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Research Article

Metal Resistant Bacteria from Tannery Effluent Discharged Soils: Isolation, Characterization and Application in Metal Bioremediation

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ABSTRACT

Background: Pollution by metals is a serious environmental and health threat due to their high toxicity, and microbial bioremediation offers an eco-friendly strategy to restore contaminated soils. This study aimed to isolate and evaluate indigenous, metal tolerant bacteria from tannery effluent contaminated soils in the Ranipet belt for their potential in eco-friendly remediation and plant growth promotion. **Materials and Methods:** Five sandy loam soils from Ranipet tannery discharge sites showed saline-alkaline conditions, elevated Fe, Mn, Ni, Cr, Zn and Cu, and N/P enrichment with macronutrient imbalance. Fifteen distinct bacterial isolates were recovered; six grew at and tolerated up to 0.75 mM Cr and none at 1 mM, whereas all tolerated Pb^{2+} up to 0.5 mM and several remained viable at 4-7 mM, revealing markedly higher Pb than Cr tolerance and a subset of multi-metal-resistant strains. **Results:** The most tolerant Isolate (Is7) was identified by 16S rRNA sequencing as *Stutzerimonas stutzeri* and, in batch assays, removed 20.14% of Pb (1.41 mM from 7 mM) and 18.67% of Cr (0.14 mM from 0.75 mM), indicating moderate dual metal bioremediation capacity with scope for process optimization. Ecotoxicity tests with *Eisenia fetida* showed that *S. stutzeri* caused no adverse effects compared with a pathogenic *Klebsiella pneumoniae* control. **Conclusion:** Furthermore, a seed germination assay with green bean demonstrated an increase in germination from 60% (control) to 75% with *S. stutzeri* treatment, confirming its plant growth-promoting and non-phytotoxic nature. Overall, the indigenous *Stutzerimonas stutzeri* isolate from tannery impacted soil emerges as a promising, safe candidate for integrated bioremediation and biofertilization in metal contaminated agro ecosystems.

Keywords: Heavy metal contamination, Metal-resistant bacteria, *Stutzerimonas stutzeri*, Tannery effluent.

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INTRODUCTION

Pollution ranks among the world's most pressing challenges, escalating daily due to urbanization and industrialization (Li *et al*., 2022). Modern industrial activities disrupt natural material cycles and introduce novel chemicals into ecosystems (Chowdhary *et al*., 2020). Water pollution emerges as a primary concern in today's world, driven by industrial expansion that also exacerbates other environmental degradation (Singh and Singh, 2017). Air and soil, the next most vital environmental components after water, remain the most neglected, mismanaged, and abused resources on land and in the atmosphere, respectively. Soil acts as the ultimate sink for organic and inorganic contaminants released into the environment (Su, 2014), serving as a critical growth medium for plants and animals. In industrialized regions, soil contamination stands out as one of the gravest environmental threats (Tadesse *et al*., 2017).

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Over recent decades, leather tanning has generated colored, foul-smelling effluents (Appiah-Brempong *et al*., 2022). Tannery effluents rank among the most polluting industrial wastes (Tadesse *et al*., 2017). The tanning process discharges three main waste types: airborne pollutants, water pollutants, and solid wastes (Awulachew, 2021). Effluent characteristics vary widely across tanneries, influenced by facility size, chemicals used, water consumption, and final product type (Sodhi *et al*., 2021).

Heavy metals, naturally occurring elements with high atomic weights and densities (Jyothi, 2020), impair plant growth and metabolism at elevated levels (e.g., Cr, Cu, Ni, Pb, Cd) (Huihui *et al*., 2020). Soil Cr contamination arises mainly from industrial processes, waste disposal, leather tanning, and agriculture (Mohanty *et al*., 2023; Fardami and Abdullahi, 2024), causing health effects like dizziness, irritation, organ poisoning, or collapse (Teklay, 2016). Tanning wastewater, rich in heavy metals (including Cr), sulfides, and POPs, severely threatens soil health.

