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Characterization and bioremediation potential of heavy metal-resistant bacteria isolated from agricultural soil

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Abstract: Heavy metal pollution is a major environmental issue that has a negative impact on soil quality and food security. As a result, heavy metal removal or remediation from hazardous sites has become mandatory. Bioremediation based on microorganisms is a promising method to remediate heavy metal-contaminated areas due to its ecofriendly, cost-effective, and highly efficient characteristics. This study aimed to isolate, identify, and characterize rhizospheric bacteria able to resist, reduce, and detoxify heavy metals [chromium (Cr), nickel (Ni), and aluminum (Al)] from agricultural soil. Two isolates were chosen due to their high level of heavy metal resistance and could serve as potential in situ remediation agents at the site of isolation. On the basis of morphological, cultural, biochemical, and molecular characterization, these two isolates were identified as *Pseudomonas aeruginosa* (S1) and *Bacillus cereus* (S2). The results revealed a minimum inhibitory concentrations (MICs) of the three heavy metals studied, ranging from 1000 to 1400 µg/mL for the two bacterial isolates. Atomic absorption spectroscopy analysis was used to evaluate the degrading potential. *B. cereus* was able to reduce Cr and Al more than *P. aeruginosa* (42% and 67.78% vs. 38.44% and 58.85, respectively). On the other hand, *P. aeruginosa* showed a higher capacity to degrade Ni than *B. cereus* (62.33% and 50.76%, respectively). The findings of the analysis revealed information regarding the use of these heavy metal-resistant bacterial isolates as potential bioremediation agents in contaminated environments. Microbial bioremediation offers sustainable alternatives to the traditional physical or chemical remediation technologies of agricultural land.

Key words: *Bacillus* sp., bioaccumulation, heavy metal, microbial remediation, *Pseudomonas* sp., soil

1. Introduction

The rapid growth of industries and technological advancement have placed an increasing burden on the environment by releasing huge amounts of hazardous waste, heavy metals, metalloids, and organic contaminants, which have significantly harmed the ecosystem (Panigrahi et al., 2019). Heavy metals are the main environmental dangers that can have serious consequences for soil biodiversity conservation (Martin et al., 2021; Yan et al., 2022; Alzuaibr, 2023). Heavy metals are resistant to biological degradation and can persist in the environment for a long time. The toxicity of heavy metals has a negative impact on soil quality, agricultural production, human health, and the environment (Zampieri et al., 2016; Christophoridis et al., 2019; Boquete et al., 2022; Ekinci et al., 2023).

Metals are naturally present at different levels in the earth's crust (Pande et al., 2022; Sahin et al., 2002), whereas when it comes to soil pollution, there are two origins. The first occurs as a result of natural processes such as parent material weathering (Chen et al., 2015; Chon et al., 2017). The other is anthropogenic activity, such as fertilizer application, wastewater irrigation, mining, and chemical manufacturing (David et al., 2016; Tang et al., 2020; De Agostini et al., 2022). While natural processes gradually release metals into the environment, anthropogenic activities have drastically accelerated their accumulation in soils. As a result, heavy metals introduced into soils,

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as well as their mobility and interactions in the soil, affect soil quality, impede plant development and productivity, resulting in reduced crop yield, and low-quality products. These latter can directly affect human health via the spread of harmful elements through the food chain (Kumar et al., 2016; Saeid et al., 2021; Wang et al., 2021).

Among the heavy metals, Nickel (Ni) is considered essential for plant development (Yusuf et al., 2011); however, excess Ni disturbs the growth of the roots and aerial parts of plants and can also cause chlorosis and necrosis and inhibit CO_2 fixation (Dohnalova et al., 2017). It can cause health troubles in humans such as heart problems and respiratory disorders. Chromium (Cr) is nonessential and toxic to plants, even at low concentrations (Pichhode and Nikhil, 2016). The consumption of contaminated foods is related with health risks in animals. Cr is associated with arterial system complications and the overproduction of red blood cells (Khan et al., 2013). Aluminum (Al) is not known to have any important role in plant metabolism, even though it is the most abundant metallic element in the earth crust. Al is recognized to inhibit root growth, which in turn, renders them inadequate in absorbing nutrients and water. In addition, it might reduce microbial activities (Singh et al., 2017).

