

2-24-2026

## Characterization and Identification of Some Indigenous Bacterial Strains for Heavy Metal Accumulation from Contaminated Soils

Aziza Usmonkulova

*Renaissance Educational University, Scientific Research Institute of the Plant Protection and Quarantine; AND Kimyo International University in Tashkent, Branch Samarkand, usmonkulova.aziza@mail.ru*

Gulchekhra Kadirova

*Institute of Microbiology of Academy Sciences of the Republic of Uzbekistan, kadirovagul@mail.ru*

Nosir Shukurov

*Institute of Geology and Geophysics named after Kh.M.Abdullaev, University of Geological Sciences, Tashkent, Uzbekistan, nosirsh@gmail.com*

Tulkin Shonakhunov

*Institute of Geology and Geophysics named after Kh.M.Abdullaev, University of Geological Sciences, Tashkent, Uzbekistan, brus\_li89@mail.ru*

Samatov Nuriddin

*Research Institute of Environment and Nature Conservation Technologies, nuriddin.samatov@ecoilm.uz*

Follow this and additional works at: <https://bsj.uobaghdad.edu.iq/home>

---

### How to Cite this Article

Usmonkulova, Aziza; Kadirova, Gulchekhra; Shukurov, Nosir; Shonakhunov, Tulkin; and Nuriddin, Samatov (2026) "Characterization and Identification of Some Indigenous Bacterial Strains for Heavy Metal Accumulation from Contaminated Soils," *Baghdad Science Journal*: Vol. 23: Iss. 2, Article 17.  
DOI: <https://doi.org/10.21123/2411-7986.5209>

This Article is brought to you for free and open access by Baghdad Science Journal. It has been accepted for inclusion in Baghdad Science Journal by an authorized editor of Baghdad Science Journal.



## RESEARCH ARTICLE

# Characterization and Identification of Some Indigenous Bacterial Strains for Heavy Metal Accumulation from Contaminated Soils

Aziza Usmonkulova<sup>1,2,3,\*</sup>, Gulchekhra Kadirova<sup>4</sup>, Nosir Shukurov<sup>5,\*</sup>,  
Tulkin Shonakhunov<sup>5,\*</sup>, Samatov Nuriddin<sup>6</sup>

<sup>1</sup> Renaissance Educational University

<sup>2</sup> Scientific Research Institute of the Plant Protection and Quarantine

<sup>3</sup> Kimyo International University in Tashkent, Branch Samarkand

<sup>4</sup> Institute of Microbiology of Academy Sciences of the Republic of Uzbekistan

<sup>5</sup> Institute of Geology and Geophysics named after Kh.M.Abdullaev, University of Geological Sciences, Tashkent, Uzbekistan

<sup>6</sup> Research Institute of Environment and Nature Conservation Technologies

## ABSTRACT

Numerous studies have reported elevated quantities of cadmium and nickel in soil, resulting in reduced plant growth, particularly in biomass, chlorophyll content, and photosynthetic traits due to their hazardous nature. Therefore, it is crucial to enhance plant resistance to heavy metal stress and reduce the toxicity of cadmium and nickel. In this study, several bacterial strains were isolated from heavy metal-contaminated soil to investigate their potential role in facilitating detrimental consequences of heavy metal stress. The capacity of the isolates to increase plant production under heavy metal stress and minimal inhibitory concentration was evaluated after the isolates were identified at the species level. Classic microbiological and molecular-genetic identification revealed that isolates #5, #18, and #11 were classified as *Enterobacter cloacae* Uz\_5, *Pseudomonas aeruginosa* 18, and *Enterobacter ludwigii* 11Uz, respectively. *P. aeruginosa* 18 produced auxin in 4, 4.8, and 5.3 times greater than the control. In contrast, *E. ludwigii* 11Uz, *Bacillus licheniformis* 10, and *Bacillus simplex* 8 produced auxin equivalent to the control at various Cd cation amounts (2.4, 4.1, and 8.2 mg/l). During the 14<sup>th</sup> day of growth, at a Ni concentration of 191.4 mg/l, *Enterobacter ludwigii* 11 produced 81 mg/l of EPS, 1.2 times higher than the control. *Bacillus atrophaeus* 4 and *Enterobacter ludwigii* 11Uz produced 25-28 mg/l and 6.12 mg/l of EPS on days 7 and 14 of culture, respectively, at Cd<sup>2+</sup> cation concentrations of 8.2 and 24.6 mg/l. These microorganisms demonstrate promising potential for bioremediation of heavy metal-polluted soils by reducing Ni<sup>2+</sup> and Cd<sup>2+</sup> toxicity and increasing the production of phytohormones and exopolysaccharides under heavy metal stress conditions.

**Keywords:** Cadmium, Exopolysaccharide, Minimal Inhibitory Concentration, Nickel, Phytohormone

## Introduction

Anthropogenic activities, technological advances, and mining activities have led to global heavy metal (HM) pollution, with cadmium (Cd) and nickel (Ni) cations becoming the predominant pollutants<sup>1</sup>. The World Health Organization (WHO) has reported nat-

ural nickel concentrations in agricultural soil ranging from 15 to 30 mg/kg and cadmium from 0.01 to 0.7 mg/kg<sup>1,2</sup>. Elevated levels of heavy metals can harm photosystems, affect nitrogen metabolism, and induce plant necrosis, chlorosis, and senescence<sup>3,4</sup>. Cd and Ni stress can also change chloroplast morphology by inhibiting chlorophyll production<sup>5</sup>. The toxic

Received 31 January 2024; revised 10 January 2025; accepted 12 January 2025.  
Available online 24 February 2026

\* Corresponding authors.

E-mail addresses: [usmonkulova.aziza@mail.ru](mailto:usmonkulova.aziza@mail.ru) (A. Usmonkulova), [kadirovagul@mail.ru](mailto:kadirovagul@mail.ru) (G. Kadirova), [nosirsh@gmail.com](mailto:nosirsh@gmail.com) (N. Shukurov), [brus\\_li89@mail.ru](mailto:brus_li89@mail.ru) (T. Shonakhunov), [nuriddin.samatov@ecoilm.uz](mailto:nuriddin.samatov@ecoilm.uz) (S. Nuriddin).

<https://doi.org/10.21123/2411-7986.5209>

2411-7986/© 2026 The Author(s). Published by College of Science for Women, University of Baghdad. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

effects of Cd and Ni on plants often suppress iron, manganese, copper, and zinc utilization, resulting in nutrient deficiency<sup>6</sup>. To mitigate oxidative damage caused by these metals, it is crucial to create efficient control solutions with antioxidant defense<sup>7</sup>. Plants rely on beneficial soil microbes to support their growth, including those that facilitate biological nitrogen fixation, organic acid production, gate hormones, phytohormones, and aminocyclopropane-1-carboxylate deaminase activity<sup>8–10</sup>. Under heavy metal stress, the biochemical and physiological mechanisms of soil bacteria can be used to improve plant development and nutrient absorption while also increasing plant hormone synthesis and exopolysaccharide production, suggesting potential future applications<sup>11</sup>. Exopolysaccharide (EPS) production is the primary mechanism bacteria use to remove inorganic contaminants and reduce stress conditions. Composite and soluble EPS are the two types of EPS produced. Although bound EPS is strongly associated with bacterial cells, soluble EPS has been detected in the aqueous phase. The different types of EPS include microbial membranes, encapsulating polymers, soluble macromolecules, colloidal layers, and mucus. EPS bacteria typically contain polysaccharides, lipids, proteins, and nucleic acids. The unique chemistry of polystyrene helps reduce environmental stress conditions<sup>12</sup>.

