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Bacterial Potency Tests for Bioaugmentation in Post-Mining Land

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Revegetated post nickel mining soils in Pomalaa, Southeast Sulawesi, are characterized by low fertility and excessive concentrations of heavy metals, particularly nickel and chromium, which severely hinder plant growth and environmental sustainability. This study aimed to isolate and characterize indigenous bacterial strains capable of reducing nickel and chromium concentrations and enhancing soil fertility through key plant growth promoting traits. A total of 72 bacterial isolates were obtained from composite soil samples collected across southern, central, and northern post mining zones. Of these, 48 isolates demonstrated growth in media containing 10 ppm $NiCl_2$ and $CrCl_2$. Ten isolates with the highest biochemical potential were further evaluated for their ability to produce indole-3-acetic acid (IAA), gibberellic acid (GA_3), solubilize phosphate, fix atmospheric nitrogen, and reduce nickel and chromium concentrations. The most active strains included TT1cr-32 (IAA, 8.0 ppm), TU2cr-41 (GA_3, 2.99 ppm), TT1cr-33 (phosphate solubilization, 12.53 ppm), and TT1ni-32 (nitrogen fixation, 1292.99 ppm). Nickel reduction was most effective in isolate TU3ni-41, achieving 97.29% and 95.55% reduction at 300 ppm and 700 ppm nickel respectively, while TS1cr-5 exhibited the highest chromium reduction (65.62% at 300 ppm and 48.27% at 700 ppm chromium. Based on Bergey's Manual, the dominant genera were *Clostridium* and *Bacillus*, both of which are known for their heavy metal resistance and soil enhancing properties. These findings suggest that native bacteria from post mining soils can serve as promising agents for bioaugmentation, offering an integrated solution for both heavy metal remediation and ecological restoration of degraded land.

Keywords: bioaugmentation, heavy metal reduction, indigenous bacteria, nickel and chromium, post mining soil.

Introduction

Revegetation is a commonly adopted method for rehabilitating post mining land. However, in the nickel mining region of Pomalaa, Southeast Sulawesi, this strategy has proven inadequate in addressing the complex issues of soil degradation and contamination. Syahri et al. (2019) reported that even after revegetation, soil samples from these areas contained alarmingly high concentrations of nickel and chromium 11.103.74 mg/kg and 4.030.17 mg/kg, respectively far exceeding the acceptable limits defined by the Indonesian National Standardization Agency (SNI, 2004), which are 0.31 µg/g for nickel and 0.5 µg/g for chromium. Additionally, nutrient deficiencies have been widely observed, particularly in potassium, calcium, iron, copper, and manganese (Widiatmaka et al., 2010), further diminishing the soil's capacity to support plant growth. These conditions not only impede vegetation recovery but also pose serious risks to environmental health and ecological balance.

To overcome these limitations, alternative and more effective remediation techniques are urgently needed. Bioaugmentation has emerged as a promising approach, involving the deliberate introduction of beneficial microorganisms capable of transforming, immobilizing, or detoxifying environmental contaminants. Hashem et al. (2023) define bioaugmentation as "an in situ biological approach to contaminant remediation in which an enriched microbial consortium is introduced directly into a polluted environment." This environmentally friendly method utilizes the metabolic activities of microbes to reduce heavy metal concentrations in soil while simultaneously enhancing soil quality. Prior studies have demonstrated that certain strains, such as *Candida tropicalis*, can completely degrade 50 mg/L of Cr (VI) within 48 hours under aerobic conditions (Purwanti et al., 2020). Similarly, *Bacillus* spp. have shown considerable potential in reducing metals like lead, cadmium, mercury, chromium, arsenic, and nickel (Wrobel et al., 2023), making them attractive candidates for soil restoration efforts.

Growing evidence supports the use of biochar immobilized *Bacillus* strains as an effective method for enhancing soil microbial activity, reducing heavy metal toxicity, and limiting metal accumulation in plants key factors for successful land restoration (Schommer et al., 2023). The use of indigenous microbial strains offers additional advantages, as these organisms are naturally adapted to the specific physicochemical conditions of contaminated sites. This enhances their survival, colonization, and functional performance in remediation efforts. As Zhang et al. (2025) emphasize, "indigenous bacterial consortia improve heavy metal tolerance and reduction through synergistic interactions." Building on this evidence, the present study aims to isolate and evaluate native bacterial strains from post nickel mining soils in Pomalaa for their dual capacity to reduce nickel and chromium concentrations and to improve soil fertility. The identification of such microbial candidates could provide a sustainable and site specific strategy for restoring ecological balance and promoting long term land rehabilitation in post mining environments.