Soil microbial biomass plays important roles in soil ecosystems by cycling nutrients, maintaining biogeochemical cycles, and increasing plant productivity (Egamberdieva et al., 2023). Microorganisms have a strong interaction with their immediate environment and can survive in metal-polluted areas using various strategies and mechanisms that regulate metal ion accumulation to avoid heavy metal toxicity (Castro et al., 2019). Indeed, plant growth-promoting (PGP) bacteria have evolved several strategies to cope with occasional or frequent exposure to toxic organics chemicals, heavy metals, and other environmental stresses (Mandal et al., 2021). These mechanisms include extracellular precipitation uptake and accumulation, exclusion by the permeability barrier, efflux pumps, active transport, and enzymatic oxidation or reduction to a non-toxic form (Mustapha and Halimoon, 2015; Johnson et al., 2019). In this context, heavy metal-tolerant bacterial isolates are applied in the bioremediation strategies of heavy metal-polluted environments, resulting in the increase of the production of agricultural crops with low input. Microorganisms can decontaminate metals by volatilization, valence transformation or extracellular chemical precipitation. Microbial cells can convert metals from one oxidation state to another, hence dropping their toxicity (Jyoti and Harsch, 2017). Secretions from microbial metabolic activities can dissolve heavy metals and soil particles holding heavy metals. Precipitation, biosorption, and enzymatic transformation are the activities used by microorganisms to destroy, decontaminate, or convert heavy metals to more stable less mobile or inert forms. In addition, biosorption is one of the best alternatives for chemical precipitation and microorganisms play a very important role in carrying out this process.

The goal of the present study was to isolate, identify, and characterize naturally occurring bacteria able to bioremediate agricultural soils contaminated with different heavy metals and thus act as PGP rhizobacteria (PGPR).

2. Materials and methods

2.1. Study region and soil samples

Samples were taken from rhizospheric soils of wheat and barley from four agricultural fields (Soils 1 to 4) located in Mascara in the western region of Algeria. The sites are located between coordinates 35°14′–35°16′ N and 0°10′–0°09′ E (Figure 1). Mascara has a temperate Mediterranean climate with hot and dry summers. The annual average temperature in Mascara is 17.2 °C and the precipitation is 393.2 mm.

The root system and bulk soil were removed to a depth of approximately 20 cm in triplicate. The rhizosphere soil was collected, packed in sterilized polyethylene bags, and transferred to the laboratory. The four random soil samples collected in each agricultural field were stored at 4 °C until investigation.

2.2. Physicochemical analysis of the soil contamination

Soil pH was determined using a glass combination electrode with a soil:water ratio of 1:2.5. The physiochemical parameters of the soil, such as temperature, electrical conductivity, organic carbon, total nitrogen, and available phosphorus, were analyzed for the four collected soil samples (Fan et al., 2018).

2.3. Isolation of bacterial strains

For isolation and enumeration of metal-tolerant bacteria, duplicate composite soil samples (1 g) were suspended in 90 mL of sterile saline solution (0.9% NaCl) in a 250-mL conical flask and mixed carefully on a magnetic agitator at 150 rpm for 5 min. Standard serial dilutions (10^{-1} to 10^{-6}) were prepared by adding 1 mL of the suspension to test tubes containing 9 mL of sterile saline solution. Then, 100 µL of each dilution was spread on the surface of nutrient agar (NA) medium and incubated at 37 °C for 48 h. Bacterial colonies that varied in shape and color were selected and purified using the streaking method on the NA medium.

2.4. Screening of the heavy metal-resistant bacteria

The isolated bacterial strains were selected for resistance to heavy metals on Luria Bertani (LB) medium using the agar dilution method (Mergeay et al., 1985) with slight modifications. Bacterial isolates were inoculated individually into 10 mL of sterile LB broth supplemented with 0.5 mM of each heavy metal [Cr(VI), Al(III), and Ni(II)]. The cultures were then incubated for 48 h at 37 °C.

