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LEAD-TOLERANT RHIZOBACTERIA ENHANCE GROWTH OF OKRA UNDER HEAVY METAL STRESS

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Keywords:

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ABSTRACT: Declining soil fertility has emerged as a critical global concern in the face of rapid population growth and increasing food demand. Among the major factors contributing to reduced soil productivity is heavy metal contamination, with lead (Pb) recognised as one of the most toxic pollutants affecting both soil health and crop yield. In this study, rhizobacteria were isolated from soils collected near battery factories and screened for their tolerance to Pb. A total of five Pb-tolerant isolates were obtained and evaluated for their maximum tolerance levels. Biochemical characterization and plant growth-promoting rhizobacteria (PGPR) trait analysis revealed that three isolates exhibited significant PGPR activities. These three promising strains were subsequently tested in pot experiments using okra (*Abelmoschus esculentus*) as a pot experiment. Inoculation with Pb-tolerant PGPR enhanced plant growth under lead stress, demonstrating their potential to mitigate heavy metal toxicity while simultaneously improving crop performance. This work highlights the dual role of Pb-tolerant rhizobacteria in restoring soil fertility and supporting sustainable agriculture in contaminated environment.

INTRODUCTION: Soil fertility is the cornerstone of agricultural productivity and global food security. Its decline has become a pressing issue in recent decades, driven by rapid population growth and the consequent rise in food demand. Intensive agricultural practices, often reliant on excessive chemical inputs and unsustainable land use, combined with industrial pollution, have accelerated soil degradation and threatened the sustainability of agroecosystems [1].

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The Food and Agriculture Organization (FAO) projects that by 2050, nearly 90% of global soil resources will be at risk due to erosion, nutrient depletion, and contamination [2]. This alarming trend underscores the urgent need for innovative strategies to restore soil fertility and ensure long-term food security.

Among the various causes of declining soil fertility, heavy metal contamination has emerged as one of the most severe threats. Industrial activities such as mining, smelting, and battery manufacturing release toxic metals into the environment, which accumulate in soils and disrupt their biological and chemical balance. Lead (Pb), in particular, is recognised as a highly toxic heavy metal due to its persistence, non-biodegradable nature, and strong affinity for soil particles. Pb contamination reduces

microbial diversity, interferes with nutrient cycling, and severely hampers plant growth. More critically, Pb enters the food chain through bioaccumulation in edible crops, posing risks to human health and food safety [3, 2]. Studies have shown that Pb exposure in plants reduces photosynthetic activity, stunts growth, and induces oxidative stress, making it a major contributor to declining soil fertility and crop productivity [4].

Plant growth-promoting rhizobacteria (PGPR) have gained significant attention as biological solutions for soil fertility restoration and heavy metal remediation. PGPR are beneficial root-associated bacteria that enhance plant growth through mechanisms such as nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis [1]. Importantly, certain PGPR strains exhibit tolerance to heavy metals and can immobilize or detoxify these pollutants, thereby reducing their bioavailability to plants. This dual role promoting plant growth while mitigating toxicity positions PGPR as promising tools for sustainable agriculture in contaminated environment [3].

The study of PGPR dates back to the early 20th century, when rhizobacterial associations were first linked to improved plant vigor. Over time, research expanded to include their role in stress tolerance, including salinity, drought, and heavy metal contamination. Recent advances highlight the potential of PGPR in ecosystem restoration, where they act as multifunctional agents for both bioremediation and biofertilization [3]. Current work emphasizes the isolation of site-specific PGPR strains from polluted soils, as native microbes are often better adapted to local stress conditions and more effective in remediation strategies [4]. Several studies have demonstrated that inoculation of crops with metal-tolerant PGPR enhances biomass, nutrient uptake, and resilience under contaminated conditions, underscoring their potential in bioremediation and sustainable agriculture [2, 1].