This study is aimed to isolate and identify bacteria from soil with high tolerance for heavy metal accumulation, determine their minimal inhibitory concentrations, and investigate how these bacteria produce phytohormones and exopolysaccharides that promote plant growth, development, and adaptation to stressors.

## Materials and methods

### *Exclusion of heavy metal-resistant bacterial isolates*

A prior investigation assessed the resilience and viability of more than 50 newly isolated strains from heavy metal-contaminated soils in the Samarkand and Kashkadarya regions to diverse HMs cations. The resistant new bacterial strains to HMs were gauged on a peptone agar medium (PAM) fortified with various amounts of Ni and Cd salts. Different concentrations of HM were used in the PAM to grow the bacterial isolates. Supplementary heavy metal salts (CdCl<sub>2</sub> and NiSO<sub>4</sub> × 7H<sub>2</sub>O) were incorporated at concentrations of 4.1 mg/l, 8.2mg/l, and 24.6 mg/l and 95.7 mg/l, 191.4 mg/l and 574.2 mg/l. Different concentrations of these salts are 5, 10, and 30 times higher than the allowable quantity of elements Ni and Cd in the soils of Uzbekistan. Allowable quantity of element Cd in

soil is 0.5 mg/kg, and for element Ni is 4 mg/kg. Therefore, varying quantities of salts containing Ni and Cd were used. A control medium that lacked metal ions was used. The strains were cultured for two days at 28°C, and the titers were compared with the control to evaluate the efficacy of cultivation.

### *Identification methods of heavy metal-resistant isolates*

Bacteria isolates with strong resistance to nickel and cadmium were identified by observing their morphological-biochemical features first (using the Biochemical Reagent Kit from Hi-Media). Through inoculation in a complicated Gissa medium, the isolates' rate of carbohydrate hydrolysis was ascertained. During the fermentation of one of the carbohydrates, acidification of the medium occurs with a change in the color of the medium from blue-green to yellow-green or yellow, as well as the formation of gas in the form of bubbles on its surface. The medium's color and gas production won't be seen if the microbes fail to hydrolyze the appropriate carbohydrates as they grow. According to the Marmur approach, the DNA of certain bacteria was extracted. Polymerase Chain Reaction (PCR) was performed on isolated DNA using universal primers 27 F and 1492R for the 16S rRNA gene<sup>13</sup>. To ascertain the degree of similarity between various bacteria, a phylogenetic tree was drawn using the 16S rRNA gene sequence accessible in the NCBI database.

### *Bacterial minimal inhibitory concentrations (MIC) determination*

Nutrient-reduced peptone agar medium supplemented with 1-7 mM concentrations of NiSO<sub>4</sub> × 7H<sub>2</sub>O and CdCl<sub>2</sub> was used to determine the MIC of bacteria in relation to Ni and Cd. A part of a known culture colony growing on the agar surface was taken, and this culture was placed in a microtube filled with 1 ml of sterile water. The bacterial suspensions were then cultivated in a nutrient medium with heavy metal concentrations of 1 to 7 mM, and the control media did not add HM. Petri plates covered with parafilm were incubated at 30°C. After being monitored for 24, 48, and 72 hours, the bacterial growth was contrasted with the control group<sup>14</sup>.

### *Analysis of auxin and gibberellin production at various HM ion concentrations*

The study utilized peptone broth with varying concentrations of heavy metals (NiSO<sub>4</sub> × 10H<sub>2</sub>O and CdCl<sub>2</sub>) ranging from 57.3 mg/l to 191.4 mg/l and

from 2.44 mg/l to 8.2 mg/l, and bacterial cultures were incubated for 3-7 days and 14 days. To prepare Salkowski's reagent, the biomass cultures were centrifuged for 30 minutes at 6000 rpm, and the reagent was made with 50 ml of 35% HClO<sub>4</sub> and 1 ml from 0.5 M FeCl<sub>3</sub>. The presence of a pink tint suggested the formation of indole-3-acetic acid (IAA), which was measured at 530 nm using a spectrophotometer after 30 minutes. Calibration curves were constructed with standard IAA values (Sigma) to estimate IAA concentrations using methods described by Ahmad and Usmonkulova<sup>15</sup>. The study also employed a spectroscopic method developed by Berrios et al. (2014) to measure gibberellin biosynthesis in bacteria after 3, 7, and 14 days.

#### Observing the ability of bacteria to synthesize exopolysaccharide

After 3, 7, and 9 days of growing, the strain was harvested from the appropriate medium by centrifuging for 30 minutes at 6000 rpm. The biomass was separated from the broth, and the remaining broth was precipitated by adding ethanol. The residue was kept at 4 °C for 24 hours and then collected and dried in a dry box by evaporating the residual ethanol. Ultimately, the weight of dried biomass was determined and compared with the control group, as reported by Sardari<sup>16</sup>.

#### Statistical analysis

The experiments were carried out in triplicate, and the mean and standard deviation (S.D.) were used to express the results. A significance level of  $P < 0.05$  was considered statistically significant. The collected data were analyzed using ANOVA, and a t-test was performed to obtain the t-values, P-values, and originality interval. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) (version 12.0, SPSS Inc., Chicago, IL).

## Results and discussion

### Isolation of bacteria with a high level of metal resistance

The concentration of heavy metals and their effect on bacterial growth were determined by counting the number of colonies grown on an agar surface. The data indicated that the type and amount of HM present influenced the growth of bacteria. After 24 hours, highly resistant bacterial colonies were observed, which were more abundant than the control group [Table 1](#). Based on the result, six samples were identified as having a high content of Ni<sup>2+</sup> and Cd<sup>2+</sup>: samples #5, #8, #10, #11, #16, and #18. Although the viability of bacteria was observed in all tested isolates at low concentrations of Cd and Ni, only resistant isolates formed colonies at high concentrations of HMs. Specifically, it was found that only #5, #11, and #18 isolates grow and form colonies at a concentration of 574.2 mg/l, which is 30 times higher than the allowable quantity of Ni<sup>2+</sup>. #5, #8, #10, #11, #16, and #18 isolates were observed to form colonies in nutritive medium containing 24.6 mg/l of Cd<sup>2+</sup>, and they were recorded as isolates with high viability and resistance to Cd<sup>2+</sup>. In particular, it was observed that the number of cells of isolates #11 and #18 at 574.2 mg/l Ni<sup>2+</sup> concentration (from  $1.6 \cdot 10^8 \pm 0,04$  to  $1.1 \cdot 10^9 \pm 0,03$ ; from  $9.6 \cdot 10^7 \pm 0,04$  to  $3.1 \cdot 10^8 \pm 0,02$ ) and isolates #8 and #16 at 24.6 mg/l Cd<sup>2+</sup> concentration were 10 times higher than the control (from  $2.4 \cdot 10^8 \pm 0,04$  to  $2.52 \cdot 10^9 \pm 0,03$ ; from  $4 \cdot 10^8 \pm 0,02$  to  $1.34 \cdot 10^9 \pm 0,04$ ).