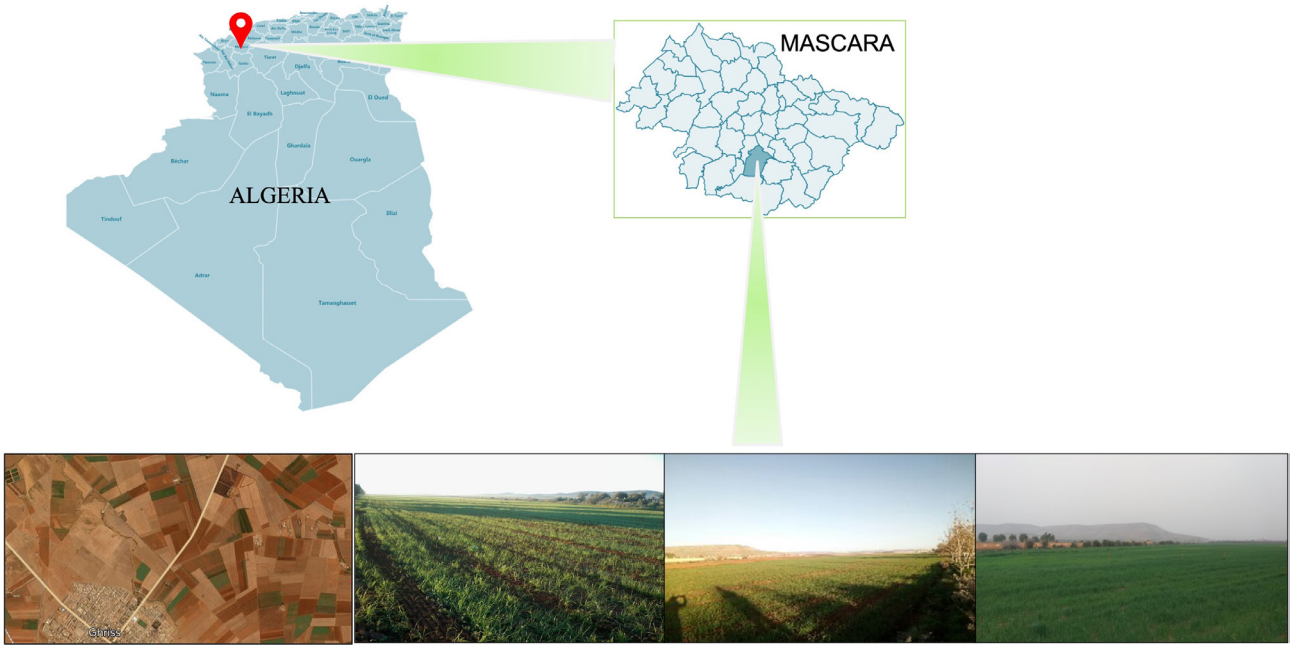

Figure 1. Map of Algeria indicating the location of the prospected site.

Tolerance was assessed based on the growth ability around the discs or the growth inhibition with halos greater than 2 mm (Vélez et al., 2021). As a negative control, the medium added with the solution without inoculation was used.

2.5. Phenotypic and molecular identification of the selected heavy metal-resistant bacteria

Heavy metal-resistant bacteria were identified based on cultural and morphological characteristics including the colony color, shape, gram staining, and motility test. Biochemical characteristics such as catalase, oxidase, nitrate reduction, glucose and lactose degradation, gas production, and hydrogen sulfide (H_2S) production were determined on Kligler iron agar based on Bergey's Manual of Determinative Bacteriology (Holt, 1994).

The genomic DNA was extracted from the bacterial isolates using the NucleoSpin Soil Kit (Macherey-Nagel Inc., Allentown, PA, USA) following the manufacturer's instructions. The quantity and purity of the DNA extracts were checked using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Amplification of the 1500 bp fragment of the 16S rRNA gene was assessed by polymerase chain reaction (PCR) using the universal bacterial primers for 16S rRNA 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Janssen, 2006).

The conditions for thermal cycling were as follows: initial denaturation at 96 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min 30 s, and a final extension at 72 °C for 5 min. The PCR products were run in gel electrophoresis using 1% agarose gel 1 × TE buffer for 45 min at 100 V. The 16S rRNA PCR products were purified using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, Wisconsin, USA) and sequenced using an ABI-PRISM 3700 DNA automated sequencer (Applied Biosystems, San Francisco, CA, USA).

