

Original Research Article

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# Phytoremediation of Al, Cr and Pb from Bauxite Mine Soil by Native Rhizobacteria Inoculated *Crotalaria juncea* L.

Ankita Tripathy and Bandana Kullu<sup>id</sup>\*

P.G. Department of Botany, Utkal University, Bhubaneswar, Vani Vihar, Odisha, India

\*Corresponding author

## ABSTRACT

Current study assessed the effectiveness of native plant growth promoting rhizobacteria of bauxite soil *Bacillus thuringensis*, *Stenotrophomonas hibiscola* and *Burkholderia cepacia* inoculation on phytoremediation potential of *Crotalaria juncea*. A greenhouse pot culture experiment was designed with single and consortium inoculation of native bacteria to *C. juncea* grown on bauxite soil. Aluminium (Al), Cadmium (Cd) and Zinc (Zn) uptake from bauxite soil was estimated. Phytoremediation potential of *C. juncea* was analysed by estimating bio-accumulation factor and metal translocation factor. Findings revealed enhanced metal uptake in both root and shoot of *C. juncea* with native rhizobacterial inoculation. Highest metal uptake was recorded with bacterial consortium, showing increase metal content in shoot and root by 45.5% and 33.2% for Al, by 109.5% and 58.7% for Cr and by 172.2% and 85% for Pb respectively. Metal uptake by *C. juncea* proportionately decreased the amount of residual soil metal content in bauxite mine soil. BAF) and translocation factor (TF) in *C. juncea* was also enhanced with native bacteria inoculation and maximum increase was observed with consortium inoculation. Bio-accumulation showed increase of 40.8%, 92.2% and 130.2% and metal translocation factor exhibited enhancement by 9.2%, 32% and 47% for Al, Cr and Pb respectively. It was concluded the biochemical attributes of native bacteria aid to enhance the metal uptake and phytoremediation potential of *C. juncea*. Future study at field level can validate the effectiveness native rhizobacteria on phytoremediation by *C. juncea*.

### Keywords

Green manure;  
Consortium; Pot  
culture;  
Bioaccumulation  
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## Introduction

Bauxite mining in Odisha has emerged as a key driver of industrial growth and economic development, supporting the expansion of aluminium industry (Layek *et al.*, 2026). The Bauxite mines at Panchpatmali, Koraput, Odisha is the largest bauxite mines of Asia. However, the open cast mining process have raised significant environmental and socio-economic concerns, because of

extensive land degradation due to excavation, removal of vegetation cover, pilling up of mine spoil and reduction of soil fertility (Xiao *et al.*, 2023).

The mining activities intensify the metal pollution which is one of environmental issues in recent decades (Ondrasek *et al.*, 2025). Elevated concentration of potentially toxic element like aluminium, lead, zinc, cadmium, arsenic, mercury resulting in soil pH alteration,

increased solubility and bioavailability of toxic metals in soil (Silva-Rego *et al.*, 2022). The physical and chemical techniques are available to manage the metal contaminated soil, but are quite expensive, labour intensive and affect the soil properties. In such context, vegetation cover and phytoremediation are alternative approaches, which have gained immense attention for sustainability and environmentally sound impact (Dhakal *et al.*, 2025). However, the soil structure and nutrient limitation of mine spoil are constraints to vegetation establishment in mine tailings.

The amalgamation of native rhizobacteria for plant growth promotion and phytoremediation of mine soil is a widely used approach (Narayanan *et al.*, 2021). The diverse functional attributes and adaptability of the rhizobacteria to local stressed environment make them suitable microbial candidate for metal accumulating plants (Thavamani *et al.*, 2017). The interaction of host plant root with rhizosphere inhabited microbes portrays a fundamental role in nutrient cycling, organic matter decomposition, transformation and mobility of toxic metal in soil system (Newsome and Falagan, 2021). Plant growth hormone production, nitrogen fixation, citrate utilization, siderophore production, phosphate and zinc solubilization are some of the attributes of bacteria responsible for plant growth promotion under stressful environment (Alonazi *et al.*, 2025). The biochemical characteristics of rhizobacteria play a role in modulating stress responsive pathways and facilitating metal immobilization, transformation and uptake (Firinca *et al.*, 2023). Hence, native rhizo-bacteria offer potential scope for effective restoration and reclamation of metal-contaminated mine soil (Newsome and Falagan, 2021).