### Results of classic microbiological and molecular genetic identification of bacterial isolates

Bacterial isolate one was initially characterized based on its morphology, physicochemical properties, and resistance to cadmium and nickel. Isolates 4, 5, 8, 10, 11, 16, and 18 were analyzed. Colonies of isolates

**Table 1.** Different Ni<sup>2+</sup> and Cd<sup>2+</sup> cation concentrations and the number of microorganisms isolated under control.

Isolates	The quantity of cells of strains with various concentrations of NiSO <sub>4</sub> × 7H <sub>2</sub> O, mg/l				The quantity of cells of strains with various concentrations of CdCl <sub>2</sub> , mg/l		
	0	95,7	191,4	574,2	4,1	8,2	24,6
#4	$4.5 \cdot 10^8 \pm 0,01$	$6.9 \cdot 10^7 \pm 0,01$	$3.7 \cdot 10^8 \pm 0,02$	0	$3.28 \cdot 10^9 \pm 0,02$	$3.12 \cdot 10^9$	0
#5	$1.6 \cdot 10^8 \pm 0,03$	$4.9 \cdot 10^7 \pm 0,03$	$2.3 \cdot 10^7 \pm 0,04$	$1.4 \cdot 10^7 \pm 0,01$	$1.80 \cdot 10^8 \pm 0,01$	$5.08 \cdot 10^8 \pm 0,05$	$3.24 \cdot 10^8 \pm 0,02$
#11	$1.6 \cdot 10^8 \pm 0,04$	$1 \cdot 10^8 \pm 0,02$	$3.4 \cdot 10^9 \pm 0,03$	$1.1 \cdot 10^9 \pm 0,03$	$7.5 \cdot 10^9 \pm 0,01$	$1.0 \cdot 10^9 \pm 0,05$	$5.4 \cdot 10^8 \pm 0,04$
#10	$4 \cdot 10^9 \pm 0,05$	$4.7 \cdot 10^9 \pm 0,03$	$3.5 \cdot 10^9 \pm 0,03$	0	$8.5 \cdot 10^8 \pm 0,02$	$9.1 \cdot 10^8 \pm 0,02$	$7.7 \cdot 10^8 \pm 0,04$
#3	$1.4 \cdot 10^7 \pm 0,05$	$5.7 \cdot 10^8 \pm 0,05$	$3.2 \cdot 10^8 \pm 0,01$	0	$3.4 \cdot 10^8 \pm 0,04$	$5.0 \cdot 10^8 \pm 0,01$	0
#15	$1.2 \cdot 10^6 \pm 0,02$	$1.4 \cdot 10^7 \pm 0,03$	$4.1 \cdot 10^8$	0	$2.1 \cdot 10^8 \pm 0,02$	$2.8 \cdot 10^8 \pm 0,05$	0
#8	$2.4 \cdot 10^8 \pm 0,04$	$1.5 \cdot 10^9 \pm 0,01$	$1.1 \cdot 10^8 \pm 0,03$	0	$4.88 \cdot 10^9 \pm 0,04$	$3.48 \cdot 10^9 \pm 0,05$	$2.52 \cdot 10^9 \pm 0,03$
#16	$4 \cdot 10^8 \pm 0,04$	$4.7 \cdot 10^9 \pm 0,02$	$3.5 \cdot 10^9 \pm 0,02$	0	$1.02 \cdot 10^9 \pm 0,01$	$1.51 \cdot 10^9 \pm 0,01$	$1.34 \cdot 10^9 \pm 0,04$
#18	$9.6 \cdot 10^7 \pm 0,04$	$1.3 \cdot 10^8 \pm 0,04$	$1.4 \cdot 10^8 \pm 0,03$	$3.1 \cdot 10^8 \pm 0,02$	$2.72 \cdot 10^9 \pm 0,03$	$1.07 \cdot 10^9 \pm 0,02$	$9.1 \cdot 10^8 \pm 0,01$

4, 5, and 18 were round, creamy, and smooth, while isolate 1 had a round shape with soft edges. #8 and #4 had wavy and smooth edges, respectively, and their cell sizes ranged from  $2.8\text{--}3.2 \times 1.6\text{--}2.5 \mu\text{m}$  and  $0.4\text{--}1.0 \times 1.0 \mu\text{m}$ , respectively. #18 had a cell size of  $0.3\text{--}0.5 \times 2.5\text{--}5.0 \mu\text{m}$ .

#8 colony had a smooth, shiny surface with slightly raised flat edges, and their size ranged from 1-5 mm. All isolates were identified as Gram-positive, rod-shaped, aerobic, motile, and spore-forming bacteria Fig. 1.

The colony morphology of isolate no. 10 exhibited a dry, wrinkled, creamy surface with a wavy edge and an irregular shape. The bacterial cells were bacilli, Gram-positive, facultative anaerobes, and sporangia.

The 11<sup>th</sup> isolated colony appeared spherical, beige, transparent, and shiny, with a slightly raised edge and a thickened center. The colony size ranged from 1-3 mm. The cells observed were small, gram-negative rods measuring  $0.6\text{--}1.0 \times 1.2\text{--}3.0 \mu\text{m}$ .

Isolate four developed sizable flat beige colonies (0.3-1.0 cm in diameter) when cultivated on bovine peptone agar with 1% glucose. Gram-positive, rod-shaped cells of  $0.8\text{--}1.5 \mu\text{m}$  in size produce central endospores Fig. 1. The colony edges are wavy, while the folds in the center are almost flat. After three to four days of incubation on a nutrient-rich medium, the colonies secrete a dark pigment.

The colonies of strain #16 were rounded and colored, with an enlarged and smooth surface, soft edges, and large volume. The short rods are gram-negative, measuring from  $0.5$  to  $1.0 \times 1.5$  to  $5.0 \mu\text{m}$ . The rod-shaped cells are gram-positive, measuring  $0.5\text{--}1.5 \mu\text{m}$  in size, and form spores. On the other hand, the colonies of no. 18 were greenish-yellow and produced a pigment that matched the substrate's color. They were spherical, flat, shiny, and smooth, measuring 0.1 to 0.5 mm. Isolate #5 exhibited round, pale colonies with flat edges and a raised, soft, shiny surface. The diameter of its colonies ranged from 0.2 to 0.6 mm. Its cells were gram-negative, short rod-shaped, unicellular, bicellular, and short chains measuring  $0.4\text{--}1.1 \times 1.1\text{--}3.0 \mu\text{m}$ .

Biochemical tests were performed on the bacterial isolates with those mentioned above morphological and cultural characteristics. According to the data presented in Table 2, all isolates demonstrated positive catalase activity. The results showed that isolates #16 and #10 could hydrolyze starch, while isolates #5, #11, and #8 do not have starch hydrolyzing activity. Isolates #16, #10, #11, #18, and #4 were recorded as isolates that have positive gelatinase activity and isolates #5 and #8 were recorded as isolates that have negative gelatinase activity.