Tannery operations, converting raw hides into leather, generate Chromium (Cr)-rich effluents that pollute soil and water, posing severe ecological and health risks and necessitating pre-treatment before disposal. Conventional physicochemical methods like chemical precipitation, ion exchange, and reverse osmosis are costly, produce sludge, and require chemical disposal, making them impractical. Bioremediation emerges as a low-cost, eco-friendly alternative, harnessing microorganisms-especially bacteria with their high surface-to-volume ratio-as efficient biosorbents. These microbes remove heavy metals like Cr via biosorption, intracellular accumulation, enzymatic redox transformations, and efflux, outperforming chemical approaches in accessibility and sustainability (Sharma and Malaviya, 2016; Pham *et al*., 2022).

Thus, bacterial bioremediation offers a cost-effective, eco-friendly solution for mitigating chromium-induced environmental hazards in tannery effluents, with promising future potential (Aquim *et al*., 2020; Desai *et al*., 2008; Saxena *et al*., 2020). Since the discovery of the first Cr(VI)-reducing microbe in the 1970s, researchers have actively isolated numerous strains capable of chromium reduction. To date, bacterial genera such as Bacillus, Shewanella, and Microbacterium have been reported to detoxify heavy metals, underscoring their bioremediation potential for Cr-contaminated wastes (Harish *et al*., 2012).

Intensive tannery operations in the Ranipet (Tamil Nadu, India) belt discharge metal-laden effluents, creating saline-alkaline soils enriched with Cr and Pb that degrade fertility, contaminate groundwater, and threaten food security. Conventional physicochemical treatments prove inadequate, costly, and sludge-generating, leaving agricultural and residential lands under chronic mental stress with ongoing health risks to communities. Urgent, safe bioremediation strategies are thus essential to remove or immobilize metals while preserving soil biota and ecosystem functions.

Although Lalith Varadhan and Mohan (2017) and Princy and Prabagaran (2022) isolated Cr-resistant bacteria from tannery effluents in Vellore and demonstrated effective Cr(VI) reduction at the wastewater level, robust metal-resistant strains suitable for soil application, together with rigorous ecotoxicological evaluation, remain poorly explored. Literature surveys show a particular scarcity of studies on metal resistant bacteria from effluent-impacted soils, on their Cr/Pb remediation performance in soil matrices, and on the safety of such isolates towards key soil invertebrates such as earthworms. Therefore, this research focuses on screening indigenous bacterial strains from tannery contaminated soils for Cr and Pb resistance, assessing their bioremediation efficacy under realistic conditions, and evaluating their toxicity to earthworms, with the goal of developing soil-applicable, environmentally safe bioremediation options for this critically polluted belt.

MATERIALS AND METHODS

Soil samples contaminated with tannery effluents were collected in quintuplicate using sterile polyethylene bags from a tanning site in Ranipet district, Tamil Nadu, India, at a 5 cm depth. Surface debris and extraneous materials were removed prior to sampling. Totally 5 soil samples collected from distinct sites within the tannery effluent-contaminated area. These were immediately transported to the Zoology Laboratory at Voorhees College, Vellore, for analysis (Ekhaise and Nkwelle, 2011).

Physico-Chemical Analysis of the Soil Samples

Physico-chemical properties of soil samples were analyzed to evaluate contamination levels and microbial suitability, targeting parameters such as color, pH, temperature, Electrical Conductivity (EC), moisture content, and heavy metal concentrations (e.g., Cr, Pb) (Rabah and Ibrahim, 2010). Measurements followed standard protocols: pH and EC via 1:2 soil: water suspension with a calibrated meter; moisture by oven-drying at 105ºC to constant weight; temperature using an *in situ* digital thermometer; color by Munsell chart or spectrophotometer; and metals through acid digestion followed by AAS or ICP-MS. These indicators highlight acidification (low pH from Cr), salinity (elevated EC), and metal burdens characteristic of tannery-impacted soils.

Isolation of bacterial isolates from soil samples

Soil samples were serially diluted up to 10^{-6} in sterile distilled water. Aliquots (100 µL) from 10^{-6} dilutions were spread-plated on nutrient agar and incubated at 37ºC for 24 hr. Post-incubation, colonies with distinct morphologies (color, texture, size, elevation) were selected using a sterile loop and streaked onto fresh nutrient agar for pure culture isolation (Rabah and Ibrahim, 2010).