To determine the identity of the sequences, they were initially edited with 4Peaks v1.8 (Nucleobytes B.V., Aalsmeer, Netherlands), and later submitted to BLASTN (National Center for Biotechnology Information; http://blast.ncbi.nlm.nih.gov/Blast.cgi), comparing them with sequences published on GenBank, according to identity ranking (>97%) and E-values (0.0). In addition, maximum likelihood clustering analysis was developed, using as the ingroup several 16S rDNA sequences of nominal species matching our isolates within a percent sequence similarity threshold of 97% (Nguyen et al., 2016). The nucleotide sequences generated during this study were deposited in the GenBank database.

2.6. Determination of the minimum inhibitory concentration (MIC)

The MIC corresponded to the concentration with no visible growth on the corresponding agar-plates after 48 h of incubation (Marzan et al., 2017; Nokman et al., 2019). The MICs of the heavy metals (Cr^{6+}, Al^{3+}, and Ni^{2+}) in varying concentrations (100 to 1800 µg/mL) against each isolate were determined via spot inoculation of 100 mL of 106 cells/mL bacterial suspension on LB agar plate amended with respective heavy metal salt. The MICs reported in the present work were the lowest concentrations of the metals that inhibited bacterial growth after an incubation of 48 h at 37 °C.

2.7. Heavy metal bioaccumulation assay

Bacterial isolates were cultured in a shake flask containing LB broth medium (pH = 7) in a rotary shaker at 150 rpm at 37 °C. After reaching an optical density at 600 nm, 100 µg/mL of sterilized metal salts (Cr^{6+}, Al^{3+}, and Ni^{2+}) was added separately to each culture flask, which were incubated for 7 days under the same conditions (Oziegbe et al., 2021). The residual concentration of each heavy metal was determined after centrifuging 10 mL of each bacterial culture at 6000 rpm for 10 min and analyzing the supernatant from each sample using atomic absorption spectrometry (AAS) (Dwivedi et al., 2012). The result was compared to the control to determine the percentage of heavy metal loss and bioaccumulation factor (%), which was calculated using the formula of Kaczorek (2012):

$$R\ (\%) = \frac{(Co - Cf)}{Co}\ x\ 100$$

Here, R is the remediation percentage (%), C_O is the initial concentration of heavy metal used (µg/mL), and C_f is the residual heavy metal concentration (µg/mL).

3. Results

3.1. Soil physicochemical parameters

Results of the physicochemical analyses of the four soil samples are presented in Table 1. Soil pH and temperature were equivalent, while the electrical conductivity, percentage of total organic carbon, percentage of total nitrogen, and available phosphorus varied among the samples.

3.2. Identification of the selected heavy metal-resistant bacteria

A total of 222 single bacterial colonies with different visible characteristics and colony forms were isolated from the soil samples. After primary screening of the bacterial colonies on LB medium supplemented with the heavy metals at a concentration of 100 µg/mL, 185 bacterial strains showed resistance to the tested heavy metals (Cr, Ni, and Al). Two isolates (S1 and S2) that varied in shape and pigment were selected for further studies.

Isolates S1 and S2 were characterized based on their cultural, morphological, and biochemical characteristics. The results showed that isolate S1 was related to members of *Pseudomonas* sp., while isolate S2 was related to *Bacillus* sp. (Figure 2, Table 2).

For bacterial identification, the 16S rRNA gene fragment of 1500 bp was amplified using universal primers, as shown in Figure 3. Sequences from isolates S1 and S2 were processed and assembled to generate the consensus sequences, which were aligned using MEGA11 software and blasted in the NCBI GenBank database to compare the raw generated sequence. Isolate S1 had nucleotide sequences with percentage identities of more than 97% to *Pseudomonas aeruginosa* from GeneBank, while isolate S2 had an identity rate of more than 97% with *Bacillus cereus* (Figure 4). The two accession numbers were OR827712 and OR827713, respectively.

