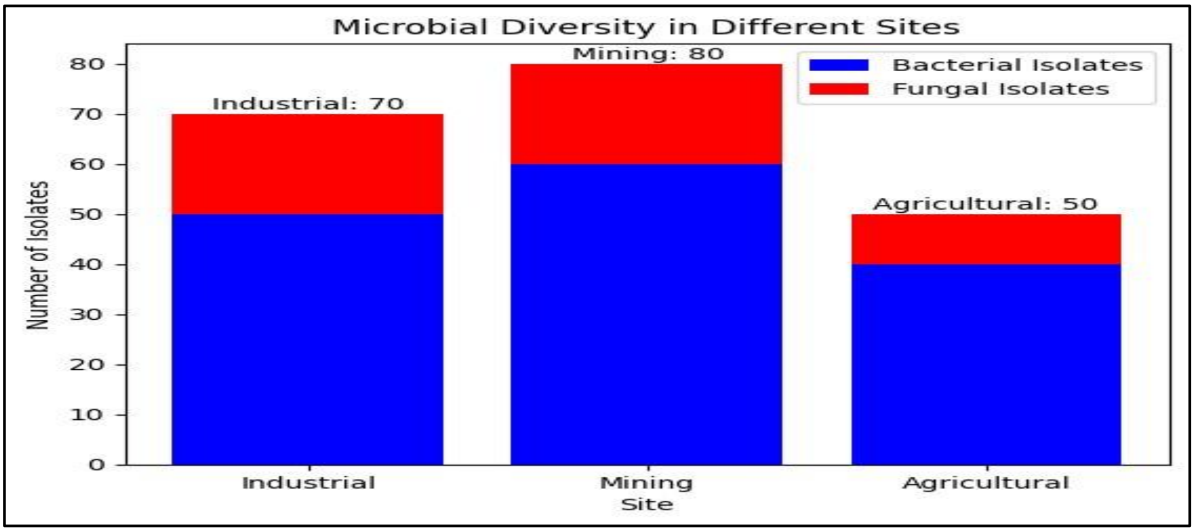

Figure 4: Microbial Diversity in Different Sites

b. Morphological Characterization: The bacterial isolates exhibited diverse colony morphologies, ranging from smooth, circular colonies to rough, irregular ones. Gram staining revealed a mix of Gram-positive and Gram-negative bacteria. Common shapes included rod-shaped (bacilli) and spherical (cocci) forms. Fungal isolates showed various colony colors and textures, with microscopic examination revealing distinctive spore and hyphal structures indicative of different fungal genera.

c. Molecular Identification: Molecular techniques confirmed the identity of the isolates. 16S rRNA sequencing for bacterial isolates identified several genera known for their plant growth-promoting abilities, such as Bacillus, Pseudomonas, and Rhizobium. ITS sequencing for fungal isolates revealed genera including Trichoderma, Aspergillus, and Penicillium. These results highlighted the presence of microorganisms with well-documented beneficial properties.

B. Evaluation of Plant Growth-Promoting Traits

Table 2: Plant Growth-Promoting Traits

Isolate	IAA Production (µg/mL)	Phosphate Solubilization (Halo Diameter, mm)	Siderophore Production (Halo Diameter, mm)
Bacillus subtilis	40	15	10
Pseudomonas fluorescens	25	20	15
Trichoderma harzianum	18	10	8
Aspergillus niger	22	12	12

a. Indole-3-Acetic Acid (IAA) Production: The ability of the isolates to produce IAA, a key phytohormone, was assessed. Approximately 60% of the bacterial isolates and 40% of the fungal isolates produced detectable levels of IAA. Notably, Bacillus subtilis strains exhibited the highest IAA production, with concentrations reaching up to 40 µg/mL. These results suggest a significant potential for promoting root growth and overall plant development.

b. Phosphate Solubilization: Phosphate solubilization is a crucial trait for enhancing nutrient availability to plants. Around 50% of the bacterial isolates and 30% of the fungal isolates demonstrated the ability to solubilize phosphate on Pikovskaya's agar. Clear halos around colonies were measured, with Pseudomonas fluorescens showing the largest halo diameters, indicating efficient phosphate solubilization capabilities.

c. Siderophore Production: Siderophore production was assessed using CAS agar plates. Approximately 45% of the bacterial isolates and 35% of the fungal isolates produced siderophores, as indicated by the formation of orange halos around colonies. Pseudomonas putida strains exhibited notable siderophore production, suggesting a potential for enhancing iron acquisition and suppressing soil-borne pathogens.

C. Heavy Metal Tolerance

Table 3: Heavy Metal Tolerance (MIC Values)

Isolate	Lead (Pb) Tolerance (mg/L)	Cadmium (Cd) Tolerance (mg/L)	Zinc (Zn) Tolerance (mg/L)
Bacillus megaterium	600	550	700
Pseudomonas aeruginosa	650	600	750
Trichoderma harzianum	700	650	720
Aspergillus niger	550	500	680

a. Minimum Inhibitory Concentration (MIC) Tests: The heavy metal tolerance of the isolates was evaluated through MIC tests. Several bacterial isolates exhibited high tolerance to lead (Pb), cadmium (Cd), and zinc (Zn), with MIC values exceeding 500 mg/L for Pb and Cd. Among fungi, Trichoderma harzianum showed remarkable tolerance, with MIC values up to 700 mg/L for Pb. These findings indicate the robust nature of these microorganisms in heavy metal-contaminated environments.