Isolation of metal tolerance isolates

Heavy metal tolerance (Cr and Pb) of bacterial isolates was assessed using the agar dilution method. Sterile nutrient agar (beef extract 10 g, peptone 10 g, NaCl 5 g, glucose 1 g, agar 20 g per liter distilled water; pH 7.5) was supplemented separately with varying concentrations of Cr and Pb salts. Plates were inoculated via streak method with bacterial suspensions and incubated at 37±2ºC for 48 hr. Metal-free plates served as controls. Growth on metal-amended plates confirmed tolerant isolates (Rabah and Ibrahim, 2010).

Identification potential isolate by sequencing

The potential isolate was selected based on superior Cr and Pb tolerance. It was identified to species level via Sanger 16S rRNA sequencing (Saranya *et al*., 2022) and phylogenetic analysis using MEGA X software (Seena *et al*., 2023), performed at Barcode Bioscience, Bangalore, Karnataka.

Bioaccumulation with bacterial isolate

In this study, *Stutzerimonas stutzeri* (IS-7) was utilized for the bioaccumulation assay. The bacterial isolate of was cultured in shake flasks containing M9 Acetate Minimal Media (0.5 g/L yeast extract, 0.2 g/L $MgSO_4$, 5.0 g/L sodium acetate, 0.001 g/L $FeSO_4$, 0.001 g/L $CaCl_2$, 0.5 g/L K_2HPO_4, and 1.0 g/L NH_4Cl) and grown in a rotary shaker at 150 rpm, while pH and temperature were maintained at 7.0 and 37ºC, respectively. After the Optical Density (OD) reached 0.6, 7mM of lead and 0.75 mM of chromium were added separately to each culture flask, and the cultures were incubated again for 7 days under the same conditions. The total culture was then centrifuged at 5000 rpm for 15 min. The supernatants were separated and mixed with double their volume of concentrated HNO_3. These mixtures were heated to 100ºC on a hotplate stirrer for acid digestion until the volume reduced to the initial supernatant volume. The extracts were filtered through Whatman No. 42 filter paper to remove insoluble materials, collected in volumetric flasks, and diluted appropriately. The resulting extracts were analyzed for total heavy metal reduction using the ICP-OES method.

Evaluation of ecotoxicity of potential isolates on earthworms (*Eisenia fetida*)

The acute Eco toxicity of the *Stutzerimonas stutzeri* was evaluated using the earthworm *Eisenia fetida*, following OECD guidelines. The assay was designed to assess the potential harmful effects of bacterial cells on *Eisenia fetida*. Sterile filter paper was affixed to the base and inner walls of plastic containers. One mL of bacterial culture (test isolate) or deionized water (control) was applied uniformly to moisten the filter paper. Prior to exposure, individual earthworms were gently rinsed with distilled H_2O to remove soil particles, blot-dried, and weighed. Two earthworms were introduced into each container, which was sealed with perforated lids for air exchange. Containers were maintained in complete darkness at 20±1ºC for 72 hr. Mortality, weight changes, behavioral alterations, and morphological abnormalities were observed at 24 hr intervals and documented photographically. The experimental setup and assessment protocol were adapted from Yuvarani *et al*. (2025) with slight modifications.

Evaluation of the efficacy of *Stutzerimonas stutzeri* in seed germination

Isolates demonstrating the most resistance to heavy metals were chosen for further investigation. To evaluate their bioefficacy, a seed germination assay was conducted using the paper towel technique. Surface-sterilized green bean seeds (n=20) were treated sequentially with 2% sodium hypochlorite for 2 min and 70% ethanol, followed by inoculation with 10^7 CFU/mL of bacterial suspension of the selected isolate for 30 min. Seeds treated with sterile distilled water served as the control group (C). The treated seeds were then transferred to sterile petri dishes containing moistened filter paper and incubated at 25±2ºC for 3 days. Germination was recorded daily starting from the 2nd day. The germination percentage was determined by dividing the number of germinated seeds by the total number of seeds per treatment. Each treatment was conducted in triplicates (Konappa *et al*., 2020).