*Crotalaria juncea* L. (Sunn hemp) belongs to Fabaceae family, is one of the fast-growing leguminous green manure plants widely used to increase soil fertility. *C. juncea* has distinctive property of rapid biomass production, nitrogen fixation, soil fertility improvement, adaptability to harsh environments, and suitable habitat for beneficial microbes. The extensive root system of *C. juncea* promotes soil structure, prevents soil erosion and facilitates microbial activity in the rhizosphere. It also aids in soil stabilization and uptake of toxic metals from mine soil (Arone *et al.*, 2024).

Previous report suggests that the native microbes of bauxite mines improve plant growth by improving soil fertility, stress tolerance of host plant and reducing toxic metal content in soil (Narayanan *et al.*, 2021). However,

the implications of native rhizospheric bacteria from the bauxite mine soil of Panchpatmali, Koraput, Odisha is yet to be explored for vegetation growth and aid to phytoremediation of the host plant.

Therefore, the current study was undertaken to assess the effect of native rhizospheric bacteria inoculation to the green manure *C. juncea* for metal uptake and phytoremediation of bauxite soil.

## Materials and Methods

### Native bacterial inoculum

The native rhizobacteria of bauxite mine soil of Panchpatmali, Koraput, Odisha the *Bacillus thuringiensis* ATCC 10972 (PV663022), *Stenotrophomonas hibiscicola* ATCC 19867 (PV342550) and *Burkholderia cepacia* UCB 717 (ATCC 25416) (PX487737) were previously isolated (Communicated). The biochemical characteristics of the bacteria were assessed.

### Gram staining

For gram staining of bacterial inoculum, 24 hr freshly cultured isolates were smeared on clean glass slides, followed by air drying and heat fixation. The prepared slides were stained with crystal violet for 1 min, followed by Gram's iodine for 1min and then decolorized with Gram's decolourizer for 10-20 s.

After rinsing through distilled water to remove the traces of decolourizer the smear was counterstained with safranin for 30-60 s, washed, airdried and observed under oil immersion (100X). Gram positive or negative were examined by appearance of pink and purple respectively (Bergey and Holt, 1994).

### MRVP test

The methyl red -Voges Proskauer test (MR-VP) was performed by inoculating the microbial inoculum into MR-VP broth and incubating at 35-37°C for 24-48 hours. 2-3 drops of methyl red indicator were added to an aliquot for methyl red test and observed through appearance of red (+ve) or yellow (-ve) colour. For Voges Proskauer test, separate aliquot was treated with VP reagent (I and II) and appearance of red or pink colour or yellow indicate positive or negative respectively (Bergey and Holt, 1994).

### **Indole production**

The bacterial inoculum was cultured on sterile 1% tryptone broth and incubated for 48hrs. Following incubation 5 drops of Kovac reagent were added to each tube including the control. Formation of cherry red ring on the top of the medium indicates positive response (Macwilliams, 2012)

### **HCN production**

For HCN production the pure culture of inoculum was streaked on nutrient agar supplemented with glycine (4.4 g/L). A Whatman filter paper soaked in picric acid solution (0.5% picric acid in 2% sodium carbonate) was placed in the lid of petri dish and sealed with parafilm to prevent the gas escape. The plates were incubated at 30-35°C for 24-48 h. Change in colour of filter paper from yellow to orange or reddish brown indicate positive (Bergey and Holt, 1994).

### **Carbohydrate fermentation**

To detect the ability of carbohydrates (glucose, sucrose, and lactose) fermentation, a loop full of fresh pure cultures were inoculated to phenol red carbohydrate broth medium (peptone-10 g, sodium chloride-5 g, phenol red-.018 g, distilled water-1000 ml, and carbohydrate-10 g, pH 7.3) independently for each carbohydrate source. The cultures were incubated at 37°C for up to 48 h. Change in colour of medium from red to yellow indicate positive (Reiner, 2012).