Methods

Study area and sample collection

Soil samples were collected from revegetated post nickel mining land in Pomalaa, Southeast Sulawesi, Indonesia, an area recognized as the largest nickel mining site in the region. The objective was to isolate indigenous bacterial strains with potential for bioaugmentation, specifically for heavy metal reduction and enhancement of soil fertility. Sampling was carried out at three major zones of the mining area: the southern (TS), central (TT), and northern (TU) sites. Within each zone, multiple sampling points were designated: TS1 to TS5 for the southern zone, TT1 to TT4 for the central zone, and TU1 to TU3 for the northern zone. At each location, soil was collected from three different depths. The samples from each site were composited into a homogeneous mixture to represent each zone.

Isolation and screening of bacterial isolates

Composite soil samples were serially diluted using sterile distilled water. Aliquots were plated on Nutrient Broth (NB) medium to cultivate indigenous bacterial colonies. After primary growth, colonies were subculture onto NB agar plates supplemented with 10 ppm of either nickel chloride or chromium chloride to assess metal tolerance. Isolates capable of growing on nickel containing media were labelled with the suffix "ni", while those tolerant to chromium were designated "cr." All cultures were incubated at 30°C for 2 to 3 days.

Colonies demonstrating growth under these selective conditions were further transferred into fresh NB media to obtain pure single colonies, which were then selected for subsequent functional assays.

Evaluation of plant growth-promoting traits

Selected bacterial isolates were screened for key plant growth promoting properties, including indole-3-acetic acid production, gibberellic acid (GA_3) synthesis, phosphate solubilization, and nitrogen fixation. Isolates showing the strongest performance in each assay were then advanced for testing their capacity to reduce nickel and chromium concentrations in vitro.

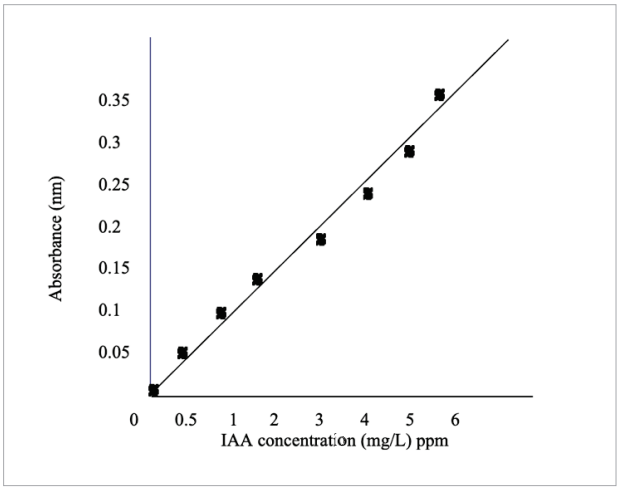
Indole-3-acetic acid (IAA) assay

Indole-3-acetic acid (IAA) production was assessed following the colorimetric method described by Gutierrez et al. (2009), with modifications. Each bacterial isolate was grown in NB medium supplemented with 1% L-tryptophan and incubated in the dark at room temperature for five days. After incubation, cultures were centrifuged, and 1 mL of the resulting supernatant was mixed with 1 mL of Salkowski reagent (0.5 M $FeCl_3$ in 35% H_2SO_4) as described by Glickman and Dessaux (1995). The mixture was incubated in the dark at 28°C for 24 hours. IAA production was indicated by the development of a pink colour. Absorbance was measured at 530 nm using a UV-VIS spectrophotometer. IAA concentrations were calculated using a standard curve prepared from IAA stock solutions (0–5.0 mg/L), based on the regression equation Y = 0.064x + 0.09 (R^2 = 0.995) (*fig. 1*). Results were expressed in mg/L of culture filtrate.

Gibberellic acid (GA3) assay

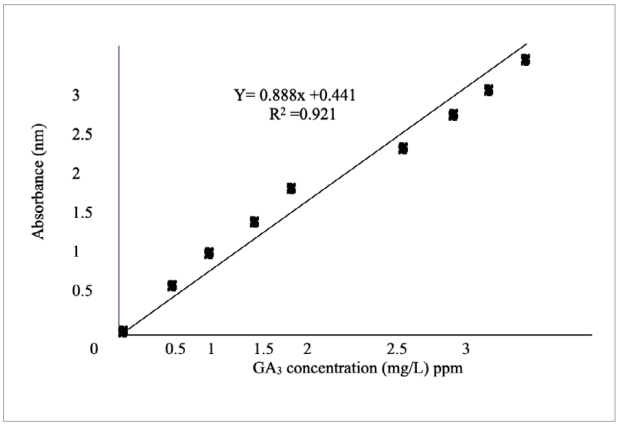
GA_3 is a key plant hormone involved in regulating various physiological processes, including stem elongation, flowering, and fruit development. The ability of bacterial isolates to produce GA_3 was assessed following the standard colorimetric method described by Borrow et al. (1995), with minor modifications. Each bacterial isolate was cultured in NB medium and incubated at 37°C for seven days under static conditions. Following incubation, the cultures were centrifuged at 8000 rpm for 10 minutes to separate the bacterial cells from the supernatant. A 15 mL aliquot of the supernatant was mixed with 2 mL of zinc acetate solution and allowed to stand for 2 minutes. This was followed by the addition of 2 mL of potassium ferricyanide solution [$K_4Fe(CN)_6$ $3H_2O$]. The mixture was then centrifuged again at 8000 rpm for 10 minutes to obtain a clarified supernatant.