Against this backdrop, the present study was undertaken to isolate Pb-tolerant rhizobacteria from soils collected near battery factories, where contamination levels are typically high. The isolates were screened for maximum tolerance to Pb, followed by biochemical characterization and evaluation of PGPR traits. Based on these analyses, three promising strains were selected for pot experiments using okra (*Abelmoschus esculentus*) as a model crop. The findings aim to demonstrate the potential of Pb-tolerant PGPR in enhancing plant growth under lead stress, thereby contributing to soil fertility restoration and sustainable crop production. By integrating microbial biotechnology with environmental remediation, this work addresses the urgent need for innovative strategies to combat declining soil fertility and ensure food security in the face of industrial pollution.

METHODOLOGY:

Collection of Soil Samples: Soil samples were collected from agricultural fields located near battery manufacturing factories in Fazalganj, Kanpur, Uttar Pradesh, India, recognised as major sources of lead (Pb) contamination. Sampling was performed at a depth of 5–15 cm to target the rhizosphere zone, where microbial activity is most pronounced. Sterile tools were used to avoid external contamination, and samples were stored in sterile polyethene bags, transported to the laboratory, and processed within 24 hours.

Isolation of Pb-tolerant Rhizobacterial Strains: For microbial isolation, 1 g of soil was serially diluted in sterile distilled water up to 10^{-6}. Aliquots were spread onto nutrient agar plates supplemented with 100 mg/L $Pb(NO_3)_2$ (enrichment concentration chosen to allow selective growth of tolerant strains while avoiding complete inhibition). Plates were incubated at 28 ± 2 °C for 48–72 h. Colonies with distinct morphologies were purified by repeated streaking and maintained on nutrient agar slants at 4 °C. Glycerol stocks (20%) were prepared for long-term storage at –80 °C [5].

Lead Tolerance Assay: Tolerance was assessed using nutrient agar plates amended with $Pb(NO_3)_2$ at concentrations ranging from 50–700 mg/L in 50 mg/L increments. Inoculum density was standardized to OD 600=0.1. Growth was defined as visible colony formation after 72 h incubation at 28 ± 2 °C, confirmed in triplicate. Maximum tolerance was recorded as the highest concentration supporting consistent growth [5].

Biochemical Characterisation: Pb-tolerant isolates were subjected to standard biochemical

assays. Gram staining was performed to determine cell wall characteristics. Catalase activity was tested by adding 3% hydrogen peroxide to fresh cultures and observing bubble formation. Oxidase activity was assessed using oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) and recording purple colour development within 30 seconds. Additional assays included indole production, methyl red and Voges–Proskauer tests following standard microbiological protocols [6, 7].

Plant Growth-promoting Assays: Pb-tolerant isolates were evaluated for PGPR traits including phosphate solubilization, IAA production, ammonia generation, and hydrogen cyanide (HCN) production.

Phosphate Solubilization: Isolates were spot-inoculated on Pikovskaya's agar medium and incubated at 28 ± 2 °C for 7 days. Clear halo zones around colonies indicated phosphate solubilization, and solubilization efficiency was calculated as the ratio of halo diameter to colony diameter [8].

Indole-3-acetic Acid (IAA) Production: IAA production was determined by inoculating isolates in Luria–Bertani broth supplemented with 0.1% L-tryptophan. Cultures were incubated at 28 ± 2 °C for 48–72 h. After centrifugation, the supernatant was mixed with Salkowski's reagent, and the development of a pink color indicated IAA production [9].

Ammonia Production: Ammonia production was tested by inoculating isolates in peptone water and incubating at 28 ± 2 °C for 48 h. After incubation, Nessler's reagent was added to the culture supernatant. Yellow to brown coloration indicated ammonia production [7].

Hydrogen Cyanide (HCN) Production: HCN production was evaluated using nutrient agar plates supplemented with glycine (4.4 g L^{-1}). Filter paper soaked in picric acid solution (0.5% picric acid in 2% sodium carbonate) was placed in the lid of each plate. Plates were sealed with parafilm and incubated at 28 ± 2 °C for 4–5 days. A color change of the filter paper from yellow to orange-brown indicated HCN production [10].