RESULTS AND DISCUSSION

Industrial development and advances in technology have increasingly impacted the environment through the release of substantial amounts of hazardous substances, including heavy metals (such as Cd, Cr, and Pb), metalloids, and organic pollutants, all of which can cause severe ecological damage. Lead is a non-essential element and is highly toxic to humans, animals, plants, and microorganisms. The aim of the present study was to isolate and identify bacterial strains capable of contributing to the natural recovery of agricultural and residential environments by remediating heavy metals in general, with a particular focus on lead contamination. The sampling sites were carefully selected as major sources of heavy metal pollution, primarily in areas influenced by tannery industries where metal-contaminated effluents are discharged onto surrounding soils, as well as other locations known or suspected to be impacted by metal-rich effluents and emission.

Presently soil samples were collected from tannery effluent discharged areas in Ranipet district, Tamilnadu. All five soils shared a sandy loam texture, a feature commonly reported for tannery influenced soils in the Vellore-Ranipet belt, where such textures improve aeration and microbial colonization but limit water and nutrient retention, thereby favouring stress adapted microbial populations rather than luxuriant plant growth. In the present study, electrical conductivity ranged from 0.32 dS/m (S2) to 3.22 dS/m (S5), reflecting a gradient from non-saline to saline conditions; similar EC elevation due to salt accumulation from tannery effluents has been documented for soils around Ambur, Vaniyambadi and Ranipet, where long-term discharge leads to progressive salinization of surface layers. The consistently alkaline pH (8.02-9.73, mean 8.73), with some samples approaching strongly alkaline conditions, is in good agreement with surveys from Vellore district reporting soil pH often between neutral and 8.5 in tannery-impacted locations, attributed to the alkaline nature of liming and dehairing chemicals in tannery wastewaters (Sinduja *et al*., 2022).

The modest moisture content (8.2-9.7%) observed, together with sandy loam texture and elevated EC in S5, aligns with earlier reports from tannery-impacted soils in Dindigul and Vellore, where long-term effluent discharge increases salinity and alters soil physical properties while maintaining only low to moderate moisture. Reviews of the Vellore-Palar basin likewise describe effluent-affected fields as characteristically saline-alkaline, with high EC and pH creating stressed but microbially active environments, indicating that the present moisture-EC pattern fits the established physicochemical profile of tannery-affected soils in this region (Yuvarani *et al*., 2019).

The soil samples in this study showed relatively high nitrogen (up to 130.3%) and moderate phosphorus (5.5-18.4%), with calcium carbonate detected only in Sample 2 (0.25%) and no detectable potassium in any sample, indicating N/P enrichment but clear macronutrient imbalance. This pattern is broadly consistent with Balasubramanian *et al*. (2017), who reported that treated tannery effluent mixed with domestic wastewater (1:3) increased available N and P and improved nutrient status in red and black soils. Together, these results reinforce concerns raised in regional studies that tannery effluents, while supplying N and P, ultimately drive salinity, alkalinity and nutrient imbalance, necessitating site-specific remediation and careful effluent management in the Vellore tannery cluster (Table 1).

The heavy metal profile of these five tannery impacted soils is characterized by iron as the most abundant element (133.36-168.69 mg/kg), followed by appreciable levels of manganese (25.7-63.97 mg/kg), nickel (33.61-63.35 mg/kg), chromium III (28.94-54.21 mg/kg), aluminium (31.87-57.10 mg/kg) and zinc (28.70-41.71 mg/kg), with copper, barium and strontium also present in the 0.66-42.41 mg/kg range (Table 2). Several highly toxic elements, including arsenic, cadmium, lead, mercury, selenium, tin, organic tin, boron and cobalt, were consistently below their respective detection limits, as indicated by values such as <10 mg/kg for Cd and Pb and <1.0 mg/kg for Hg. This pattern fits well within the range reported for the Vellore-Ranipet tannery belt, where Sinduja *et al*. (2017) and co-authors showed that soils in tannery-influenced regions of Walajapet and surrounding Vellore district are heavily contaminated with chromium and enriched in Zn and other metals, but often exhibit relatively low to moderate levels of Pb and Cd, similar to the detectable but no extreme Zn and no detectable Pb and Cd observed here.