3.3. Assessment of the MIC against heavy metals

On solid media, the MICs of isolates S1 and S2 for each heavy metal were determined, ranging from 100 to 1800 µg/mL. Isolate S1 exhibited significant resistance to high concentrations of Cr, Ni, and Al; however, it was more tolerant to Al and Ni than Cr, with MICs against Cr, Ni, and Al reaching 1200, 1400, and 1400 µg/mL, respectively. Isolate S2 showed significantly less activity than isolate S1. The MICs of Cr and Ni were the same with a value of 1000 µg/mL, while the MIC of Al for isolate S2 was 1200 µg/mL (Table 3).

3.4. Bioremediation of heavy metals

The treated samples were tested using AAS and compared to the controls to determine the total heavy metal bioaccumulation ability. The results revealed that isolate S2 was more efficient at removing Cr and Al, with a bioaccumulation rate ranging from 42% and 67.78%, respectively, while isolate S1 showed a bioaccumulation rate of 38.44% and 58.85%, respectively (Figure 5). Conversely, isolate S1 had a higher capacity to remove Ni with an estimated rate of 62.33%, while isolate S2 exhibited a level of 50.76% (Figure 5).

Table 1. Physicochemical characteristics of soil at the four different sites (mean ± SE).

Parameters	Soil 1	Soil 2	Soil 3	Soil 4
pH	7.68 ± 0.05	7.45 ± 0.09	7.14 ± 0.06	7.28 ± 0.06
Temperature (°C)	39	41	42	41
Electrical conductivity (µs/cm)	0.16	0.17	0.11	0.08
Total organic carbon (%)	0.77 ± 0.16	0.82 ± 0.11	0.51 ± 0.21	0.59 ± 0.09
Total nitrogen (%)	0.35 ± 0.01	0.18 ± 0.00	0.12 ± 0.00	0.16 ± 0.02
Available phosphorus (mg/kg)	10.50 ± 0.09	9.01 ± 0.05	10.04 ± 0.07	6.99 ± 0.1

SE: Standard error

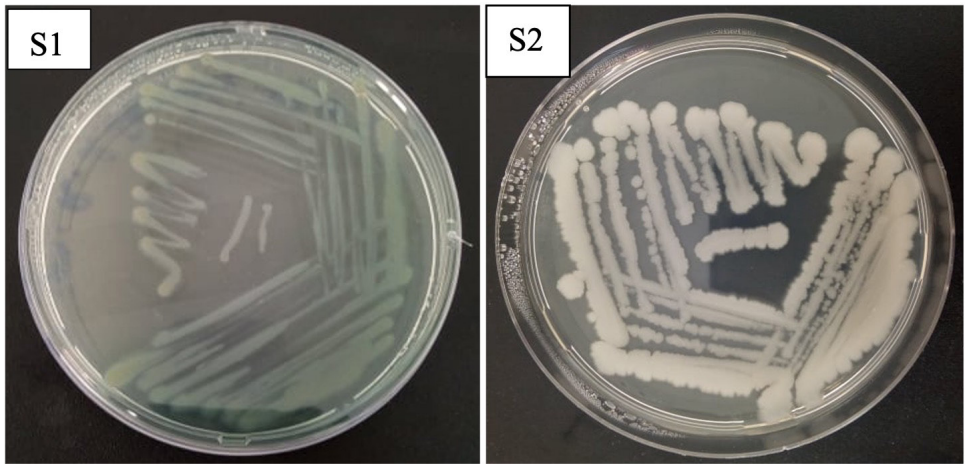

Figure 2. Macromorphological appearance of bacterial isolates S1 and S2 in the NA medium.

Table 2. Morphological and biochemical characteristics of the bacterial heavy metal-resistant isolates S1 and S2.

	Isolates	
	S1	**S2**
Morphological characteristics		
Colony color	Green	White
Cell shape	Rod	Rod
Gram staining	-	+
Motility test	+	+
Biochemical characteristics		
Oxidase	+	-
Catalase	+	+
Nitrate	+	+
Citrate	+	+
Glucose	+	+
Lactose	-	-
Gas production	-	+
H_2S	-	-
Bacteria provisionally identified		
	Pseudomonas sp.	*Bacillus* sp.