Isolates that showed high resistance to high concentrations of  $\text{Ni}^{2+}$  and  $\text{Cd}^{2+}$  were identified with the molecular-genetic method. The molecular identification results confirmed the morphological, microscopic, and biochemical identification results. According to the results, isolates #5, #11, and #18 were found to be *Enterobacter cloacae*, *Enterobacter ludwigii*, and *Pseudomonas aeruginosa*, respectively. The 16S rRNA nucleotide sequences of identified strains were submitted to GenBank from NCBI, USA, and registered with accession number: *Pseudomonas aeruginosa* 18 (OQ932917.1); *Enterobacter ludwigii* 11UZ (OQ932957.1) and *Enterobacter cloacae* Uz\_5 (OQ932923.1) were registered.

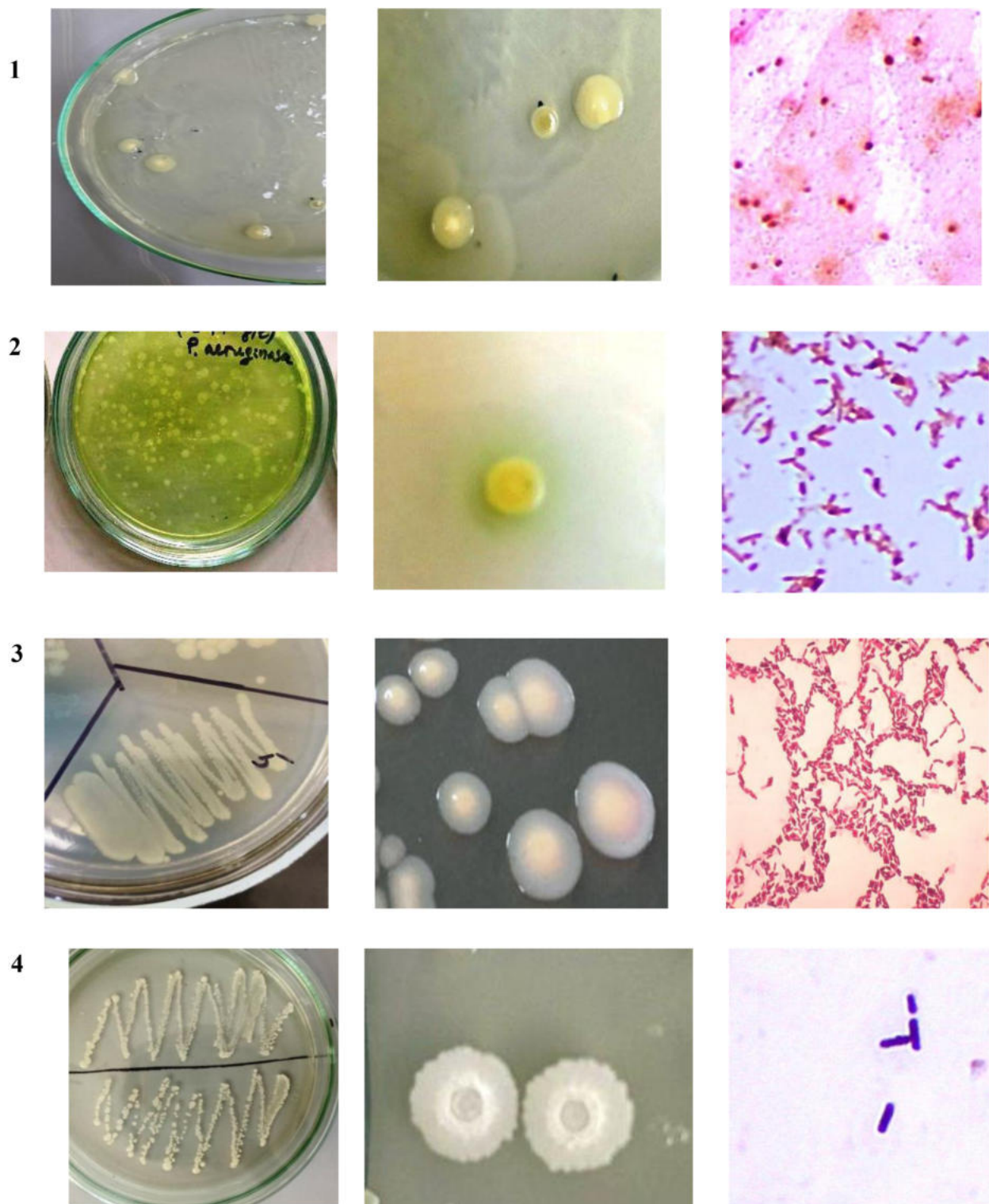
A phylogenetic tree of the 16S rRNA genes of the isolates sequenced using MEGA-64 was constructed Fig. 2.

### Bacterial minimal inhibitory concentration

The investigation of the minimal inhibitory concentration of bacteria for  $\text{Ni}^{2+}$  was carried out on the fourth day of culture. All bacterial species studied, notably *Enterobacter ludwigii* 11Uz, *Bacillus licheniformis*10, *Bacillus atrophaeus* 4, *Enterobacter cloacae* Uz\_5, and *Bacillus subtilis*, continued to grow even after 48 hours and demonstrated growth at one mM Ni concentration. After 4<sup>th</sup> day of cultivation, the MIC value for Ni was two mM in the *Pseudomonas aeruginosa*18 strain and 3 mM in *Enterobacter ludwigii*11Uz and *Enterobacter cloacae* Uz\_5 strains. The MIC value of other resistant strains against Ni was discovered to be 1 mM. Within 24 to 72 hours, the MIC of active strains was ascertained for various Cd concentrations. Based on the data obtained, only two bacterial cultures, *Enterobacter ludwigii* 11Uz and *Bacillus simplex* 8, showed growth after 24 hours of incubation at 1 mM Cd concentration. Bacterial cultures of *Bacillus megaterium* 16, *Bacillus licheniformis*10, *Enterobacter ludwigii* 11Uz, and *Enterobacter cloacae* Uz\_5 were able to grow and reproduce in Cd concentrations ranging from 1 to 2 mM. The appearance of growth was observed within 3 days. During the fourth day of growth, *Pseudomonas aeruginosa* 18 exhibited up to 4 mM for  $\text{Cd}^{2+}$ . Additionally, a strain of *Bacillus licheniformis*10 demonstrated a high MIC of 2 mM for  $\text{Cd}^{2+}$  cation.

### Analysis of the production of auxin and gibberellin

Soil bacteria produce exopolysaccharides, phytohormones such as auxins, cytokines, gibberellins, and other metabolites crucial for plant development, nutrition, and biotic and abiotic stress tolerance. The concentration of  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$  in soil negatively



**Fig. 1.** (A) The overall appearance of the colony. (B) Magnified view of the colony. (C) Microscopic view of cell (magnification  $10 \times 100$ ): 1- Isolate #5; 2- Isolate #18; 3- Isolate #11; 4- Isolate #4; 5- Isolate #8; 6- Isolate #10.