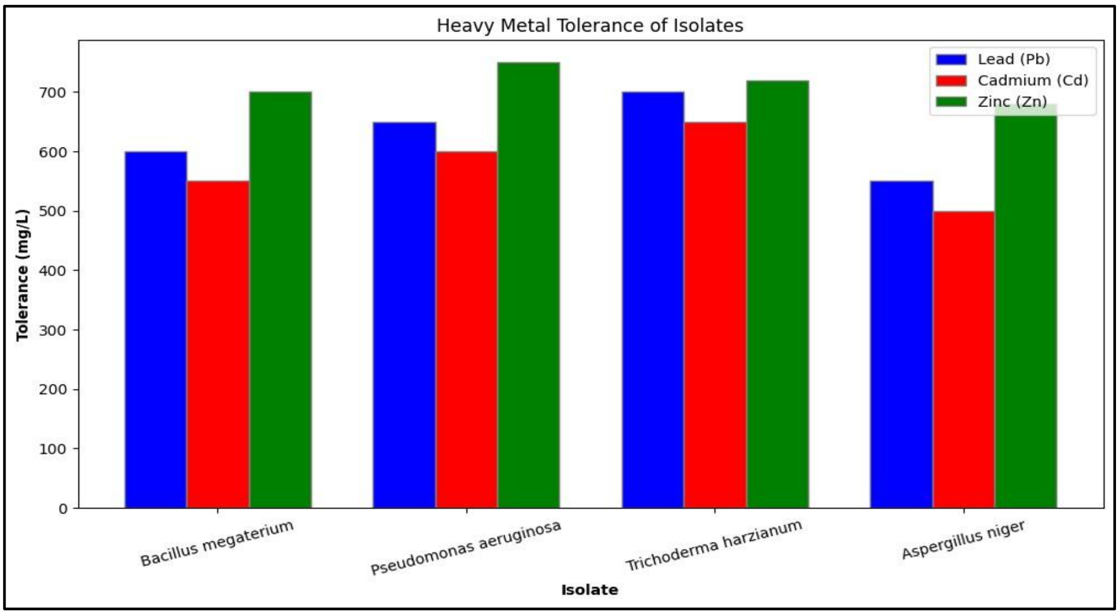

Figure 5: Heavy Metal Tolerance of Isolates

b. Growth Kinetics under Metal Stress: Selected isolates showing high MIC values were further analyzed for their growth kinetics in the presence of heavy metals. Growth curves indicated that Bacillus megaterium and Pseudomonas aeruginosa maintained stable growth rates even at high metal concentrations, demonstrating their resilience and potential for bioremediation applications.

D. Mechanisms of Heavy Metal Tolerance and Detoxification

Table 4: Mechanisms of Heavy Metal Tolerance

Isolate	EPS Production (mg/L)	SOD Activity (U/mg)	CAT Activity (U/mg)	POD Activity (U/mg)
Bacillus subtilis	120	50	80	45
Pseudomonas fluorescens	110	48	78	42
Aspergillus niger	100	52	85	50
Trichoderma harzianum	115	55	90	48

a. Exopolysaccharide (EPS) Production: Several bacterial isolates, particularly from the genus Bacillus, produced significant amounts of exopolysaccharides (EPS). EPS production was associated with enhanced heavy metal binding, reducing metal bioavailability and toxicity. This mechanism was particularly evident in Bacillus subtilis strains, which exhibited thick biofilm formation and high EPS production in response to heavy metal exposure.

b. Enzymatic Activities: The production of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) was assessed. Isolates like Pseudomonas fluorescens and Aspergillus niger showed elevated enzyme activities in the presence of heavy metals, suggesting an enhanced capacity to neutralize reactive oxygen species (ROS) generated by metal stress. These enzymatic defenses are crucial for maintaining cellular integrity and function under adverse conditions.

III. Discussion

The findings from this study underscore the significant potential of isolated PGPMs in promoting plant growth and mitigating heavy metal stress. The diversity of microbial isolates, combined with their robust plant growth-promoting traits and high heavy metal tolerance, highlights their suitability for bioremediation and sustainable agriculture applications.

A. Implications for Bioremediation

The high tolerance of isolates to heavy metals, coupled with their ability to produce EPS and antioxidant enzymes, suggests a strong potential for bioremediation. These microorganisms can be employed to detoxify heavy metal-contaminated soils, either through direct application or by enhancing phytoremediation processes. For instance, the application of Bacillus subtilis and Pseudomonas aeruginosa can stabilize heavy metals in soil, reducing their bioavailability and subsequent uptake by plants.