The predominance of Fe and Mn, together with Ni, Cu and Zn in the present samples, corresponds to the suite of metals typically associated with chrome tanning and mixed industrial inputs identified in recent geochemical and speciation studies from the same area. At the same time, the non-detection of Cr(VI), As, Cd, Pb and Hg at the applied detection limits suggests that, although these soils are clearly metal enriched, the most acutely toxic species may occur at lower concentrations or in less bioavailable forms, consistent with the findings of Kistan and Kanchana (2020), who reported very high Cr but only slight Cd and Pb contamination in Ranipet tannery soils.

In tanning, metals are mainly used to produce high-quality leather-chromium (III) sulfate crosslinks collagen to give strong, flexible, water- and heat-resistant hides, and because the process is fast and efficient, about 80% of global leather is chrome tanned. Other metals (Fe, Mn, Ni, Cu, Zn) enter indirectly as impurities in chemicals and by leaching from equipment, and 30-40% of Cr(III) plus much of these metals end up in effluent and sludge, which, when poorly managed, are discharged onto land and into waterways (Yakubu *et al*., 2018; El-Gawad *et al*., 2023).

In Vellore district, such metal-bearing effluents have produced saline-alkaline, metal-rich soils enriched in Cr, Fe, Mn, Ni, Cu and Zn with high EC and pH, as shown in chromium speciation and soil contamination studies from Walajapet and nearby tannery zones. These conditions disturb N-P-K balance, degrade soil structure, reduce fertility, contaminate groundwater and the Palar River, and contribute to ecological damage, crop losses and health impacts documented in regional reviews and legal assessments of tannery pollution in Vellore. In this context, there is an urgent need to remediate metal-contaminated soils using alternative approaches based on bacterial isolates. Therefore, in the present study, bacterial strains were isolated from native tannery effluent-impacted soils in Ranipet district and evaluated for their potential to remediate the accumulated metals.

The recovery of 15 distinct cultivable bacterial isolates from soil samples in the present study confirms that chromium-exposed environments host diverse microbial communities with varying metal tolerance. Similar patterns of variable microbial recoverability were reported from chromium-impacted soils in the Vellore Palar River basin, where Silambarasan and Abraham (2013) isolated multiple metal-tolerant strains showing differential resistance to heavy metals due to localized contamination stress.

Table 1: Physiochemical characterization of tannery effluent discharged soil samples.

Sl. No.	Name of the test	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1.	Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
2.	EC	0.85%	0.32%	0.62%	1.15%	3.22%
3.	pH	8.02	8.58	8.58	9.73	
4.	Moisture	8.20%	8.30%	8.90%	9.20%	9.70%
5.	Calcium carbonate	-	0.25%	-	-	-
6.	Nitrogen	82.10%	36.80%	25.50%	-	130.30%
7.	Phosphorus	5.50%	18.40%	8.10%	9.40%	6.80%
8.	Potassium	-	--	-	-	-

Table 2: Determinations of heavy metals on tannery effluent discharged soil samples.

Sl. No.	Name of metals	Result in mg/kg				
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1.	Aluminium	31.87	57.1	36.4	54.7	55.4
2.	Antimony	<2.5	-	-	-	<2.5
3.	Arsenic	<2.5	<1.8	<2.5	<2.5	<2.5
5.	Barium	26.41	24.1	18.3	11.2	0.66
5.	Boron(B)	<2.5	<2.5	<2.5	-	-
6.	Cadmium	<10	-	-	-	-
7.	Chromium III	34.83	41.16	28.94	54.21	36.41
8.	Cobalt	<2.5	<2.5	<2.5	-	-
9.	Copper	37.26	28.7	11.45	14.74	11.24
10.	Lead	<10	<10	<10	<10	<10
11.	Manganese	63.97	54.7	41.25	25.7	33.6
12.	Mercury	<1.0	<1.0	<1.0	<1.0	<1.0
13.	Nickel	62.25	33.61	47.72	63.35	34.52
14.	Selenium	<2.5	<2.5	<2.5	<2.5	<2.5
15.	Strontium	42.41	25.41	27.11	35.51	44.72
16.	Tin (Sn)	<2.5	-	-	<2.5	<2.5
17.	OrganicTin	<2.0	<2.0	<2.0	<2.0	-
18.	Zinc (Zn)	41.23	35.78	28.70	41.71	39.45
19.	Iron	133.36	156.4	151.22	114.2	168.69