Pot Trial Experiment: Pot experiments were conducted to evaluate the effect of Pb-tolerant PGPR isolates on okra (*Abelmoschus esculentus*) under lead stress. Seeds were surface sterilized with 0.1% mercuric chloride for 2 min, followed by repeated washing with sterile distilled water. Soil was autoclaved to eliminate native microbial populations, and Pb contamination was simulated by amending soil with $Pb(NO_3)_2$ to achieve a final concentration of 20 mg kg^{-1}. Four treatments were prepared: (i) Soil (uninoculated control), (ii) Soil + Pb (Pb-contaminated control), (iii) Soil + PGPR (PGPR inoculated without Pb), and (iv) Soil + Pb + PGPR (Pb-contaminated soil with PGPR inoculation). PGPR inoculation was performed by seed coating with bacterial suspensions (10^8 CFU mL^{-1}) and soil drenching at sowing. Each treatment was replicated four times, and pots were maintained under greenhouse conditions at 25–28 °C with regular watering using autoclaved water. Plant growth parameters, including shoot length, root length, and biomass, were recorded after 30 days to assess the effect of Pb-tolerant PGPR on okra performance under lead stress [12].

RESULTS:

Collection of Soil Samples: Soil samples were collected from agricultural fields located near battery manufacturing factories in Fazalganj, Kanpur, Uttar Pradesh, India, recognised as major sources of lead (Pb) contamination. Sampling was performed at a depth of 5–15 cm to target the rhizosphere zone, where microbial activity is most pronounced. Sterile tools were used to avoid external contamination, and samples were stored in sterile polyethylene bags, transported to the laboratory, and processed within 24 hours. To ensure traceability, soil sampling was conducted at five distinct sites located within a 2 km radius of major battery manufacturing and recycling factories. Sampling was carried out during the post-monsoon season (October 2024) to capture conditions after peak effluent discharge and leaching events. At each site, composite samples were prepared by pooling three subsamples collected within a 10 m radius, homogenized to minimize spatial variability.

Soil Characterization: The physicochemical properties of the collected soils were analyzed to establish baseline characteristics and assess anthropogenic impacts.

- Soil pH (7.5–8.8): Ranged from slightly to strongly alkaline, reflecting the influence of industrial salts and lime residues.
- Electrical Conductivity (0.8–2.5 dS/m): Moderate to high values indicated elevated dissolved salt concentrations, consistent with effluent runoff.
- Organic Carbon (0.3–0.55%): Low levels were observed, typical of industrial soils with suppressed microbial activity and disrupted nutrient cycling.
- Soil Texture: Sandy loam to loam, conferring high permeability that facilitates leaching of contaminants into the Chandraiya aquifer.
- Lead (Pb) Concentration: Measured concentrations ranged from 180–650 mg/kg,

Isolation of Pd-tolerant PGPR: Soil samples collected from Pb-contaminated mentioned sites of Kanpur, yielded several morphologically distinct bacterial colonies when plated on nutrient agar. From these, five pure isolates FZL-1, FZL-2, FZL-3, FZL-4 and FZL-5 were successfully obtained after repeated streaking and purification **Table 1**. The isolates exhibited diverse colony morphologies, including variations in size, pigmentation, and margin characteristics. All isolates were maintained on nutrient agar slants at 4 °C for short-term storage, and glycerol stocks were prepared for long-term preservation.

Lead Tolerance Assay: All five isolates (FZL-1 to FZL-5) obtained from Pb-contaminated soil were able to grow on nutrient agar plates supplemented with $Pb(NO_3)_2$, though their tolerance levels varied. FZL-1 and FZL-4 showed growth up to 300 mg L^{-1}, while FZL-2 tolerated concentrations as high as 500 mg L^{-1}. The most resistant strains, FZL-3 and FZL-5, exhibited visible growth even at 700 mg L^{-1}, indicating a high Pb tolerance. Colony growth was progressively reduced with increasing Pb concentrationas shown in **Table 1.** Then these lead tolerant isolates were subjected for their biochemical characterization and summarized in **Table 2.**