Fig. 1. *IAA standard curve line equation*



The reaction mixture was prepared by incubating 5 mL of the clarified supernatant with 30% hydrochloric acid (HCl) at 27°C for 75 minutes to initiate color development for GA_3 detection. A 5% HCl solution was used as the blank control. Absorbance was measured at 254 nm using a UV VIS spectrophotometer. The concentration of GA_3 in each sample was determined using a standard calibration curve prepared from serial dilutions of a GA_3 stock solution (ranging from 0 to 2.25 mg/L). The regression equation used for quantification was Y = 0.888x + 0.441 (R^2 = 0.921) (*fig. 2*). Final concentrations were expressed in mg/L.

Fig. 2. *GA3 standard curve line equation*



Phosphate solubilization assay

Phosphorus is an essential macronutrient required for plant growth and development, however, it is often present in insoluble forms that limit its availability to plants. This assay evaluated the ability of indigenous bacterial isolates to solubilize phosphate and convert it into a plant available form. Each bacterial isolate was inoculated into Pikovskaya's medium to assess its phosphate-solubilizing capability. The medium consisted of (per liter) namely, 10 g glucose, 5 g $Ca(H_2PO_4)_2{\cdot}2H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.2 g KCl, 0.005 g $MnSO_4$, 0.005 g $FeSO_4$, 0.2 g NaCl, and 0.5 g yeast extract, all dissolved in 1 L of ion free water. The cultures were incubated on a rotary shaker at room temperature for seven days.

Following incubation, the cultures were filtered using Whatman No. 1 filter paper to obtain the cell free supernatant. The filtrate was centrifuged at 1000 rpm for 15 minutes to remove residual cells and debris. A 5 mL aliquot of the supernatant was then mixed with 0.5 mL of a freshly prepared color developing reagent, which consisted of 0.53 g of ascorbic acid dissolved in 50 mL of concentrated phosphate reagent and diluted with ion-free water. The concentrated phosphate reagent was prepared by dissolving 12 g of ammonium molybdate $[(NH_4)_6Mo_7O_{24}{\cdot}4H_2O]$ in 100 mL of distilled water, followed by the addition of 140 mL of concentrated sulfuric acid (H_2SO_4) and 0.227 g of potassium antimony (III) oxide tartrate $[K(SbO)C_4H_4O_6{\cdot}0.5H_2O]$, then diluted to 1 L with ion free water. The final reaction mixture was allowed to stand for 30 minutes to complete color development.

The absorbance of the solution was measured at 693 nm using a UV VIS spectrophotometer. The concentration of solubilized phosphate was calculated based on a standard calibration curve and expressed in milligrams per liter (mg/L).

Nitrogen fixation assay

Nitrogen is a critical macronutrient required in large quantities by plants, especially during the vegetative growth phase. This assay aimed to evaluate the ability of indigenous bacterial isolates to fix atmospheric nitrogen using nitrogen free Burk's medium, as described by Stella and Suhaimi (2010). The nitrogen free Burk's medium was prepared with the following composition (per liter) namely, 20 g sucrose, 0.04 g K_2HPO_4, 0.16 g KH_2PO_4, 0.20 g $MgSO_4{\cdot}7H_2O$, 0.20 g NaCl, 0.05 g $CaSO_4{\cdot}2H_2O$, and 15 g agar. In addition, 5.0 mL of 0.05% sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$) and 5.0 mL of 0.3% iron sulfate ($FeSO_4{\cdot}7H_2O$) were filter sterilized and added separately to the medium after autoclaving. The pH of the medium was adjusted to 7.0 prior to sterilization.

Each bacterial isolate was pre cultured in NB medium and then inoculated onto solidified Burk's nitrogen free medium. The inoculated plates were incubated at room temperature for 24 hours. Bacterial growth on nitrogen free media was taken as an indication of the isolate's ability to fix atmospheric nitrogen. The total nitrogen content fixed by the bacteria was subsequently quantified using the Kjeldahl method. This involved digestion, distillation, and titration steps to determine the amount of nitrogen present, expressed as milligrams per liter (mg/L).