Chromium and lead were selected for further study because they are globally recognized priority toxic metals with strong carcinogenic, neurotoxic and systemic effects, and are therefore considered higher risk than essential metals such as Fe, Mn, Zn and Cu. Several studies from the Vellore-Ranipet/Walajapet tannery belt show that soils, sludge and groundwater are heavily contaminated with chromium, with total Cr in nearby agricultural fields often exceeding permissible limits, making Cr the signature pollutant in this region that requires urgent remediation. Tannery effluent discharged into the Palar basin has been shown to be cytotoxic, genotoxic and phytotoxic, with elevated chromium and related metals identified as major contributors to this toxicity. Although Pb was below detection in the present soil batch, lead is a strictly non-essential, bioaccumulative metal frequently reported in mixed industrial wastes and is consistently grouped with Cr, Cd, As and Hg as a priority public health contaminant (Tchounwou *et al.*, 2012; Balali-Mood *et al.*, 2021). Focusing the experiments on Cr and Pb therefore targets the most hazardous and policy-relevant metals, ensuring that any demonstrated bacterial remediation has clear environmental and health significance.

When tested for chromium tolerance, all isolates in this study grew at 0.25 mM, and most at 0.5 mM-with inhibition starting at higher chromium concentrations. This trend aligns with findings of a previous study, which isolated chromium-resistant bacteria from tannery effluents in the Vellore district and noted that although several strains tolerated chromium, their growth declined with increasing Cr(VI) levels (David, 2016).

At 0.75 mM, only six isolates in the current study (1, 4, 7, 11, 12, and 15) continued to grow, while none survived at 1 mM, highlighting a clear upper limit to chromium tolerance under experimental conditions (Figure 1). Similarly, Verma *et al.* (2001) documented a decline in survival with increased chromium concentration and found that only a subset of isolates from tannery effluent retained significant growth at high Cr levels, indicating that chromium resistance mechanisms are often operative only up to defined thresholds.

In this study, all 15 soil bacterial isolates tolerated Pb^{2+} up to 0.5 mM, most still grew at 0.75 mM, and several isolates (4, 7, 9, 11-15) remained viable at 4-7 mM before inhibition at 8 mM, indicating markedly higher tolerance to lead than to chromium and revealing a distinct group of highly Pb-resistant, multi-metal-tolerant strains. This tolerance range is comparable to earlier reports where lead-resistant bacteria from contaminated soils grew at millimolar Pb levels and were proposed as bioremediation agents, such as the lead-resistant isolates described by Sapale *et al.* (2015) from Pb-polluted soils, which showed robust growth and were suggested for remediation use. Likewise, a recent investigation of bacteria from lead-contaminated soil recovered

26 Pb-tolerant isolates, with three strains tolerating up to 1500 mg/L Pb and exhibiting strong bioaccumulation, demonstrating that environmental communities can harbour highly Pb-resistant phenotypes similar in behaviour to the most tolerant isolates identified here.

In this study, all isolates tolerated Pb^{2+} up to 0.5 mM, almost all at 0.75 mM, and a subset (4, 7, 9, 11-15) survived up to 4-7 mM before inhibition at 8 mM, indicating higher tolerance to lead than to chromium and revealing multi-metal-tolerant candidates for bioremediation (Figure 2). Similar selection for metal-resistant bacteria has been reported from tannery-impacted sites in Vellore district: surveys in the Palar River basin showed that a large proportion of native isolates from tannery-polluted locations were tolerant to multiple metals including Pb, Zn, Ni, Fe and Cd, reflecting long-term exposure to mixed metal loads from tannery effluents (Andiyappan *et al*., 2017). Likewise, a recent investigation of bacteria from lead-contaminated soil recovered 26 Pb-tolerant isolates, with three strains tolerating up to 1500 mg/L Pb and exhibiting strong bioaccumulation, demonstrating that environmental communities can harbour highly Pb-resistant phenotypes (Siswanto *et al*., 2025).