TABLE 1: LEAD TOLERANCE BY ISOLATED RHIZOBACTERIAL STRAINS

Isolated PGPR Code	Colony Morphology	Growth Range (mg L^{-1} Pb)	Maximum Tolerance Level (mg L^{-1})
FZL-1	White, pinpoint	50–300	300
FZL-2	Large, white, sticky	50–500	500
FZL-3	Yellow, circular, small	50–700	700
FZL-4	Irregular, opaque, pinkish	50–300	300
FZL-5	Smooth, pale	50–700	700

TABLE 2: BIOCHEMICAL CHARACTERISATION

Isolates	Gram Reaction	Catalase	Oxidase	Indole	Methyl Red	Voges–Proskauer
FZL-1	Positive cocci	+	–	–	+	–
FZL-2	Negative cocci	+	+	+	–	–
FZL-3	Negative cocci	+	+	–	+	–
FZL-4	Negative small rods	+	–	+	–	–
FZL-5	Positive rods	+	+	–	–	+

PGPR Activity: Among the five Pb-tolerant isolates, only FZL-2, FZL-4, and FZL-5 demonstrated plant growth-promoting activities. Phosphate solubilization was observed in FZL-2 and FZL-5, while FZL-4 showed weak activity. IAA production was moderate in FZL-2 and FZL-4, with FZL-5 producing the highest levels. Ammonia production was positive in all three isolates, whereas HCN production was detected only in FZL-2 and FZL-5.

TABLE 3: PGPR ACTIVITIES OF PB-TOLERANT ISOLATES

Isolates	Phosphate Solubilization	IAA Production	Ammonia Production	HCN Production
FZL-1	-	-	-	-
FZL-2	+++	++	++	++
FZL-3	-	-	-	-
FZL-4	+	+++	++	–
FZL-5	+++	++	++	++

Effect of Pb-resistant Rhizobacterial Strains on Okra Plant Growth: Based on maximum Pb tolerance and PGPR activities, isolates FZL-2, FZL-4, and FZL-5 were selected for pot experiments with Okra (*Abelmoschus esculentus*) plant. In the control (seed only), okra seedlings showed moderate growth with root length of 7.51 cm, shoot length of 9.08 cm, and relatively low biomass. Pb treatment alone reduced growth, with shoot length decreasing to 10.38 cm and dry shoot weight dropping sharply to 0.11 g. Inoculation with the selected PGPR strains significantly improved plant performance under Pb stress. FZL-2 enhanced shoot length (13.45 cm) and shoot biomass (dry weight 0.567 g), while FZL-4 produced the longest roots (11.16 cm) and improved shoot dry weight (1.71 g). FZL-5showed the strongest effect on shoot growth, with maximum shoot length (15.4 cm) and highest shoot dry weight (2.274 g). Overall, PGPR inoculation mitigated Pb toxicity, with FZL-4 and FZL-5 showing the most pronounced growth-promoting effects. The detailed growth parameters are summarized in **Table 4,** the values represented in the table are mean of triplicate readings, all replicates yielded almost similar values without significant differences.

TABLE 4: EFFECTS OF BIOINOCULANTS ON OKRA PLANT

Treatment with PGPR strains	Root Length (cm)	Shoot Length (cm)	Wet Weight Root (g)	Wet Weight Shoot (g)	Dry Weight Root (g)	Dry Weight Shoot (g)
Soil	7.51	9.08	0.267	2.6	0.138	0.932
Soil + FZL-2	8.29	13.82	0.8	12.3	0.92	1.25
Soil + FZL-4	10.68	11.89	1.16	1.16	0.892	1.69
Soil + FZL-5	8.92	15.07	1.08	18.02	0.96	2.17
Soil + Pb	7.56	10.38	0.9	7.97	0.26	0.11
Soil + Pb + FZL-2	8.59	13.45	0.19	13.7	0.08	0.567
Soil + Pb + FZL-4	11.16	12.91	1.20	14.78	0.324	1.71
Soil + Pb + FZL-5	8.04	15.4 ±	1.2	19.3	0.34	2.274