4. Discussion

Agricultural lands where activities such as the exceptional use of agrochemicals and long-term application of urban sewage sludge, waste incineration, industrial waste disposal, and vehicle exhausts have resulted in heavy metal accumulation, which is furthered by absorption and accumulation by plants and finally, ingestion by humans via the food chain (Marin and Rusanescu, 2023). Looking at the consequences of heavy metals on different components of the food chain highlighted the quest for cost-effective, durable, and ecological friendly solutions for their clean-up. In the recent past, numerous biological means have been considered, in which microbes have emerged as essential alternatives in terms of efficiency and performance (Mitra et al., 2022). This study highlighted the isolation and characterization of heavy metal-tolerant bacteria from agricultural polluted sites and further screened for their tolerance against three heavy metals that are frequently encountered in the surroundings.

The soil is a resource that is not renewable and supports ecosystems and human life by providing a habitat for the majority of species on earth and acts as a food production medium (Hou et al., 2020). Industry expansion and heavy metal accumulation in the soil damage human health and the environment (Riseh et al., 2023). Microorganisms are abundant in agricultural soils and can be naturally resistant to heavy metals, allowing them to persist under heavy metal stress conditions and contribute to metal removal from the

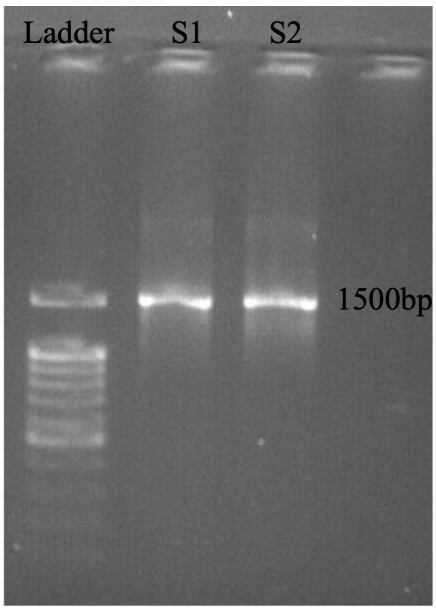

Figure 3. Bands for the 16S rRNA gene fragment for isolates S1 and S2.

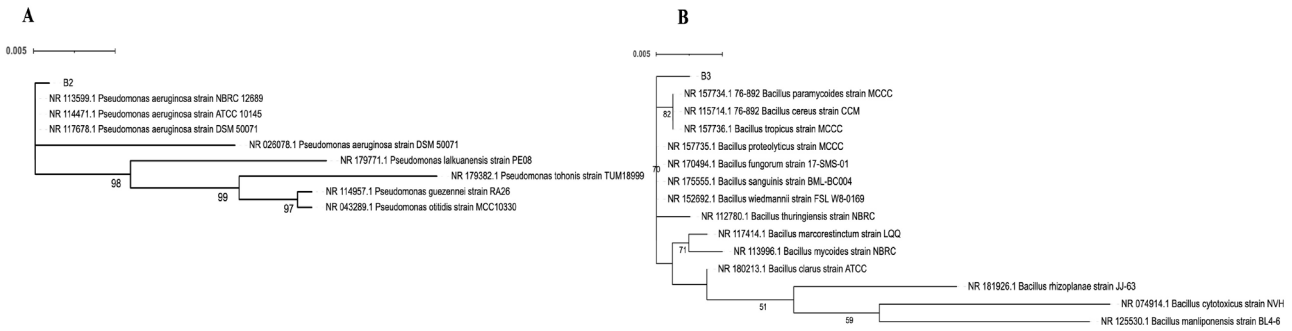

Figure 4. Phylogenetic tree of B2 and B3 at the bootstrap test of 1000 replicates using MEGA 11 software.

Table 3. Comparative MIC values of heavy metals obtained by strains S1 and S2.