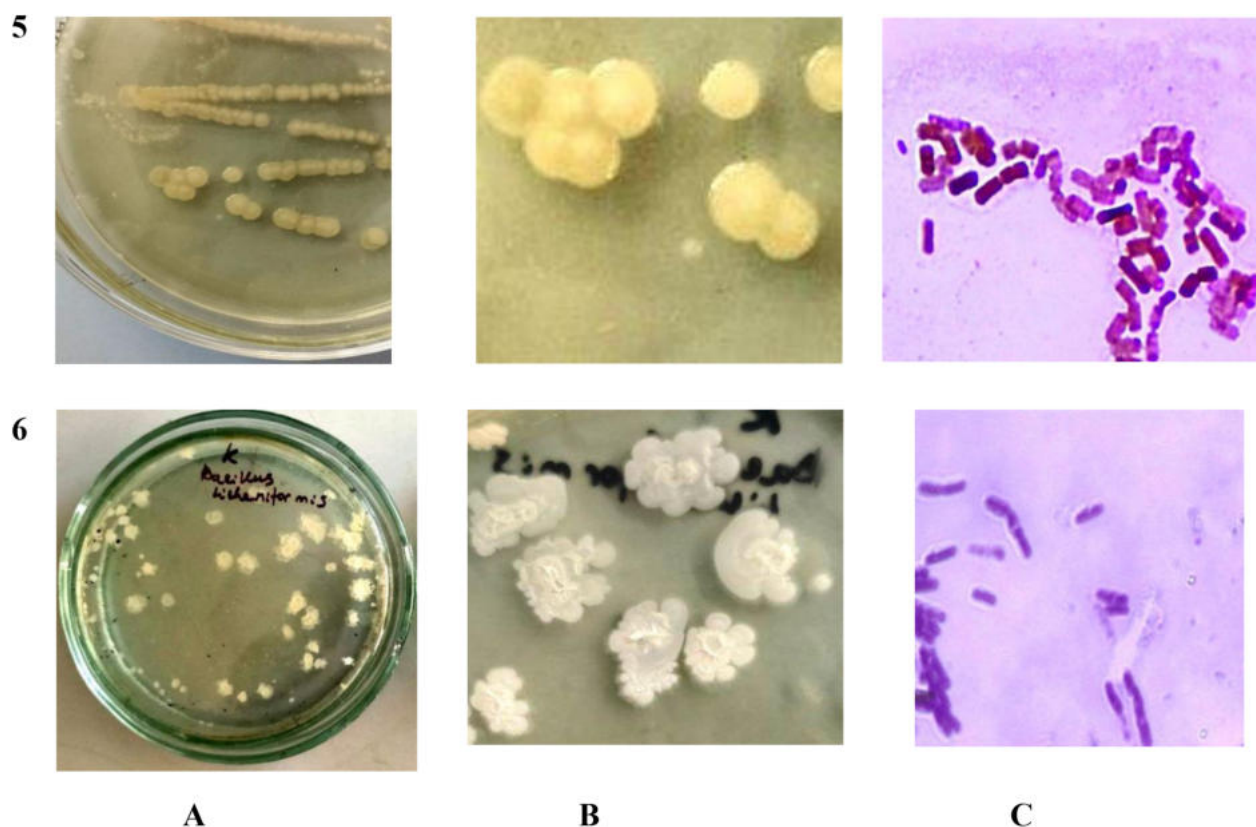


Fig. 1. Continued.

**Table 2.** Bacterial biochemical properties attributing to an extreme tolerance to high  $\text{Ni}^{2+}$  and  $\text{Cd}^{2+}$  cation concentrations.

Characteristics	#5	#16	#11	#10	#18	#4	#8
Starch hydrolysis	-	+	-	+	-	-	-
Casein hydrolysis	-	+	+	-	-	+	+
Tyrosine hydrolysis	-	-	-	-	-	-	+
Gelatine hydrolysis	-	+	+	+	+	+	-
Sucrose assimilate	+	-	+	-	+	-	-
D-mannose Assimilate	+	-	+	-	+	-	-
Rhamnose Assimilate	+	-	-	-	+	-	-
Lactose generation of acid	+	-	-	+	+	-	-
D-glucose generation of Acid	+	+	-	+	+	-	-
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	+	-	-
Citrate disposal	+	+	-	+	-	-	+
Nitrate reduction	+	+	-	+	+	+	-
Oxygen Relationship	Facultative anaerobic	Aerobic	Facultative anaerobic	Facultative anaerobic	Aerobic	Facultative anaerobic	Aerobic

\*Note “+” – positive result, “-” – negative result.

affects gibberellin production in plants. However, *Pseudomonas aeruginosa* 18 and *Enterobacter ludwigii* 11Uz synthesized high amounts of gibberellin, up to 390  $\mu\text{g}/\text{mL}$ , as shown in Table 3. *Pseudomonas aeruginosa* 18 produced 390  $\mu\text{g}/\text{mL}$  gibberellin on the seventh day of growth. In contrast, *Enterobacter ludwigii* 11 produced 375  $\mu\text{g}/\text{mL}$  and 380  $\mu\text{g}/\text{mL}$  at a concentration of 4.2 mg/L of  $\text{Cd}^{2+}$  and 95.7 mg/L

of  $\text{Ni}^{2+}$ , respectively. *Bacillus licheniformis* 10 and *Enterobacter ludwigii* 11Uz synthesized more gibberellin than the control on the seventh day, regardless of the concentration of  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$ . During the fourth day of growth, the strains *Enterobacter cloacae* Uz\_5 and *Bacillus simplex* 8 had higher gibberellin concentrations than the control, regardless of the heavy metal concentration.