Molecular identification of isolate Is7 was carried out by sequencing the 16S rRNA gene. High-quality genomic DNA was isolated, and an approximately 1407 bp fragment of the 16S rRNA gene was successfully amplified and sequenced. The 16S rRNA sequencing identified the most metal-tolerant isolate Is7 as belonging to the Stutzerimonas/Pseudomonas stutzeri group, showing 99.93% identity to S. stutzeri, which agrees with previous genomic analyses indicating that Stutzerimonas species (Accession No. PP373728.1). A previous study by Saravanan *et al*. (2025) reported Stutzerimonas stutzeri from coal fly ash-contaminated soil as a multi-metal-tolerant and plant growth-promoting bacterium. Another study by Ridene *et al*. (2023) also isolated Pseudomonas stutzeri from lead-contaminated soil samples.

In this study, Stutzerimonas stutzeri removed 20.14% of Pb (1.41 mM from 7 mM) and 18.67% of Cr (0.14 mM from 0.75 mM), indicating moderate but clear dual-metal bioremediation capacity. Similarly, a consortium of Pseudomonas stutzeri LBR and Cupriavidus metallidurans LBJ removed more than 70% of Pb from polluted soil, whereas single strains showed only 55-67% Pb reduction, suggesting that the 20% removal observed here reflects both strain-specific capacity and the absence of consortia or process optimization (Kumar *et al*., 2024). The same isolate showed only modest chromium removal (18.67%) from the media. Compared to previous studies, the present reduction was lower; Hossan *et al*. (2020) reported 72.2% reduction of Cr(VI). Taken together, these comparisons show that the isolate of Stutzerimonas stutzeri behaves similarly to other members of this group, acting as a microorganism capable of removing lead (Pb) and Chromium (Cr). However, its currently observed removal efficiency is close to the baseline values reported before process optimization or the use of microbial consortia. With this in mind, it can be proposed as a promising bioremediation candidate whose performance could be significantly enhanced by tuning factors such as pH, biomass load, contact time and co-culturing strategies.

Using Eisenia fetida to evaluate the ecotoxicological impact of the present study showed that Stutzerimonas stutzeri is environmentally safer than Klebsiella pneumoniae under the tested conditions. Earthworm-based bioassays are widely recognized as sensitive tools for detecting soil toxicity, and behavioural symptoms such as coiling, swelling, excessive mucous secretion and growth inhibition are classical indicators of acute or sub-lethal stress in E. fetida exposed to contaminated soils or toxicants. In several ecotoxicity and bioremediation studies, earthworms have exhibited strong avoidance, reduced biomass and visible body abnormalities in polluted soils, whereas remediated or less toxic treatments supported normal survival and growth, mirroring the strong adverse responses observed

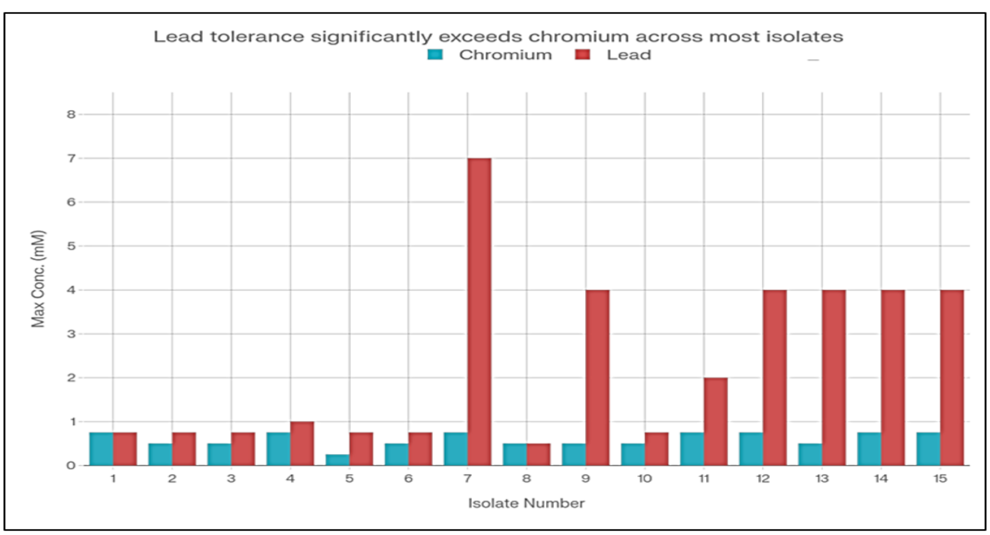

Figure 1: Metal tolerance varies across bacterial isolates.