### **Citrate utilization**

To perform the citrate utilization of bacterial inoculum, pure culture of the strains was streaked on Simmon's citrate agar slants and incubated at 35-37°C for 24-48 h. Change in colour of the medium from green to blue along with visible growth of culture indicate positive result where as no change in colour indicate negative result (Bergey and Holt, 1994).

### **IAA Production**

The IAA production of the isolated strains was detected in yeast mannitol broth medium (YMB) fortified with 0.5 g L-Tryptophane (Ehmann 1977). Pink colouration of the culture filtrate with the addition of Salkowski reagent confirmed the positive result of the qualitative assay.

### **Ammonia production**

Ammonia production was tested in peptone water medium with Nessler's reagent. The formation of yellow-brown precipitate in culture filtrate indicated ammonia production (Cappuccino and Sherman 2013).

### **Siderophore production**

The siderophore production of the isolates was performed in Tryptone yeast extract salt medium (TYES). Reddish-brown coloration of the culture filtrate (24-48 h incubation) by adding FeCl<sub>3</sub> (2%) indicated positive results (Dave *et al.*, 2006).

### **Phosphate and Zn solubilization**

Phosphate solubilization efficiency was assessed through Pikovskaya's agar media containing tricalcium phosphate (5%), whereas the Zn solubilization was tested in zinc solubilizing agar media containing zinc oxide (0.1%). The formation of a transparent halo zone around the bacterial colony indicates P / Zn solubilization by the bacteria (Premono *et al.*, 1996; Sharma *et al.*, 2012).

### **ACC (1-aminocyclopropane-1-carboxylate) deaminase activity**

The bacterial isolates were inoculated on sterile minimal DF (Dworkin and Foster) salt media amended with 3 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as the sole source of nitrogen for the screening of ACC deaminase activity (Dworkin and Foster 1958).

### **Nitrogen-fixation**

The Norris's glucose nitrogen-free media (Himedia) was used to screen the nitrogen fixation by the bacterial isolates. Inoculated plates were incubated for 2-3 days, and a clear halo zone around the colony indicated a positive result (Ranganayaki and Mohan 1981).

### **Pot Culture Experiment**

The potting soil for pot culture experiment was constituted of bauxite mine soil (70%) and organic manure (30%). The native bacteria were supplemented to the experimental plant *C. juncea* by seed inoculation method. *C. juncea* seeds were surfaced sterilised with mercury chloride (0.1%) followed by rinsing with sterile

distilled water 4-5 times. The seeds were germinated on Petri plate over moistened sterile cotton. The bacteria *B. thuringiensis*, *S. hibiscicola* and *B. cepacia* were inoculated to sterilized nutrient broth media separately and incubated in a bacteriological incubator for 24 hours at 25° C. The bacteria were inoculated into the germinated seeds of *C. juncea* by soaking in bacterial culture for 5-6 hours. The bacteria inoculated seeds (5 seeds per pot) were transferred to pot containing autoclaved potting mix. The experimental plants were grown under a greenhouse for 30 days. Treatment for pot culture experiment was described in Table 1, and physiochemical properties of the potting mix were presented in Table 2.

### Assessment of metal content in *C. juncea* and potting mix

Metal uptake from bauxite mine soil by *C. juncea* was assessed after 30 days of plant growth. The Al, Cr and Pb content in root and shoot of *C. juncea* and potting soil (initial and residual) was estimated by acid digestion of the dried plant tissue / soil in nitric acid and perchloric acid (4:1 v/v) using a microwave digester. (Zasoski and Burau, 1977). The metal content acid digested filtrate analysed by ICP-OES (Model: Avivo 200, Perkin Elmer).

### Phytoremediation potential of *C. juncea*

Phytoremediation potential of *C. juncea* was assessed by estimating bioaccumulation factor (BAF) and translocation factor within plant (TF). Metal bioaccumulation factor (BAF) corresponds to the concentration of metal in plant tissues with respect to metal concentration of mine soil. The translocation of metals from root to shoot within the plant is expressed as translocation factor (TF) (Chandra *et al.*, 2017).