Heavy metal reduction assay for nickel and chromium

The final phase of this study involved evaluating the ability of selected indigenous bacterial isolates to reduce nickel and chromium concentrations in solution, following successful outcomes from previous IAA, GA_3, phosphate solubilization, and nitrogen fixation tests. This assay was adapted from the method described by Rahadi et al. (2020), with modifications. Selected bacterial isolates were inoculated into liquid NB medium supplemented with either nickel chloride or chromium chloride at concentrations of 300 ppm and 700 ppm, respectively. The cultures were incubated in a shaking incubator at 37°C and 220 rpm for 24 hours to promote bacterial growth and metal interaction. Following incubation, the cultures were centrifuged at 10.000 rpm for 5 minutes to separate bacterial cells from the supernatant. To assess chromium reduction, a chromium complex was formed by combining 12.5 mL of distilled water (adjusted to pH 3) with 250 µL of diphenyl carbazide solution (1 g/400 mL acetone) in a test tube. For nickel reduction, a nickel complex was prepared by mixing 12.5 mL of distilled water (pH 3) with 250 µL of dimethylglyoxime solution and 25 mL of chloroform. The absorbance of the resulting metal complexes was measured using a UV VIS spectrophotometer at a wavelength of 540 nm. The concentrations of residual nickel and chromium were determined using a calibration curve based on the regression equation

y = 0.1057x + 0.0025. Reduction efficiency was expressed as a percentage relative to the initial metal concentration.

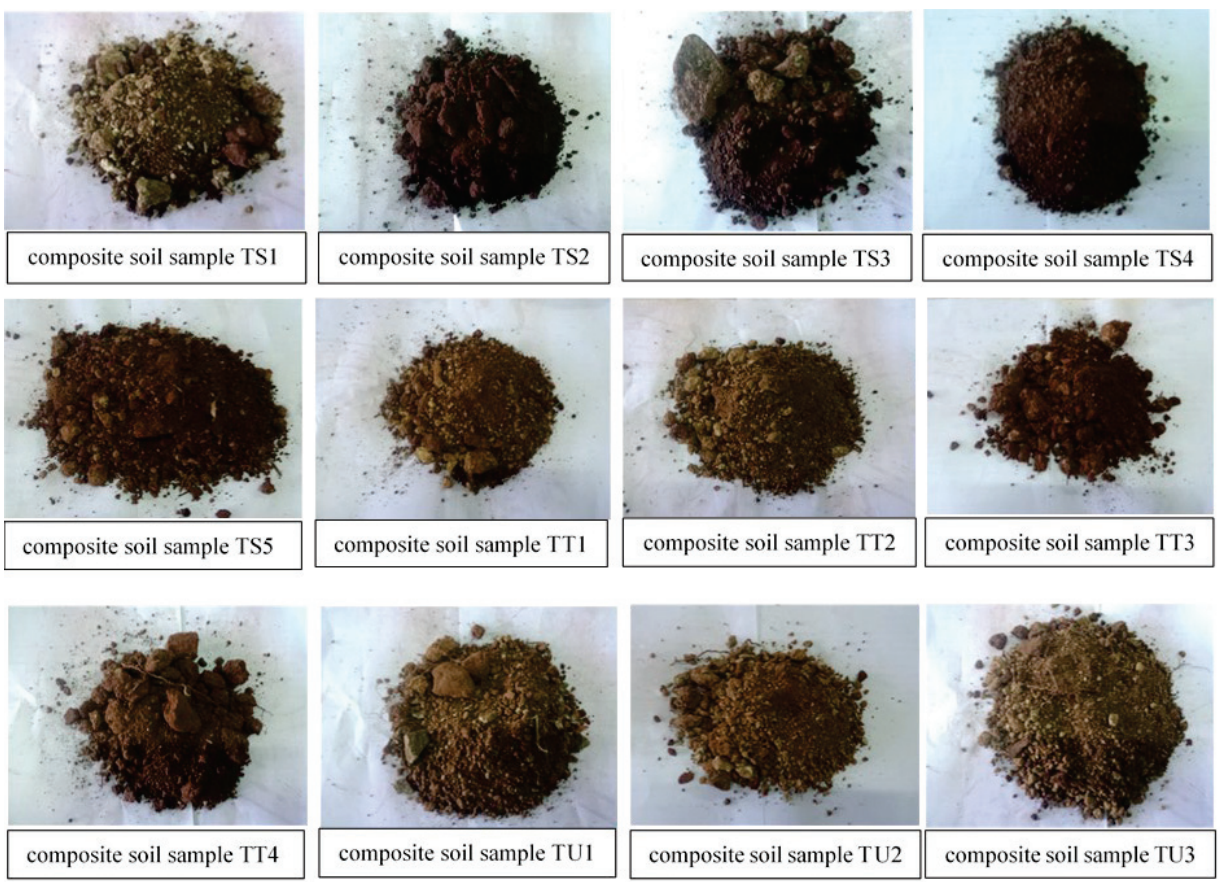
Results and Discussion

Isolation and screening of indigenous bacteria

Soil samples were collected from twelve locations across revegetated post nickel mining areas and composited into a representative sample for microbial analysis. *Figure 3* depicts composite soil samples collected at 12 sampling points. The composite soil samples were serially diluted using sterile distilled water to achieve concentrations of 10^{-1} to 10^{-5}. Suspensions from the 10^{-3}, 10^{-4}, and 10^{-5} dilutions (50 mL each) were plated in duplicate onto NB agar media for initial bacterial isolation.