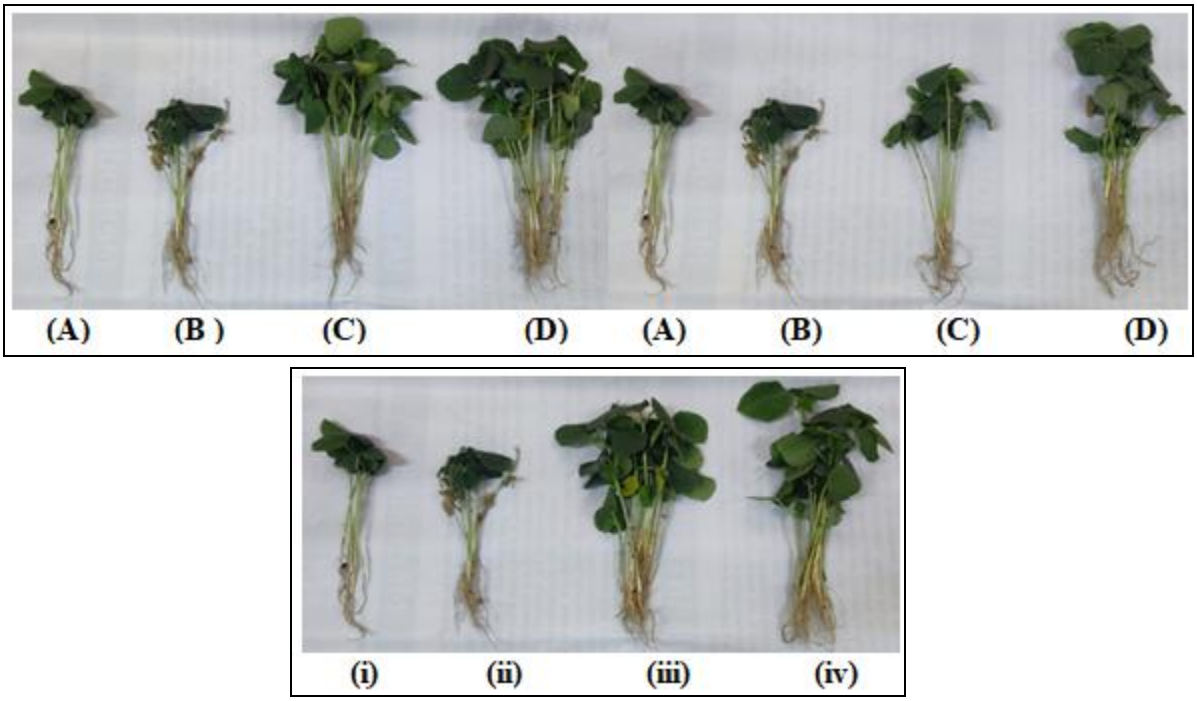

FIG. 1: EFFECTS OF BIOINOCULANTS ON OKRA PLANT: A) SOIL, (B) SOIL + PB, (C) SOIL + FZL-2, (D) SOIL + PB + FZL-2; (A) SOIL, (B)) SOIL + PB, (C) SOIL + FZL-4, (D) SOIL + PB + FZL-4 AND (I) SOIL, (II) SOIL + PB, (III) SOIL + FZL-5, (IV) SOIL + PB + FZL-5

DISCUSSION: The present investigation demonstrated that isolates FZL 2, FZL 4, and FZL 5 exhibited maximum Pb tolerance while expressing multiple PGPR traits, making them suitable candidates for pot experiments. Their ability to grow under high Pb concentrations reflects adaptive mechanisms that enable survival in contaminated soils. Similar findings were