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{metal concentration of total plant tissues}}{\text{metal concentration of mine soil}}$$

$$\text{Translocation factor within plant (TF)} = \frac{\text{metal concentration of shoot tissues}}{\text{metal concentration of root tissues}}$$

### Statistical analysis

For pot culture experiment 3 replicate pot for each treatment were maintained. Data obtained for each parameter was presented as mean (n = 3) ± standard error

of mean (SEM). Statistical significance of the mean values in each treatment was tested by Duncan's multiple range test (DMRT) at  $p \leq 0.05$  in SPSS 30.0 software.

## Results and Discussion

### Characterization of Native Bacterial inoculum

The characterization of bacterial inoculum revealed *S. hibiscicola* and *B. cepacia* were Gram negative, whereas *B. thuringiensis* was Gram positive (Fig 1). *B. thuringiensis* showed positive result for MRVP test, indole and HCN production, glucose and sucrose fermentation. *S. hibiscicola* and *B. cepacia* were positive for indole and HCN production, sucrose fermentation and citrate utilization. All the three bacteria exhibited positive result for IAA, ammonia and siderophore production, whereas positive result for ACC deaminase activity and phosphate solubilization recorded for *S. hibiscicola* and *B. cepacia* (Table 3)

The methyl red test indicates the production of organic acids during glucose fermentation, leading to a drop in pH observed through a change in colour of the media from yellow to red (Andy *et al.*, 2020). The bacteria showing positive response for the Voges Proskauer (VP) test, indicating acetyl methyl carbinol production from glucose fermentation (Andy *et al.*, 2020). MRVP and citrate utilization test are part of the IMVIC test, widely used to classify and characterize the bacteria (Abdallah *et al.*, 2016).

The HCN production by bacteria is important for pathogen control in plants and increasing P availability by metal chelation (Rijavec and Lapanje 2016). The findings of the current study indicate that all the isolates were capable of glucose fermentation, demonstrating their ability to produce organic acid by breaking down the glucose, which helps in metal immobilisation (Su *et al.*, 2024).

Production of phytohormone IAA and siderophore the iron-chelator contributes towards plant growth under stress condition (Singh *et al.*, 2022). Ammonia production, ACC deaminase activity and nitrogen fixation promote host plant growth by providing antioxidant defence, ethylene level management, enhanced biomass and nitrogen fixation (Alonazi *et al.*, 2025), highlighting the importance of native bacterial for growth on bauxite mine.

### Metal uptake from bauxite mine soil by *C. juncea*

The Al, Cr and Pb uptake in root and shoot tissues of *C. juncea* with bacterial inoculum from bauxite mine soil were analysed (Fig 2). Compared to the control (B0), Al uptake was observed to be increased by 19.7%, 17.7%, 30.2% and 45.6% in shoot tissue under B1, B2, B3 and B4. In the root tissue, Al uptake was enhanced by 12.6%, 11.5%, 21.1%, 33.2% with B1, B2, B3 and B4 treatment, respectively. Cr uptake in shoot and root was recorded to be enhanced by 56.4% and 29.9% for B1, 50% and 24.7% for B2, 63.3% and 31.9% for B3, 109.5% and 58.7% for B4, respectively. Pb uptake in the shoot and root tissue was increased by 75% and 26.9% for B1, 63.9 and 25.4% for B2, 88.8% and 32.8% for B3, 172.2 and 85.1% for B4, respectively. Among Al, Cr and Pb, uptake of Al was highest in both root and shoot in all the bacterial treatments. For Al, Cr and Pb, the highest level of metal accumulation was observed with B4 inoculation (consortium) followed by B3, B1 and B2 in both shoot and root.

*B. thuringensis* was reported to influence the uptake and translocation of Cd and Pb in wheat (Zhu *et al.*, 2024) and Pb remediation in tomato plants by *B. cepacia* (Janaki *et al.*, 2024) support our study. *Stenotrophous maltophilia*, one of the closely related bacteria of *Stenotrophous hibiscola*, reported enhanced Cd uptake from mine soil by *Capsicum annum* L. (Liaquat *et al.*, 2020), supporting the current finding. The combined inoculation of plant growth-promoting rhizobacteria (PGPR) *Bacillus gibsoni* and *Bacillus xiamenesis*, facilitated the metal uptake potential of *Sesbania sesban* (Zainab *et al.*, 2021), which was similar to the present findings. The protective role of *Burkholderia sp.* under stress conditions has been previously reported (Pal *et al.*, 2022).