The bacterial isolates selected from initial screening were further evaluated for their tolerance to heavy metals. A total of 57 isolates that successfully grew on NB agar were inoculated onto selective media containing 10 ppm nickel chloride and 10 ppm chromium chloride. The screening process aimed to assess the ability of these isolates to grow under heavy metal stress. Out of the 57 isolates, 48 exhibited strong and consistent growth on the metal supplemented media, indicating a high level of tolerance to both nickel and chromium. The remaining isolates showed limited or no growth, suggesting sensitivity to heavy metal exposure. These findings suggest that a majority of the indigenous bacteria in post mining soil have developed adaptive traits, likely due to continuous exposure to elevated heavy metal concentrations.

Fig. 3. *Composite soil samples at each post mining location*



The metal tolerant isolates identified in this study hold significant potential for use in bioaugmentation strategies aimed at remediating nickel and chromium contaminated soils. Bacteria that are resistant to heavy metals have survival mechanisms such as bioaccumulation, bioprecipitation, methylation, and bioreduction (Atlas and Bartha, 1998)

These findings suggest that a significant proportion of indigenous bacteria present in post mining soil have adapted to survive in metal contaminated environments. The ability of 48 isolates to grow under nickel and chromium stress makes them strong candidates for further evaluation in bioaugmentation based soil rehabilitation programs. These results are consistent with studies indicating that long term exposure to metal contaminated soils can lead to the natural selection of metal tolerant microbial communities (Wuana and Okieimen, 2011).

Indole-3-acetic acid (IAA) and gibberellic acid (GA3) production by indigenous bacterial isolates

IAA and GA_3 are critical phytohormones that regulate a wide range of plant developmental processes. IAA influences root elongation, cell division, and differentiation, while GA_3 promotes stem elongation, seed germination, and flowering. In this study, 48 indigenous bacterial isolates from post nickel mining soils were evaluated for their capacity to produce IAA and GA_3.

IAA production was assessed using the Salkowski colorimetric method, where a pink coloration results from the formation of a complex between IAA and iron (Fe^{3+}) in an acidic environment. This color development reflects both complexation and redox reactions, and the intensity of the pink hue corresponds to the IAA concentration produced by the bacteria (Kovacs, 2009). Eight isolates TS3ni^{-31}, TS3ni^{-34}, TS5ni^{-32}, TU2cr^{-41}, TT1cr^{-4}, TT2cr^{-32}, TT2cr^{-35}, and TT4cr^{-5}, did not grow in NB medium supplemented with 1% L-tryptophan, likely due to limited metabolic adaptation to the medium or precursor uptake.

GA_3 production was quantified spectrophotometrically after incubation in NB medium. One isolate (TT2cr^{-36}) failed to grow under the test conditions, while TT3cr^{-51} produced the lowest detectable GA_3 concentration at 2.204 ppm. Among the remaining 47 isolates, ten were identified as the highest producers of either IAA or GA_3 (*Table 1*). Notably, isolates TT1ni^{-32} and TS1ni^{-41} were distinguished by their ability to produce high levels of both phytohormones.

Table 1. *IAA and GA3 Production by indigenous bacterial isolates*

No	Bacterial isolates	IAA (ppm)	Bacterial isolates	GA_3 (ppm)
1	TT1cr^{-32}	8 ppm	TU2cr^{-41}	2.99 ppm
2	TT2cr^{-33}	2.34 ppm	TS1ni^{-41}	2.75 ppm
3	TT1ni^{-32}	2.03 ppm	TT2cr^{-31}	2.735 ppm
4	TS5ni^{-31}	1.95 ppm	TS4ni^{-32}	2.731 ppm
5	TT1cr^{-33}	1.79 ppm	TU3ni^{-4}	2.70 ppm
6	TS1ni^{-41}	1.09 ppm	TS1cr^{-5}	2.68 ppm
7	TS3cr^{-31}	0.93 ppm	TS1ni^{-52}	2.66 ppm
8	TT3cr^{-52}	0.92 ppm	TS2ni^{-5}	2.63 ppm
9	TT1ni^{-34}	0.90 ppm	TT1ni^{-41}	2.62 ppm
10	TU2ni^{-5}	0.89 ppm	TT1ni^{-32}	2.62 ppm

The distribution of high performing isolates showed that the majority of strong IAA producers originated from the central post mining area (TT zone), followed by isolates from the southern zone (TS). Only one isolate from the northern zone (TU2ni^{-5}) ranked among the top IAA producers. For GA_3, TU2cr^{-41} (2.99 ppm) and TS1ni^{-41} (2.75 ppm) were the most productive. These dual ability isolates hold strong potential as plant growth promoting bacteria (PGPB) for bioaugmentation applications in degraded or heavy metal contaminated soils.

These findings are consistent with recent research that demonstrates the significant role of phytohormone producing microbes in plant development and stress resilience. Olagunju et al. (2025) reported that *Bacillus cereus* and *Providencia rettgeri* strains with high IAA production enhanced seedling growth under stress conditions. Similarly, Shah et al. (2023) emphasized the relevance of GA_3 in plant stress signaling pathways, highlighting its role in enhancing plant resilience under abiotic stress.