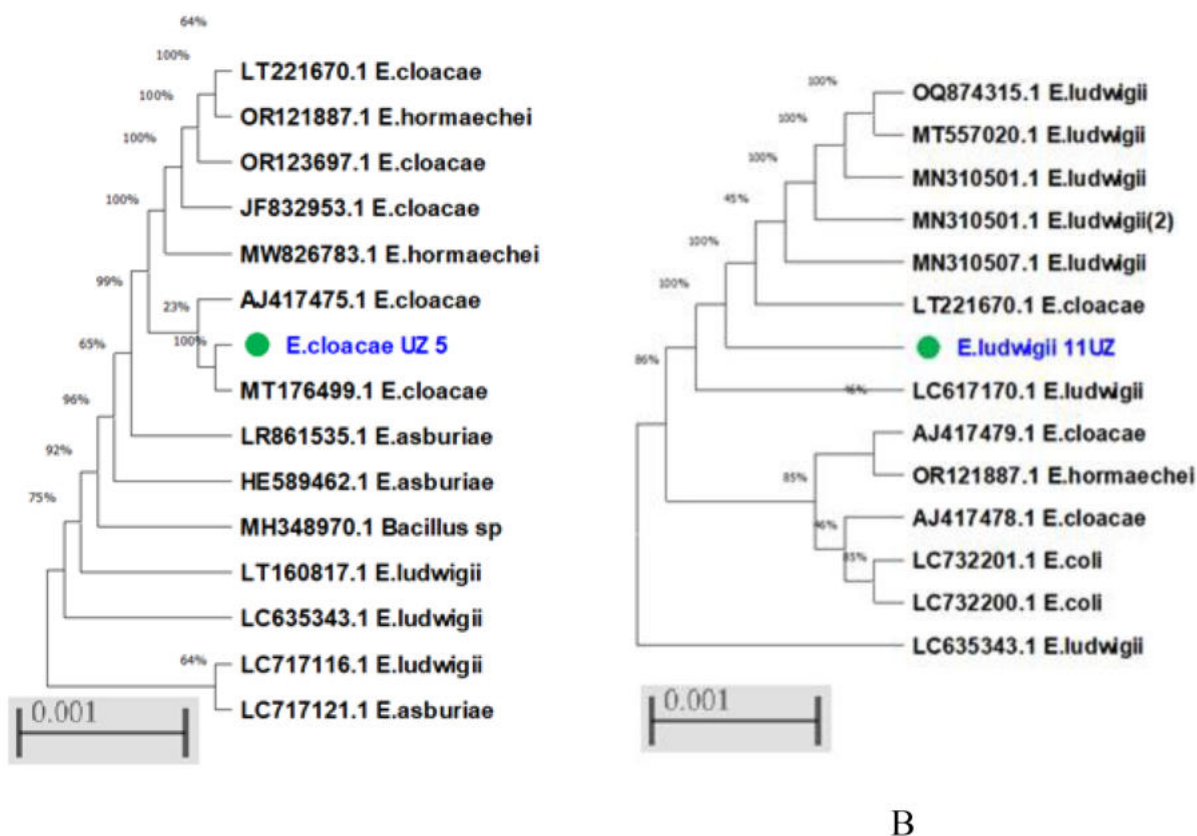


Fig. 2. Phylogenetic tree of *Enterobacter cloacae* Uz\_5 (A), *Enterobacter ludwigii* 11UZ (B).

The results showed that the tested bacterial strains produced significantly more auxin than the control group in the presence of HM. The auxin content synthesized by *Enterobacter ludwigii* 11Uz, *Bacillus licheniformis* 10, and *Bacillus simplex* 8 was similar to the control. In contrast, the *Pseudomonas aeruginosa* 18 strain produced 4, 4.8, and 5.2 times more auxin than the group under control, depending on the concentration of Cd<sup>2+</sup> cations (2.4, 4.1, and 8.2 mg/these strains). The auxin synthesis increased during the third day of growth in these strains. On the seventh day of growth, the *Pseudomonas aeruginosa* 18 strain produced more auxin than the control. On the fourteenth day, the *Bacillus atrophaeus* 4 strain produced more auxin in different Ni<sup>2+</sup> cations concentrations in contrast to the group under control. Additionally, during the third day of growth at a Ni concentration of 95.7 mg/l, the *Bacillus licheniformis* 10 strain produced auxin at a rate 1.6 times greater than the control rate.

#### Study of exopolysaccharides synthesized by bacteria under different conditions of heavy metals

The findings suggest that exopolysaccharide (EPS) production increases as heavy metal concentration increases. *Bacillus* strains showed a 1.2-1.4 times

increase in EPS formation under cationic stress. *Pseudomonas aeruginosa* 18 and *Enterobacter ludwigii* 11UZ produced more EPS than the control group during the seventh day of growth. *Enterobacter ludwigii* 11 produced the highest amount of EPS as a reaction to Ni<sup>2+</sup> stress. During the 14th day of growth, at a Ni concentration of 191.4 mg/l, the strain produced 81 mg/l of EPS, 1.2 times higher than that of the control group. *Bacillus atrophaeus* 4 and *Enterobacter ludwigii* 11Uz produced 25-28 mg/l and 6.12 mg/L of EPS on days 7 and 14 of culture, respectively, at Cd<sup>2+</sup> cation concentrations of 8.2 and 24.6 mg/l. *Pseudomonas aeruginosa* 18 produced 79 mg/l EPS during seven days of growth, with a Cd<sup>2+</sup> cation concentration of 24.6 mg/l. Because of the negative charge on their cell membrane, EPS-producing bacteria offer promise as chelating agents for removing positively charged HM ions. These findings suggest that EPS production contributes to microbial fitness and stress resistance<sup>17</sup>.

Studies have demonstrated the importance of plant growth-promoting bacteria in improving plant growth and yields under organic stress<sup>18,19</sup>. These bacteria employ various mechanisms, such as enzyme production, biological nitrogen fixation, amino acid synthesis, and changes in nutrient processing, to enhance nutrient availability in the rhizosphere