Organic acids secretion by PGPR reduces soil pH, which enhances metal bioavailability and adsorption through the root (Zhao *et al.*, 2024), corroborating the findings. The synergistic effects of bacteria in the consortium containing *B. thuringiensis*, *S. hibiscicola* and *B. cepacia* justify the highest metal uptake in B4.

### Residual metal content in bauxite mine soil

The residual Al, Cr and Pb content in bauxite mine soil (potting soil) was analysed after pot culture experiment and compared with that of the initial level (Table 4). Compared to the initial metal level of Al, the residual

content was reduced by 9.1% B0, by 10.8% in B1, by 10.6% in B2, by 11.5% in B3, and by 12.7% in B4. For Cr, residual content was decreased to be 8.1% in B0, 11.1% in B1, 10.6% in B2, 11.6% in B3 and 14.4% in B4 than initial level. For Pb, the decline in residual metal content than initial level was 2.6% in B0, 3.6% in B1, 3.3% in B2, 3.6% in B3 and 5.4% in B4. The maximum decrease in residual metal content for Al, Cr and Pb was recorded in B4. The *C. juncea* grown on mine soil in pot culture take up metal from the potting mix. Native bacterial inoculation enhanced the metal uptake and highest uptake was recorded with B4 inoculation. As the metal gets accumulated in *C. juncea* root and shoot, the residual metal content proportionally decreased in the bauxite mine soil. Similar observation of decline in residual metal content in mine soil with metal uptake by plant (Narayanan *et al.*, 2021) supports our study.

### Phytoremediation potential of *C. juncea* with native bacteria inoculation

The phytoremediation potential of *C. juncea* was represented as bioaccumulation factor (BAF) and translocation factor from root to shoot (TF) for Al, Cr, and Pb with native bacterial inoculation after 30 days of plant growth (Fig 3). Inoculation of *B. thuringensis* (B1), *S. hibiscicola* (B2), and *B. cepacia* (B3) enhanced the BAF and TF for Al, Cr, and Pb in *C. juncea* compared to non-inoculated (B0). The BAF for Al was enhanced by 16.9% in B1, 15.3% in B2, 26.6% in B3 and 40.8% in B4. For Cr, BAF showed an increase of 47.3% in B1, 52.6% in B2, 52.6% in B3 and 92.3% in B4. Enhancement of BAF for Pb was 51.7% in B1, 45.3% in B2, 61.8% in B3 and 130.2% in B4. The combined inoculation of *B. thuringensis* + *S. hibiscicola* + *B. cepacia* (B4) to *C. juncea* significantly enhanced the TP and BAF. Elevation in BAF and TF in *C. juncea* followed an order of B4 > B3 > B1 > B2 among bacterial inoculum treatments.

The enhancement of heavy metal BAF and TF for Al, Cr and Pb in *C. juncea* by PGPR inoculation was supported by the similar findings in *Brassica juncea* for Pb and Ni (Sharma and Saraf, 2023) and *Jatropha curcas* for Pb and Cd (Narayanan *et al.*, 2021). Bacteria in heavy metal-contaminated soil evolve metal resistance strategies to sustain (Patil *et al.*, 2025). Increase in bioaccumulation factor with bacterial inoculum indicate enhanced metal uptake potential of *C. juncea* due to enhanced bioavailability of metals in rhizosphere through acidification or chelating agent (Wu *et al.*, 2006).