Phosphate solubilization and nitrogen fixation by indigenous bacterial isolates

Phosphorus is a key macronutrient required for plant growth, yet it is often unavailable in soils due to its presence in insoluble forms such as Fe-phosphate and Al-phosphate in acidic conditions, or $Ca_3(PO_4)_2$ in alkaline environments. Soil microorganisms, particularly phosphate-solubilizing bacteria (PSB), contribute to phosphorus availability by secreting organic acids

(e.g., citric, malic, oxalic) that solubilize bound phosphates and convert them into plant available forms (Cunningham and Kuiack, 1992). While, nitrogen fixation is the process of reducing dinitrogen (N_2) to ammonia (NH_3) catalyzed by the enzyme nitrogenase. Nitrogen fixation can only be performed by prokaryotic organisms because the nitrogenase enzyme is encoded only in the prokaryotic genome (Fay, 1992).

In this study, 48 indigenous bacterial isolates from post nickel mining soils were tested for phosphate solubilization using spectrophotometry. Among these, 10 isolates demonstrated significant phosphate dissolution capabilities (*Table 2*). The isolate TT1cr^{-33} exhibited the highest solubilization at 12.53 ppm, while TS2ni^{-33} showed the lowest among the top performing group, at 8.84 ppm.

Table 2. *Phosphate solubilization and nitrogen fixation by indigenous bacterial isolates*

No	Bacterial isolates	Phosphate dissolution (ppm)	Bacterial isolates	Nitrogen fixation (ppm)
1.	TT1cr^{-33}	12.53	TS1cr^{-5}	921.55
2.	TT1ni^{-31}	11.20	TS2ni^{-5}	887.38
3.	TT2cr^{-31}	9.67	TS4ni^{-32}	984.01
4.	TT1ni^{-41}	9.63	TT1ni^{-32}	1292.99
5.	TU2ni^{-5}	9.58	TT1ni^{-41}	639.56
6.	TS3ni^{-32}	9.18	TT1cr^{-32}	891.96
7.	TS4ni^{-42}	9.12	TT1cr^{-33}	620.18
8.	TS1ni^{-42}	8.89	TT2cr^{-31}	860.74
9.	TS4ni^{-32}	8.86	TU2ni^{-5}	983.69
10.	TS2ni^{-33}	8.84	TU3ni^{-41}	925.01

The visual change in the supernatant to a blue colour during testing indicated successful phosphate solubilization, which was attributed to the secretion of organic acids by the bacterial isolates, consistent with previous findings (Zuluaga et al., 2023). These solubilization capabilities support the potential of these isolates as biofertilizers for improving soil phosphorus availability, especially in degraded post mining areas.

Nitrogen fixation, the biological conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), is another essential microbial function for plant nutrition. This process is catalysed by the enzyme nitrogenase, which is encoded exclusively in prokaryotic genomes (Lee et al., 2022). All 48 isolates in this study were assessed for nitrogen fixing ability. Among the top ten nitrogen-fixing isolates (*Fig. 2*), TT1ni^{-32} recorded the highest fixation activity at 1292.99 ppm, while TT1cr^{-33} recorded the lowest at 620.18 ppm.

The distribution of high performing PSB highlights the dominance of isolates from the central post mining zone (TT), such as TT1cr^{-33}, TT1ni^{-31}, TT2cr^{-31}, and TT1ni^{-41}. These isolates demonstrated higher solubilization values, possibly due to prolonged adaptation to the low phosphorus, metal contaminated conditions of the Pomalaa post mining soil. This is consistent with prior studies suggesting that environmental stress, particularly heavy metal presence, can enhance the selective pressure for microbial populations capable of solubilizing mineral bound nutrients (Shrivastava et al., 2020). Isolates from the southern zone (TS3ni^{-32}, TS1ni^{-42}, TS4ni^{-32}) and northern zone (TU2ni^{-5}) also showed substantial phosphate solubilizing activity, indicating that beneficial microbes are distributed across varied micro ecological niches within the post mining landscape.