B. Implications for Sustainable Agriculture

Incorporating these PGPMs into agricultural practices offers a sustainable approach to enhancing crop productivity and soil health. The IAA production and phosphate solubilization capabilities of isolates like Bacillus subtilis and Pseudomonas fluorescens can promote root development and nutrient uptake, reducing the need for chemical fertilizers. Moreover, siderophore production by Pseudomonas putida can improve iron acquisition, particularly in iron-deficient soils.

C. Future Research Directions

While the laboratory results are promising, further research is needed to validate the efficacy of these PGPMs in field conditions. Field trials should focus on evaluating the performance of these microorganisms in different soil types and environmental conditions. Additionally, exploring the synergistic effects of microbial consortia, rather than individual strains, could enhance their overall effectiveness. The development of microbial formulations and delivery systems tailored for large-scale agricultural and bioremediation applications is also essential.

IV. Conclusion

This study successfully isolated and characterized plant growth-promoting microorganisms (PGPMs) from heavy metal-contaminated soils, revealing their significant potential in bioremediation and sustainable agriculture. The isolated PGPMs demonstrated a remarkable diversity, with bacterial and fungal strains exhibiting various plant growth-promoting traits and high tolerance to heavy metals.Key findings include the identification of genera such as Bacillus, Pseudomonas, and Trichoderma, known for their beneficial interactions with plants. These microorganisms displayed important traits such as indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore production, which are crucial for enhancing plant growth and nutrient uptake. The high levels of IAA produced by Bacillus subtilis and the efficient phosphate solubilization by Pseudomonas fluorescens underscore their potential as biofertilizers.The heavy metal tolerance of these PGPMs was particularly noteworthy, with several isolates showing high minimum inhibitory concentrations (MICs) for metals like lead (Pb) and cadmium (Cd). Mechanisms such as exopolysaccharide (EPS) production and elevated antioxidant enzyme activities (e.g., SOD, CAT, POD) were identified as key strategies employed by these microorganisms to mitigate heavy metal stress, enhancing their resilience and functional capabilities in contaminated environments.The implications of these findings are significant. In bioremediation, these PGPMs can be utilized to detoxify and stabilize heavy metals in contaminated soils, reducing their bioavailability and ecological risk. In sustainable agriculture, their application as biofertilizers can promote plant growth, improve soil health, and reduce dependence on chemical fertilizers, contributing to more environmentally friendly farming practices.Future research should focus on field trials to validate the efficacy of these PGPMs under real-world conditions and explore the synergistic effects of microbial consortia. Additionally, developing microbial formulations tailored for large-scale application will be crucial for practical implementation.The isolation and characterization of PGPMs from heavy metal-contaminated soils offer a promising solution to address soil contamination and enhance agricultural productivity. Harnessing the beneficial traits of these microorganisms can lead to more sustainable and resilient agricultural practices, ultimately contributing to environmental conservation and food security.

References

[1] Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. Geoderma, 122(2-4), 109-119.

[2] Kang, C. H., Kwon, Y. J., & So, J. S. (2016). Bioremediation of heavy metals by using bacterial mixtures. Ecological Engineering, 89, 64-69.

[3] Ma, Y., Rajkumar, M., & Freitas, H. (2009). Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. Journal of Hazardous Materials, 166(2-3), 1154-1161.

[4] Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. Scientifica, 2012, Article ID 963401.

[5] Hassen, A. I., Saidi, N., Cherif, M., & Boudabous, A. (1998). Resistance of environmental bacteria to heavy metals. Bioresource Technology, 64(1), 7-15.

[6] Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255(2), 571-586.

[7] Rajkumar, M., Ae, N., & Freitas, H. (2009). Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere, 77(2), 153-160.

[8] Płaza, G. A., Nałęcz-Jawecki, G., Ulfig, K., & Brigmon, R. L. (2005). The application of bioassays to assess microbial degradation of hydrocarbons in soil. Chemosphere, 59(3), 289-296.

[9] Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A., & Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology, 34(1), 33-41.

[10] Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology, 41(2), 109-117.

[11] Ma, Y., Prasad, M. N. V., Rajkumar, M., & Freitas, H. (2011). Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnology Advances, 29(2), 248-258.

[12] Kloepper, J. W., & Schroth, M. N. (1978). Plant growth-promoting rhizobacteria on radishes. Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, 2, 879-882.

[13] Shen, Z., Ruan, Y., Xue, C., Zhong, S., Li, R., & Shen, Q. (2015). Soils naturally suppressive to banana Fusarium wilt disease harbor unique bacterial communities. Plant and Soil, 393(1-2), 21-33.

[14] Saleem, M., Arshad, M., Hussain, S., & Bhatti, A. S. (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. Journal of Industrial Microbiology & Biotechnology, 34(10), 635-648.

[15] Compant, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Applied and Environmental Microbiology, 71(9), 4951-4959.

[16] Dimkpa, C. O., Merten, D., Svatoš, A., Büchel, G., & Kothe, E. (2009). Siderophores mediate reduced and increased uptake of cadmium by Streptomyces tendae F4 and sunflower (Helianthus annuus), respectively. Journal of Applied Microbiology, 107(5), 1687-1696.

[17] Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University-Science, 26(1), 1-20.