**Table.1** The bacterial inoculation treatments of the pot experiment

Treatments	Bacterial inoculation
B0	Control (no inoculation of bacteria)
B1	<i>Bacillus thuringiensis</i>
B2	<i>Stenotrophomonas hibiscicola</i>
B3	<i>Burkholderia cepacia</i>
B4	<i>B. thuringiensis</i> + <i>S. hibiscicola</i> + <i>B. cepacia</i> (consortium)

**Table.2** Physiochemical properties of the potting mix

Parameters	Value
pH	5.8
EC	0.23 dsm <sup>-1</sup>
N	173 mg kg <sup>-1</sup>
P	7.1 mg kg <sup>-1</sup>
K	140 mg kg <sup>-1</sup>
WHC	43%

**Table.3** Biochemical characteristics of native rhizobacteria

Test	<i>Bacillus thuringiensis</i>	<i>Stenotrophomonas hibiscicola</i>	<i>Burkholderia cepacia</i>
Gram stain	+ve, bacilli	-ve, bacilli	-ve, bacilli
Methyl Red test	+	-	+
Voges Proskeur test	+	+	-
Indole production	+	+	+
HCN production	+	+	+
Glucose fermentation	+	+	-
Sucrose fermentation	+	+	+
Lactose fermentation	-	-	+
Citrate utilization	-	-	+
Indole acetic acid (IAA) production	-	+	+
Phosphate solubilization	-	+	+
Zn solubilization	-	-	+
Siderophore production	+	+	+
Ammonia production	+	+	+
ACC deaminase activity	-	+	+
Nitrogen fixation	-	-	+

**Table.4** Metal content in potting soil before and after pot culture of *C. juncea*

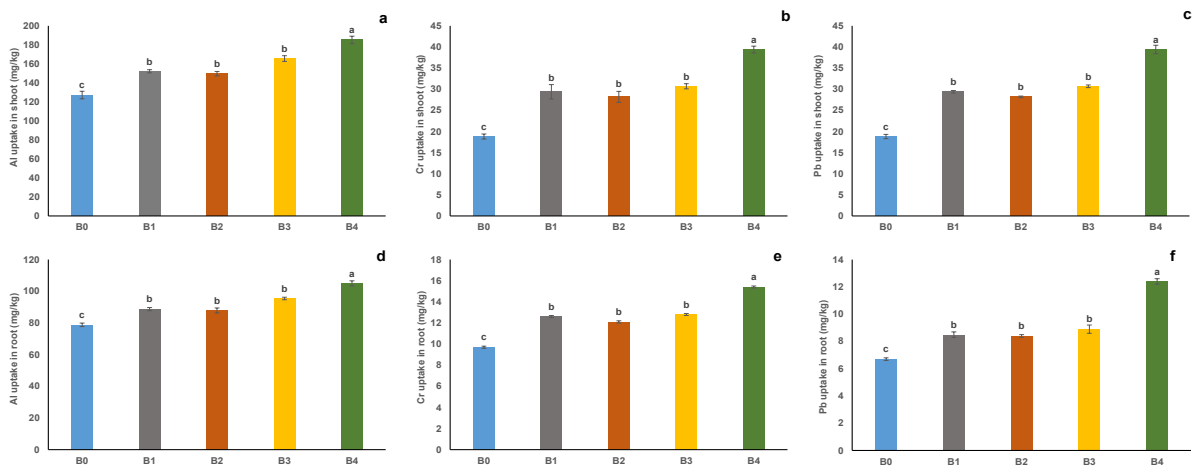
Initial soil metal content (mg kg <sup>-1</sup> )	Residual soil metal content (mg kg <sup>-1</sup> )					
	B0	B1	B2	B3	B4	
Al	2245	2039.9 ± 6.2 <sup>a</sup>	2002.5 ± 6.5 <sup>b</sup>	2006.1 ± 1.8 <sup>b</sup>	1984.6 ± 2.3 <sup>c</sup>	1958.9 ± 2.4 <sup>d</sup>
Cr	378.8	347.8 ± 4.4 <sup>a</sup>	336.4 ± 1.7 <sup>b</sup>	338.6 ± 2.8 <sup>b</sup>	334.8 ± 2.0 <sup>b</sup>	324.1 ± 2.3 <sup>c</sup>
Pb	587.5	571.8 ± 3.1 <sup>a</sup>	565.9 ± 1.7 <sup>b</sup>	568.1 ± 2.6 <sup>b</sup>	565.8 ± 1.9 <sup>b</sup>	555.3 ± 3.3 <sup>c</sup>

Al: Aluminium, Cr: Chromium, Pb: Lead. Data presented are mean ± SEM. Different alphabet in along a row represent significant difference tested through DMRT.