Metal concentration (µg/mL)	Strain S1			Strain S2		
	Cr	Ni	Al	Cr	Ni	Al
200	++	++	++	++	++	++
400	++	++	++	++	++	++
600	++	++	++	++	++	++
800	++	++	++	++	++	++
1000	++	++	++	+	+	++
1200	+	+	++	-	-	+
1400	-	+	+	-	-	-
1600	-	-	-	-	-	-
1800	-	-	-	-	-	-
MIC (µg/mL)	1200	1400	1400	1000	1000	1200

- Absent; + Moderate; ++High growth

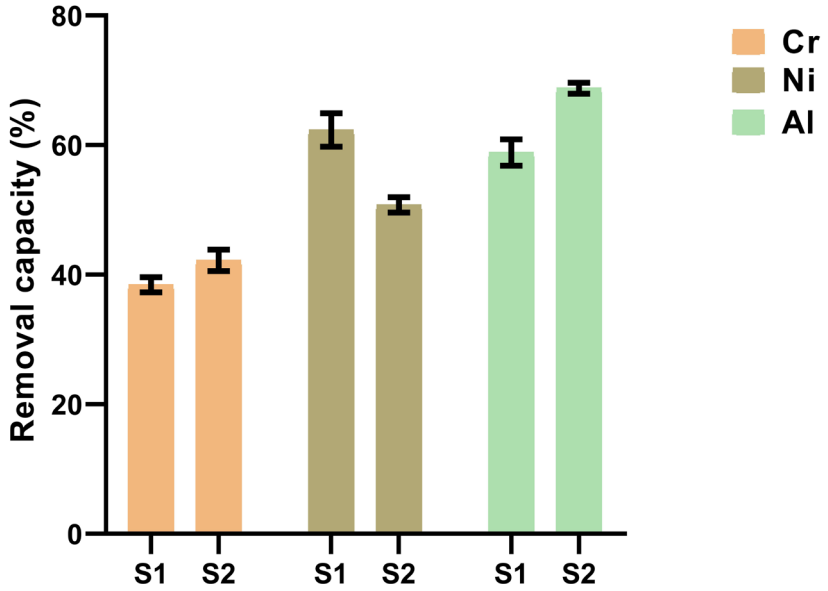

Figure 5. Heavy metal removal capacity by isolates S1 and S2.

environment (Mohan et al., 2022). This may occur due to improved or balanced plant growth under heavy metal stress, or by elevating the bioavailability of metals for easy uptake by microbial cells and plants. The physiological requirement of the biological systems to survive the toxicity of heavy metals is enhanced by balancing the resistance mechanism and the normal cellular metal metabolism, which allows the cell to accumulate metal for the conservation of metal-dependent activities while reducing excess metal concentrations.

Based on the morphological, biochemical, and molecular identification, two isolates were identified as *P. aeruginosa* (S1) and *B. cereus* (S2). *P. aeruginosa* is a gram-negative *Enterobacteriaceae* bacterium (Nguyen et al., 2016), which is the most prominent microorganism in the environment as well as in clinical settings such as hospitals. *Bacillus* spp. belong to the phylum Firmicutes. They are gram-positive, rod-shaped bacteria, and spore-forming in nature. They are commonly categorized as soil microorganisms that can be aerobic or anaerobic but can also be found in several sources such as air, water, food, and the human gut (Alotaibi et al., 2021).

The order of the MICs for isolate S1 was Ni > Al ≥ Cr, while the order for isolate S2 was Al > Ni ≥ Cr. More precisely, the results revealed that *P. aeruginosa* was characterized with MICs against Cr and Al at 1200 and Ni at 1400 µg/mL. On the other hand, *B. cereus* was found to have MICs against Cr, Ni, and Al reaching 1000, 1000, and 1200 µg/mL, respectively. Such findings are in agreement with previous works. As an example, Nayak et al. (2018) reported the tolerance of *Bacillus* sp. to 1500 mg/L of Cr, while Abdul Hussain and Al Saadi (2021) attested that *Pseudomonas* sp. and *Bacillus* sp. strains tolerated Cr at 1500 mg/L. Regarding Al, *Pseudomonas* sp. and *Bacillus* sp. isolates remain active at concentrations greater than 1000 µg/mL. In this context, Purwanti et al. (2019) demonstrated the ability of *Pseudomonas* spp. to tolerate Al concentrations up to 500 µg/mL, while Dhanarani et al. (2016) demonstrated *Bacillus* spp. tolerance to 100 mg/L of Al. Finally, *P. aeruginosa* BC15 showed resistance against 700 mg/L of Ni (Raja et al., 2006). The tolerance to numerous heavy metals by *P. aeruginosa* has been related with diverse mechanisms such as membrane protein pumps that are encoded by either genomic DNA or bacteria plasmid, which passes in and out of metals through the cell membrane by active or passive mechanisms. This can be completed through resistance-nodulation-cell family transporters as well as by exopolysaccharides, as reported in many gram-negative bacteria (Kang and Gross, 2005). These bacteria have been reported as one of the bioremediation agents for cyclic and aromatic hydrocarbons in contaminated areas. In addition, *Bacillus* spp. are considerably investigated for their role in the mitigation of heavy metals from polluted environments via biosorption and bioaccumulation, among numerous other methods, because contaminated sites are frequently dominated by gram-positive bacteria, owing to their versatile metabolic properties and better biosorption abilities (Alotaibi et al., 2021).