**Table 3.** Auxin and gibberellin content synthesized by bacteria at various concentrations of heavy metals.

Strains	Heavy metals mg/l	Auxin content, mg/ml			Gibberellin content, mg/ml		
		3 <sup>rd</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	3 <sup>rd</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day
<i>Pseudomonas aeruginosa</i> 18	Control	136 ± 0,2	75 ± 0,23	195 ± 0,4	155 ± 0,3	375 ± 0,18	233 ± 0,24
	Cd <sup>2+</sup> 2,4	556 ± 0,25	176 ± 0,41	172 ± 0,33	157 ± 0,5	262 ± 0,29	282 ± 0,28
	Cd <sup>2+</sup> 4,1	656 ± 0,45	122 ± 0,24	95 ± 0,31	151 ± 0,2	266 ± 0,31	250 ± 0,21
	Cd <sup>2+</sup> 8,2	724 ± 0,13	125 ± 0,34	273 ± 0,19	365 ± 0,35	250 ± 0,22	271 ± 0,46
	Ni <sup>2+</sup> 57,3	150 ± 0,18	165 ± 0,32	35 ± 0,24	168 ± 0,5	300 ± 0,37	233 ± 0,14
	Ni <sup>2+</sup> 95,7	156 ± 0,24	85 ± 0,29	36 ± 0,15	192 ± 0,42	390 ± 0,26	268 ± 0,36
	Ni <sup>2+</sup> 191,4	127 ± 0,41	83 ± 0,43	34 ± 0,21	138 ± 0,49	332 ± 0,25	228 ± 0,27
<i>Bacillus atrophaeus</i> 4	Control	180 ± 0,11	73 ± 0,2	38 ± 0,15	222 ± 0,43	300 ± 0,39	258 ± 0,13
	Cd <sup>2+</sup> 2,4	151 ± 0,21	76 ± 0,22	33 ± 0,14	235 ± 0,27	292 ± 0,29	225 ± 0,23
	Cd <sup>2+</sup> 4,1	127 ± 0,15	125 ± 0,34	65 ± 0,46	212 ± 0,22	302 ± 0,33	193 ± 0,45
	Cd <sup>2+</sup> 8,2	296 ± 0,31	156 ± 0,16	238 ± 0,19	235 ± 0,31	197 ± 0,18	203 ± 0,43
	Ni <sup>2+</sup> 57,3	215 ± 0,42	124 ± 0,25	139 ± 0,5	282 ± 0,28	380 ± 0,12	230 ± 0,29
	Ni <sup>2+</sup> 95,7	237 ± 0,38	167 ± 0,21	236 ± 0,41	275 ± 0,24	164 ± 0,17	256 ± 0,21
	Ni <sup>2+</sup> 191,4	176 ± 0,12	86 ± 0,19	220 ± 0,25	247 ± 0,38	288 ± 0,23	248 ± 0,35
<i>Enterobacter cloacae</i> Uz_5	Control	351 ± 0,41	175 ± 0,27	198 ± 0,36	218 ± 0,21	360 ± 0,29	345 ± 0,45
	Cd <sup>2+</sup> 2,4	305 ± 0,5	174 ± 0,42	105 ± 0,23	228 ± 0,43	300 ± 0,17	360 ± 0,33
	Cd <sup>2+</sup> 4,1	234 ± 0,23	255 ± 0,37	136 ± 0,4	226 ± 0,31	282 ± 0,48	267 ± 0,15
	Cd <sup>2+</sup> 8,2	257 ± 0,17	165 ± 0,22	36 ± 0,2	252 ± 0,47	373 ± 0,32	257 ± 0,22
	Ni <sup>2+</sup> 57,3	274 ± 0,46	115 ± 0,49	215 ± 0,39	184 ± 0,24	268 ± 0,19	316 ± 0,3
	Ni <sup>2+</sup> 95,7	267 ± 0,38	124 ± 0,3	85 ± 0,15	236 ± 0,35	293 ± 0,44	203 ± 0,48
	Ni <sup>2+</sup> 191,4	376 ± 0,26	185 ± 0,25	56 ± 0,47	230 ± 0,28	300 ± 0,18	254 ± 0,34
<i>Bacillus licheniformis</i> (10)	Control	231 ± 0,21	125 ± 0,35	31 ± 0,43	319 ± 0,45	183 ± 0,13	210 ± 0,38
	Cd <sup>2+</sup> 2,4	277 ± 0,3	91 ± 0,22	160 ± 0,11	212 ± 0,49	248 ± 0,33	290 ± 0,15
	Cd <sup>2+</sup> 4,1	253 ± 0,14	65 ± 0,43	35 ± 0,25	270 ± 0,37	333 ± 0,46	223 ± 0,24
	Cd <sup>2+</sup> 8,2	476 ± 0,26	83 ± 0,41	93 ± 0,32	385 ± 0,18	238 ± 0,24	242 ± 0,3
	Ni <sup>2+</sup> 57,3	296 ± 0,31	35 ± 0,1	132 ± 0,29	280 ± 0,12	234 ± 0,41	251 ± 0,11
	Ni <sup>2+</sup> 95,7	391 ± 0,46	125 ± 0,20	35 ± 0,36	328 ± 0,44	252 ± 0,36	165 ± 0,34
	Ni <sup>2+</sup> 191,4	173 ± 0,39	135 ± 0,13	56 ± 0,49	290 ± 0,23	300 ± 0,19	256 ± 0,42
<i>Enterobacter ludwigii</i> 11Uz	Control	353 ± 0,32	187 ± 0,1	72 ± 0,24	375 ± 0,16	256 ± 0,48	361 ± 0,23
	Cd <sup>2+</sup> 2,4	370 ± 0,45	305 ± 0,25	195 ± 0,4	382 ± 0,37	372 ± 0,47	355 ± 0,31
	Cd <sup>2+</sup> 4,1	434 ± 0,13	152 ± 0,35	120 ± 0,28	365 ± 0,22	301 ± 0,14	242 ± 0,15
	Cd <sup>2+</sup> 8,2	350 ± 0,33	125 ± 0,29	30 ± 0,25	380 ± 0,45	365 ± 0,42	245 ± 0,27
	Ni <sup>2+</sup> 57,3	318 ± 0,2	122 ± 0,34	40 ± 0,13	377 ± 0,12	290 ± 0,26	352 ± 0,13
	Ni <sup>2+</sup> 95,7	257 ± 0,46	242 ± 0,17	25 ± 0,21	364 ± 0,18	380 ± 0,38	263 ± 0,4
	Ni <sup>2+</sup> 191,4	275 ± 0,19	160 ± 0,44	26 ± 0,36	376 ± 0,43	369 ± 0,15	251 ± 0,1
<i>Bacillus simplex</i> 8	Control	125 ± 0,5	145 ± 0,11	200 ± 0,45	135 ± 0,21	328 ± 0,13	258 ± 0,26
	Cd <sup>2+</sup> 2,4	166 ± 0,43	142 ± 0,15	25 ± 0,16	166 ± 0,5	303 ± 0,22	238 ± 0,39
	Cd <sup>2+</sup> 4,1	146 ± 0,34	155 ± 0,25	33 ± 0,2	208 ± 0,24	304 ± 0,29	282 ± 0,27
	Cd <sup>2+</sup> 8,2	156 ± 0,1	147 ± 0,33	27 ± 0,27	198 ± 0,26	242 ± 0,26	242 ± 0,4
	Ni <sup>2+</sup> 57,3	115 ± 0,19	136 ± 0,17	190 ± 0,12	170 ± 0,44	331 ± 0,33	252 ± 0,18
	Ni <sup>2+</sup> 95,7	71 ± 0,35	84 ± 0,41	132 ± 0,31	155 ± 0,3	318 ± 0,5	228 ± 0,23
	Ni <sup>2+</sup> 191,4	126 ± 0,14	125 ± 0,28	37 ± 0,25	150 ± 0,28	290 ± 0,15	174 ± 0,48

Values in each column represent the mean ± standard error of three replicates.

and stimulate plant growth. As a result, using bacteria has become a crucial strategy in sustainable agriculture to reduce plant osmotic stress, as highlighted by Mowafy<sup>20</sup>. In heavy metals, bacteria such as *Enterobacter Ludwig 11Uz*, *Pseudomonas aeruginosa 18*, and *Enterobacter cloacae Uz.5* produce plant hormones and exopolysaccharides. As noted, stress-induced plant growth stress<sup>21</sup>. Stress-induced hormones such as auxins and gibberellins are also secreted by bacteria such as pseudo-alcaligenes, which have been observed in numerous studies, such as *Bacillus pumilus*, as noted in Jha<sup>11</sup>. As observed in multiple studies, heavy metal stress in plants can

delay development and nutrient utilization, leading to adverse effects such as enzyme inhibition<sup>17,20</sup>.

Furthermore, Cd and Ni stress can affect metabolic processes and respiration rates, which can overwhelm the plant's antioxidant defense mechanisms and exacerbate stress, according to Ghori and El-Monem<sup>22</sup>.