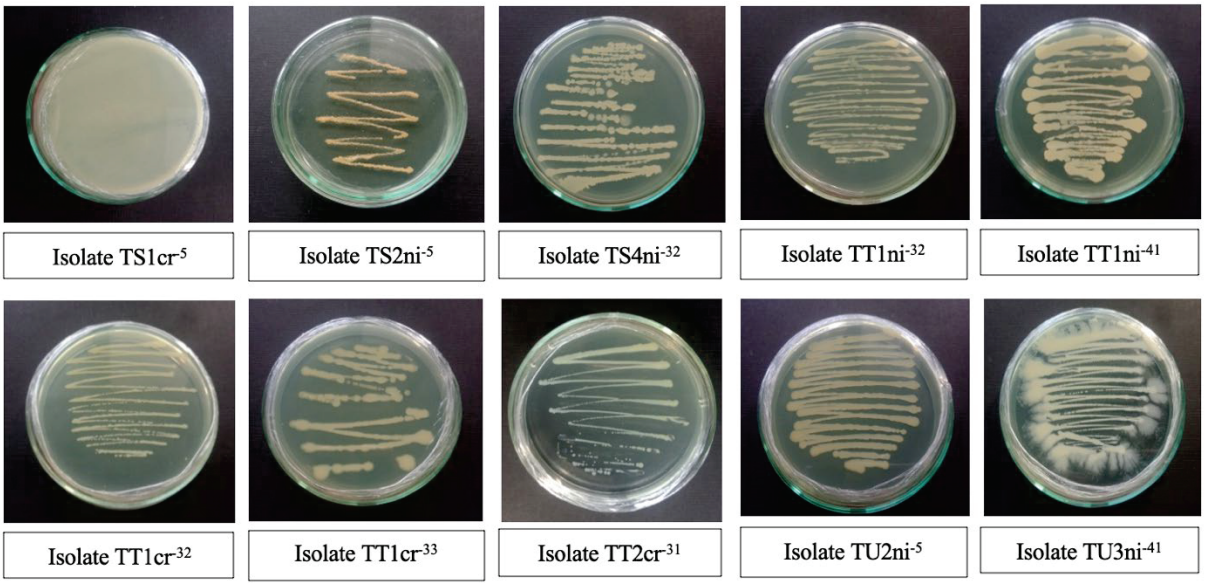
The comparison of nitrogen fixing capabilities among isolates also reveals noteworthy patterns. Isolates such as TT1ni^{-32} and TU2ni^{-5}, which scored highly in both nitrogen fixation and phosphate solubilization, stand out as multifunctional biofertilizer candidates. TT1ni^{-32}, in particular, exhibited the highest nitrogen fixation value (1292.99 ppm) and also ranked among the top phosphate solubilizers (9.63 ppm), showcasing its strong potential for integrated soil fertility enhancement. This dual capability is especially valuable for restoring degraded post-mining soils, where both nitrogen and phosphorus are typically deficient. The ability of certain strains to perform both functions efficiently reflects microbial versatility and adaptability, traits emphasized in recent reviews of rhizosphere microbiome roles in sustainable agriculture (Lee et al., 2022). These traits make such isolates excellent candidates for further greenhouse and field trials targeting bioaugmentation of post mining lands.

Reduction capability of indigenous bacteria towards nickel and chromium

This study evaluated the bioremediation potential of ten indigenous bacterial isolates namely, TS1cr^{-5},

TS2ni^{-5}, TS4ni^{-32}, TT1ni^{-32}, TT1ni^{-41}, TT1cr^{-32}, TT1cr^{-33}, TT2cr^{-31}, TU2ni^{-5}, and TU3ni^{-41}, selected based on their superior biochemical capabilities in IAA and GA_3 production, phosphate solubilization, and nitrogen fixation. These isolates were tested for their ability to reduce nickel and chromium concentrations in liquid NB enriched with 300 ppm and 700 ppm of $NiCl_2$ or $CrCl_2$ (*Fig. 4*).

Fig. 4. *ten bacterial isolates for nickel and chromium reduction ability test*



The results at *Tables 3* and *4* below, showed that isolate TU3ni^{-41} achieved the highest nickel reduction, with 97.29% at 300 ppm and 95.55% at 700 ppm. For chromium, isolate TS1cr^{-5} exhibited the greatest reduction efficiency at both 300 ppm (65.62%) and 700 ppm (48.27%). These findings confirm the significant reduction potential of specific indigenous bacteria under heavy metal stress, highlighting their value for potential application in contaminated environments.

Further analysis of the nickel reduction data reveals that isolate TT1ni^{-32}, identified as a *Bacillus* species, also demonstrated excellent performance, achieving 84.0% and 86.02% reduction at 300 and 700 ppm, respectively. TT1ni^{-41}, a *Clostridium* isolate, also showed promising results, though with a decline at higher concentration (77.19% at 300 ppm and 57.89% at 700 ppm), suggesting a potential threshold effect in metal tolerance. While most isolates displayed greater efficiency at 300 ppm, only TU3ni^{-41} and TT1ni^{-32} maintained consistently high reduction across both concentrations. These patterns are consistent with the known adaptability and resistance mechanisms of *Bacillus* spp. to nickel stress (Mohan et al., 2023).

Taxonomic identification using *Bergey's Manual of Determinative Bacteriology* indicated that nine of the ten isolates belonged to the genus *Clostridium*, while TT1ni^{-32} was identified as *Bacillus*. These two genera are widely reported for their heavy metal resistance through mechanisms such as bioaccumulation, bioprecipitation, enzymatic reduction, and biosorption (Juwarkar et al., 2023). *Bacillus* spp., in particular, are known to produce extracellular enzymes and siderophores, enhancing metal chelation and detoxification, while *Clostridium* spp. exhibit high tolerance to anaerobic and metal rich environments.