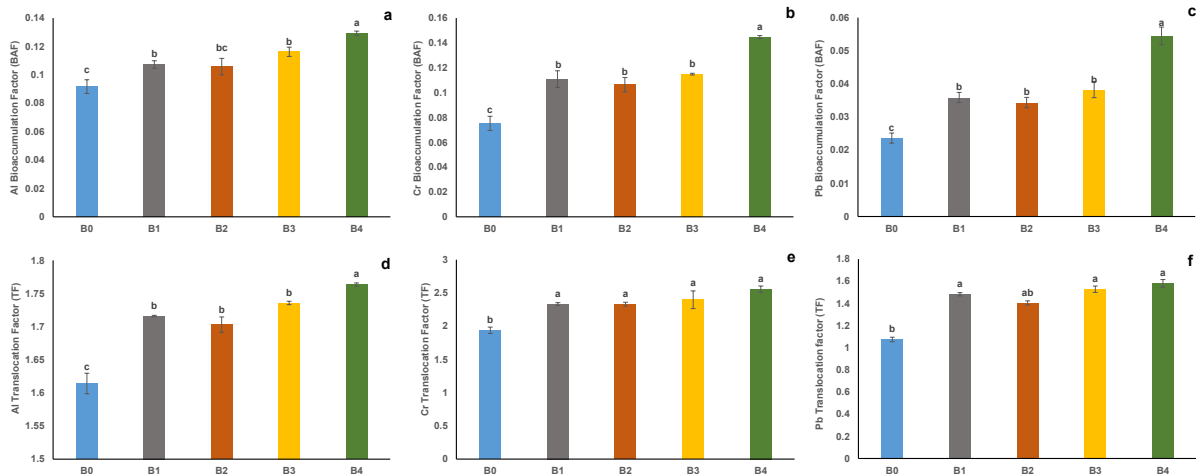
**Fig.1** Pure culture and Gram staining of bacterial inoculum



**Fig.2** Metal uptake by *C. juncea* from bauxite mine soil. (a) Al uptake in shoot, (b) Cr uptake in shoot, (c) Pb uptake in shoot, (d) Al uptake in root, (e) Cr uptake in root and (f) Pb uptake in shoot. Data presented are mean  $\pm$  SEM. Different alphabet on the bars represent significant difference tested through DMRT.



**Fig.3** Phytoremediation potential of *C. juncea*. (a) Al bioaccumulation factor, (b) Cr bioaccumulation factor, (c) Pb bioaccumulation factor, (d) Al translocation factor, (e) Cr translocation factor and (f) Pb translocation factor. Data presented are mean  $\pm$  SEM. Different alphabet on the bar represent significant difference tested through DMRT.



Bioavailability of metals can be enhanced by metal-mobilizing bacteria in soil and increase the phytoremediation efficiency of plants (Kar and Palit, 2019).

Improvement of Pb and Cd phytoremediation efficiency of tall fescue and red clover plants by *B. cepacia* inoculation (Jin *et al.*, 2013) justifies our findings.

As the PGPR consortium significantly enhanced the Cr, Pb and Al bio-accumulation in *C. juncea*, it can be efficiently used for phytoremediation and stabilization of bauxite mine soil.

In conclusion, the study outcome suggests native bacterial inoculation facilitate the metal uptake from bauxite mine soil and enhance phytoremediation potential of *C. juncea*. The native bacterial consortium containing *B. thuringensis* + *S. hibiscicola* + *B. cepacia* was most efficient to increase phytoremediation potential of *C. juncea*. Phytoremediation of toxic metals Al, Cr and Pb from bauxite mine soil decline the residual metal content in which can lower metal pollution in surrounding environment. However, successful implementation of phytoremediation requires validation through on site field study on bauxite mine tailings of Panchpatmali.

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### Author contributions

Bandana Kullu, Ankita Tripathy: Conceptualization, experimental design; Ankita Tripathy: Experimental work and data analysis; Bandana Kullu: Supervision; Bandana Kullu, Ankita Tripathy: Draft manuscript, Editing and finalization of manuscript. All authors have read and agreed to the final manuscript

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflicting Interests** The authors declare no competing interests.

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