According to Ahmed et al. (2005), bacteria may adapt to high quantities of heavy metals in their environment by developing multiple resistance mechanisms. These methods could prove useful in removing heavy metals from contaminated locations. Bioaccumulation studies

have shown that *Bacillus* sp. have the capability to bioaccumulate Cr and Al, at a rate of 42% and 67.78%, respectively, whereas *Pseudomonas* sp. has a lower bioaccumulation capacity of 38.44% and 58.85%, respectively. *Pseudomonas* sp. and *Bacillus* sp. removed Ni at a rate of 62.33% and 50.76%, respectively, attesting that *Pseudomonas* sp. was more efficient. Research conducted by Purwanti et al. (2019) showed the potential of Al removal by *Pseudomonas* spp. up to 45.04% from an initial concentration of 100 mg/L and Dhanarani et al. (2016) discovered that *Bacillus* spp. presented a maximum biosorption of Al of 79 mg/L at optimum temperature. According to Rajkumar et al. (2005), *Pseudomonas* spp. was able to remove more than 87% of Cr(VI) at an initial concentration of 200 mg/L, while several studies have shown that *Bacillus* sp. could reduce Cr(VI) by 80% at a concentration of 40 µg/mL (Elangovan et al., 2006) and 93% at a starting concentration of 64 mg/L (Wróbel et al., 2023). High Cr(VI) concentrations have a deleterious effect on microbial growth, primarily due to oxidative stress and DNA and protein damage in bacterial cells (Nayak et al., 2018). However, several investigations have indicated that the two primary physiological mechanisms for removing Cr(VI) were extracellular reduction (75% removal rate) and adsorption by the cell wall (24% removal rate) (Pang et al., 2022). Regarding Ni, Naskar et al. (2020) discovered that *B. cereus* M16 can absorb up to 80% of Ni(II) in aqueous solution. In a study by Raja et al. (2006), they observed that *P. aeruginosa* had a biosorption capacity of 93% with Ni at a starting concentration of 100 mg/L. The rate of bioremediation should be strongly dependent on the cell population and their intrinsic resistance mechanisms, which eventually permit metal absorption, transit, and efflux in and out of the cell (Guo et al., 2010). Overall, the results herein confirmed that in bacterial uptake and tolerance to heavy metals, *P. aeruginosa* and *B. cereus* recorded potential roles in the bioremediation of different heavy metals with great removal rates compared with other bacterial strains.

5. Conclusion

P. aeruginosa and *B. cereus* rhizospheric bacterial isolates collected from an agricultural area of Mascara in the western region of Algeria were resistant to high concentrations of heavy metals (Cr, Ni, and Al) and enclosed considerable biotechnological potential for the removal of these metals. Microorganisms in metal-polluted soils develop various strategies to resist heavy metal stress. These identified metal-tolerant microorganisms, which also promote plant growth, provide a foundation for their use as effective bioremediation agents. These metal-resistant, PGP bacteria can be developed into bioformulations for remediating and utilizing metal-contaminated soils.

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Conflict of interest

The authors have no relevant financial or nonfinancial interests to disclose.

Author contributions

Material preparation, data collection, and analysis were performed by KHEIRA DAHNOUN. Software was assessed by BADIAA ESSGHAIER, CHAHNEZ NACCACHE, and NADIA ZITOUNA. The first draft of the manuscript was written by KHEIRA DAHNOUN, HEDIA BOURGUIBA, and İSMAIL BAYYİĞİT. All authors have read and approved the final manuscript.

Data availability statement

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

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