reported by Kaushal *et al.*[11], who showed that rhizobacteria such as *Pseudomonas* and *Bacillus* tolerated elevated Pb and Cd levels while maintaining growth, thereby contributing to soil remediation. Gavande *et al.*[12] also documented PGPR isolates from crop rhizospheres in India that retained growth-promoting traits under heavy metal stress, reinforcing the potential of such strains in contaminated environments. Biochemical assays confirmed diverse metabolic capabilities among the isolates. FZL 2 and FZL 5 were oxidase positive and citrate utilizing, while FZL 4 showed moderate carbohydrate fermentation. These traits are consistent with the metabolic versatility described by Wahab *et al.*[13], who emphasized that IAA biosynthesis, phosphate solubilization, and citrate utilization are critical pathways enabling PGPR to support plant growth under abiotic stress. The ability of the isolates to produce ammonia and HCN further suggests biocontrol potential, in line with Al Turki *et al*.[14], who demonstrated that PGPR can also suppress pathogens.

PGPR activities were particularly pronounced in FZL 2, FZL 4 and FZL 5, with phosphate solubilization and IAA production being strongest in FZL 5. These traits are directly linked to improved nutrient uptake and root elongation, as highlighted by Gavande *et al.*[12], who reported similar outcomes in crop rhizospheres where PGPR enhanced plant vigor through phosphate solubilization and hormone production. Ammonia and HCN production observed in FZL 2 and FZL 5 further suggest roles in nitrogen cycling and pathogen suppression, consistent with the dual growth-promoting and biocontrol functions of PGPR described by Karničnik B [15]. Comparable results were reported by Singh *et al*.[16], who found that PGPR strains producing IAA and ammonia significantly improved root architecture and biomass in maize under heavy metal stress.

Pot experiments confirmed that inoculation with the selected isolates mitigated Pb toxicity in okra. Pb-only treatments reduced shoot length and biomass drastically, whereas PGPR inoculation restored growth parameters. FZL 5 produced the highest shoot length (15.4 cm) and dry shoot weight (2.274 g), while FZL 4 contributed maximum root length (11.16 cm), and FZL 2 enhanced shoot biomass. These outcomes align with Kaushal *et al*.[11], who demonstrated that PGPR inoculation enhances biomass and chlorophyll content under heavy metal stress. Wahab *et al*.[13] similarly emphasized that PGPR improve plant resilience by modulating biochemical pathways, including IAA biosynthesis and phosphate solubilization. In addition, Sharma *et al*.[17] reported that PGPR inoculation in Pb-contaminated soils improved wheat growth by increasing shoot length and chlorophyll content, paralleling the improvements observed in our okra trials. The dual capacity of FZL 2, FZL 4, and FZL 5 to tolerate Pb and express PGPR traits underscores their potential as biofertilizers for contaminated soils. This aligns with the broader consensus that PGPR are vital for soil fertility restoration, crop productivity, and sustainable farming practices [14, 13]. By integrating heavy metal tolerance with PGPR activities, these isolates represent promising candidates for bioremediation strategies aimed at improving food safety and soil health.

Our findings therefore, contribute to the growing body of evidence that PGPR can simultaneously detoxify heavy metals and promote plant growth, supporting their application in sustainable agriculture.

CONCLUSION: This study demonstrated that among the five Pb-tolerant bacterial isolates, FZL-2, FZL-4, and FZL-5 exhibited maximum tolerance to lead and expressed multiple PGPR traits, including phosphate solubilization, IAA production, ammonia release, and HCN production. Biochemical assays confirmed their metabolic versatility, while pot experiments with okra revealed that inoculation with these strains significantly mitigated Pb toxicity and enhanced plant growth compared to Pb-only treatments. FZL-5 showed the strongest effect on shoot length and biomass, FZL-4 contributed most to root development, and FZL-2 improved shoot biomass, highlighting complementary roles among the isolates. These findings emphasize the dual capacity of PGPR to tolerate heavy metals and promote plant growth, supporting their potential application as biofertilizers in Pb-contaminated soils, and further need 16S rRNA sequencing for specification of such effective PGPR isolated strains. Overall, the selected strains represent promising candidates for sustainable

bioremediation strategies aimed at restoring soil fertility, improving crop productivity, and safeguarding food security in heavy metal polluted environments.

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CONFLICT OF INTEREST: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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