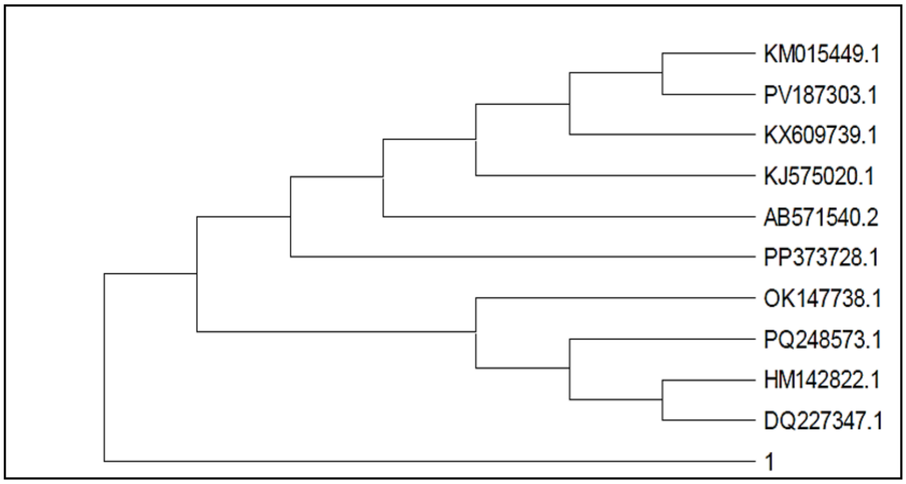

Figure 2: Phylogenetic tree analysis of *Pseudomonas stutzeri*.

with *K. pneumoniae* (Akhila and Keshamma, 2023). Similarly, Vílchez *et al*. (2016) determined the toxicity of bacterial isolates on earthworms.

CONCLUSION

In this study, seed treatment with the soil-derived Stutzerimonas stutzeri isolate increased green bean germination from 60% to 75%, indicating a clear plant growth-promoting effect without phytotoxicity. This aligns with reports that Pseudomonas/ Stutzerimonas stutzeri acts as a plant growth-promoting rhizobacterium: strains MJL19 enhanced soybean and rice germination and seedling growth via root colonization, biofilm formation and nitrogen fixation, while the multi-metal-tolerant strain WA4 improved growth and biomass of several crops in metal-rich soils and aided phytoextraction of Cu, Ag and Pb (Lami *et al*., 2020). A similar line of study was carried out by Yuvarani *et al*. (2025), who determined the ecotoxicity of beneficial bacterial isolates on earthworms. Together, these findings suggest that the S. stutzeri isolate similarly combines metal tolerance with no toxicity and is a suitable, safe inoculant for bioremediation-linked biofertilization in contaminated soils.

It may be concluded that the indigenous Stutzerimonas stutzeri isolate from tannery-impacted soil exhibits concurrent tolerance and bioaccumulation of Pb and Cr as adaptive traits for survival under metal stress. Under the tested conditions, these traits supported moderate dual-metal removal, and, together with its non-toxic response in Eisenia fetida and promotion of seed germination, confirm its suitability as a safe bioremediation agent. It is evident that appropriate optimization of environmental and process parameters governing its metal accumulation and plant growth-promoting properties could turn this isolate into an effective tool for remediation of Pb- and Cr-contaminated agroecosystems.

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None.

ABBREVIATIONS

Cr: Chromium; **Pb:** Lead; **Fe:** Iron; **Mn:** Manganese; **Ni:** Nickel; **Cu:** Copper; **Zn:** Zinc; **EC:** Electrical conductivity; **N:** Nitrogen; **P:** Phosphorus; **K:** Potassium; **AAS:** Atomic Absorption Spectroscopy; **ICP-MS:** Inductively Coupled Plasma Mass Spectrometry; **ICP-OES:** Inductively Coupled Plasma Optical Emission Spectrometry; **rRNA:** Ribosomal Ribonucleic Acid; **OD:** Optical Density; **CFU:** Colony Forming Units; **OECD:** Organisation for Economic Co-operation and Development.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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