Chromium reduction trends were more variable. Although TS1cr^{-5} consistently outperformed other isolates, TT2cr^{-31} showed an unexpected spike at the higher concentration, with a reduction of 61.79% at 700 ppm, compared to 25.0% at 300 ppm. This

Table 3. *Reduction efficiency of selected indigenous bacterial isolates on nickel at 300 ppm and 700 ppm concentrations*

No	Bacterial osolates	Concentration (ppm)	24-hour initial concentration (ppm)	48-hour final concentration (ppm)	Total reduction (ppm)	Percentage of reduction
1	TS2ni^{-5}	300	0.034	0.003	0.031	91.17
		700	0.043	0.014	0.029	67.44
2	TS4ni^{-32}	300	0.065	0.015	0.050	76.92
		700	0.076	0.031	0.045	59.21
3	TT1ni^{-32}	300	0.075	0.012	0.063	84
		700	0.093	0.013	0.080	86.02
4	TT1ni^{-41}	300	0.057	0.013	0.044	77.19
		700	0.057	0.024	0.033	57.89
5	TU2ni^{-5}	300	0.061	0.015	0.046	75.40
		700	0.073	0.017	0.056	76.71
6	TU3ni^{-41}	300	0.037	0.0003	0.036	97.29
		700	0.045	0.002	0.043	95.55

Table 4. *Reduction efficiency of selected indigenous bacterial isolates on chromium at 300 ppm and 700 ppm concentrations*

No	Bacterial Isolates	Concentration (ppm)	24-hour initial concentration (ppm)	48-hour final concentration (ppm)	Total reduction (ppm)	Percentage of reduction
1	TS1cr^{-5}	300	0.032	0.011	0.021	65.62
		700	0.058	0.030	0.028	48.27
2	TT1cr^{-32}	300	0.037	0.017	0.020	54.05
		700	0.079	0.040	0.039	49.36
3	TT1cr^{-33}	300	0.035	0.035	0	0
		700	0.089	0.038	0.051	57.30
4	TT2cr^{-31}	300	0.040	0.030	0.010	25
		700	0.089	0.034	0.055	61.79

suggests that certain bacterial pathways for Cr (VI) reduction may be concentration dependent or inducible under high oxidative stress, a mechanism previously observed in metal reducing bacteria (Sreedevi et al., 2022). In contrast, TT1cr^{-33} showed no detectable chromium reduction, potentially due to either genetic limitations or inhibited growth under chromium exposure. These findings underscore the need to match remediation strategies to specific bacterial profiles and environmental conditions.

In addition to their heavy metal resistance, these isolates have demonstrated beneficial plant growth promoting traits. Their ability to produce phytohormones (IAA and GA_3), solubilize phosphate, and fix atmospheric nitrogen makes them attractive candidates for integrated bioremediation and soil fertility enhancement in post mining areas. Nursyirwani and Yoswaty (2021) highlighted that such microbial traits not only aid in detoxification but also facilitate ecosystem restoration through organic acid production and metabolic transformations that reduce pollutant toxicity. Therefore, *Clostridium* and *Bacillus* species isolated from metal-contaminated post mining soils show strong promise for use as bioaugmentation agents in future bioremediation programs. According to Tiquia-Arashiro, (2018) bacteria have various resistance mechanisms

that can be used to reduce heavy metal concentrations in the environment, which include biosorption, precipitation, bioaccumulation, and binding with siderophores.

Conclusions

This study identified and evaluated indigenous bacterial isolates from post nickel mining lands in Pomalaa for their dual capabilities in promoting soil fertility and reducing heavy metal concentrations. From 72 initial isolates, 48 showed metal tolerance and 10 were selected for advanced testing based on their biochemical traits. Key strains demonstrated strong performance in IAA and GA_3 hormone production, phosphate solubilization, and nitrogen fixation, important functions for plant growth and nutrient cycling. Among these, TT1cr^{-32} (IAA), TU2cr^{-41} (GA_3), TT1cr^{-33} (phosphate), and TT1ni^{-32} (nitrogen fixation) stood out as the highest producers in their respective categories. Additionally, TU3ni^{-41} and TS1cr^{-5} showed exceptional ability to reduce nickel and chromium concentrations, respectively, with TU3ni^{-41} achieving over 95% nickel reduction even at high concentrations. These strains were identified as *Clostridium* and *Bacillus* spp., which are well documented for their adaptive resistance to heavy metals via biosorption, bioprecipitation, and enzymatic reduction pathways. The combined ability of these indigenous bacteria to mitigate toxic metal levels and enhance soil nutrient content highlights their strong potential as bioaugmentation agents for bioremediation and land restoration. Their adaptability, metabolic versatility, and multifunctionality offer a sustainable, ecologically sound strategy to rehabilitate degraded post mining landscapes. Field trials and molecular studies are recommended to validate their performance in large scale applications and assess their compatibility with native plant species and soil conditions.

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