The research findings indicate that increased heavy metal concentrations decrease bacterial cell count, and exposure to low nickel and cadmium reduces exopolysaccharide production in bacteria. However, the maximum development of biofilm and exopolysaccharide production occurred in bacteria treated with 191.4 mg/l Ni. Moderate nickel concentrations may

weaken defense mechanisms, reverse membrane polarization, and increase cell hydrophilicity, promoting biofilm adhesion and enhancing survival. Bacteria have developed metal tolerance systems to avoid cell damage, such as producing or sequestering toxic metals, taking intracellular ions to neutralize, and releasing poisonous ions by the drainage system, as reported by Jayakumar and Wang<sup>23,24</sup>. Studies show that bacteria can gather Ni and Cd, and biofilm cell walls, cell membranes, and extracellular polymer components can absorb these harmful metals. Bacteria produce many EPS to protect themselves from the abundant concentrations of Ni and Cd, which leads to increased biomass and production of plant hormones such as gibberellin, auxin, and exopolysaccharides. EPS can bind HM, as noted by Zainab<sup>17</sup>. Strains of *Pseudomonas aeruginosa* and *Enterobacter ludwigii* durable to significant concentrations of Ni<sup>2+</sup> and Cd<sup>2+</sup> have been observed to produce auxin, gibberellin, and exopolysaccharides on the third and seventh days of culture. Gibberellins have been found to mitigate the adverse influences of HM in the soil by limiting oxidative damage and activating the antioxidant system of plants, as Gong reported<sup>25</sup>. When gibberellin was added to young wheat plants, it was observed to reduce the adverse influences of nickel-induced oxidative stress on growing, chlorophyll content, and carbonic anhydrase activity. Notably, the presence or absence of heavy metals in the growth medium or the growth conditions did not affect bacterial production of auxin and gibberellin. EPS-producing bacteria have been shown to have a higher tolerance to metals than non-EPS-producing bacteria, with the latter being more sensitive to Cr (VI) ions, according to Mohite<sup>26</sup>. *Pseudomonas aeruginosa* BU1 and BU2 have been reported to be susceptible to EPS-mediated metal resistance and tolerance to high environmental copper levels, as per Mativanan<sup>27</sup>. Furthermore, *Enterobacter cloacae* SUKCr1D exhibited increased metal tolerance and EPS production when cultured in media containing various concentrations of Cr (VI) in 5-50 mg/l and 100 mg/l, according to Kailasam<sup>12</sup>. Hence, enhancing EPS production can significantly improve microbial remediation and reduce HM contamination.

## Conclusion

As a result of our observational studies, for the first time, local strains of *Pseudomonas aeruginosa* 18, *Enterobacter ludwigii* 11Uz, and *Enterobacter cloacae* Uz 5 with high resistance and viability to Ni<sup>2+</sup> and Cd<sup>2+</sup> cations were isolated from soil samples contaminated

with heavy metals and identified with molecular genetic method. Even under metallic conditions, auxin synthesized by bacteria reduces the effects of stress by increasing plant uptake of nutrients and water and changing the root system's structure. Gibberellin alleviates metal toxicity by reducing Cd and Ni uptake by plants and lipid peroxidation, changing hormonal balance, and regulating the activities of proteases, catalase, and peroxidase. EPS synthesized by bacteria strongly binds to metals and forms organic metal complexes, increasing plants' resistance to toxic metals. These secondary metabolites are crucial in reducing stress by converting heavy metals into stable compounds.

Thus, according to the conducted research and the obtained results, the microorganisms we observed have unique abilities to alleviate the stress conditions, restore productivity in soils contaminated with Ni and Cd, and increase the yield of plants by ensuring their adaptability to heavy metals.

## Acknowledgment

The authors would like to express their deep gratitude to the Ministry of Innovation department of Uzbekistan and Institute of Microbiology of Academy Sciences of the Republic of Uzbekistan for supporting this study.

## Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the Institute of Microbiology of Academy Sciences of Uzbekistan approved the project

## Authors' contribution statement

AU. collected the sample and analyzed all parameters, interpretation, GK. Conception, TSh. analysis, acquisition of data, NS. design, drafting the MS, NSh. interpretation, revision and proofreading. All authors read and approved the manuscript.

## References

- Saleh SR, Kandeel MM, Ghareeb D, Ghoneim TM, Talha NI, Alaoui-Sossé *et al.* Wheat biological responses to stress caused by cadmium, nickel and lead. *Sci Total Environ.* 2020;706:136013. <https://doi.org/10.1016/j.scitotenv.2019.136013>.
- Hamad AA, Alamer KH, Alrabie HS. The Accumulation Risk of Heavy Metals in Vegetables which Grown in Contaminated Soil. *Baghdad Sci J.* 2021Sep 1;18(3):0471. <http://dx.doi.org/10.21123/bsj.2021.18.3.0471>.
- Manzoor D, Sharma M, Khursheed W. Heavy metals in vegetables and their impact on the nutrient quality of vegetables: A review. *J Plant Nut.* 2018;41:1–20. <https://doi.org/10.1080/01904167.2018.1462382>.
- Usmonkulova A, Shonakhunov T, Kadirova G. Activity of nitrogen-fixing cyanobacteria under salinity and heavy metals stress. *J Pharm Neg Resul.* 2022;13(3):355–363, <https://doi.org/10.17762/sfs.v10i1.465>.
- El-Sheshtawy HS, Mahdy HM, Sofy AR, Sofy MR. Production of biosurfactant by *Bacillus megaterium* and its correlation with lipid peroxidation of *Lactuca sativa*. *Egyptian J Petroleum.* 2022;31:1–6. <https://doi.org/10.1016/j.ejpe.2022.03.001>.
- Amjad M, Raza H, Murtaza B, Abbas G, Imran M, Shahid M, Naeem MA, Zakir A. & Iqbal MM. Nickel Toxicity Induced Changes in Nutrient Dynamics and Antioxidant Profiling in Two Maize (*Zea mays* L.) Hybrids. *Plants.* 2020;9:5. <https://doi.org/10.3390/plants9010005>.
- Abu-Shahba M, Mansour M, Mohamed H. Sofy M. Effect of biosorptive removal of cadmium ions from hydroponic solution containing indigenous garlic peel and mercurized garlic peel on lettuce productivity. *Sci Hortic.* 2022;293:110727, <https://doi.org/10.1016/j.scienta.2021.110727>.
- Ahmad M, Naseer I, Hussain A, Zahid Mumtaz M, Mustafa A, Hilger TH, *et al.* Appraising endophyte - Plant symbiosis for improved growth, nodulation, nitrogen fixation and abiotic stress tolerance: An experimental investigation with chickpea (*cicer arietinum* L.). *Agronomy.* 2019;9(10):621. <https://doi.org/10.3390/agronomy9100621>.
- Manoj SR, Karthik C, Kadirvelu K, Arulselvi PI, Shanmugasundaram T, Bruno B, *et al.* Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. *J Environ Manag.* 2020; 15(254):109779 <https://doi.org/10.1016/j.jenvman.2019.109779>.
- Usmonkulova AA, Kadirova GKH, Khusanov TS, Shonakhunov TE, Shukurov N. Determination of local bacteria synthesizing ACC deaminase on plant growth indicators under nickel and cadmium stress conditions. *SABRAO J Breed Genet.* 2024;56(5):2033-2044. <https://doi.org/10.54910/sabrao2024.56.5.26>.
- Jha Y. Endophytic Bacteria as a Modern Tool for Sustainable Crop Management Under Stress. *Biofertilizers for Sustainable Agriculture and Environment.* *Soil Biology.* 2019;55:203-223. [https://doi.org/10.1007/978-3-030-18933-4\\_9](https://doi.org/10.1007/978-3-030-18933-4_9).
- Kailasam S, Sundaramanickam A, Balaji K, Kanth S. Adsorption of chromium by exopolysaccharides extracted from lignolytic phosphate solubilizing bacteria. *Inter J Biolog Macromol.* 2022;206:788-798. <https://doi.org/10.1016/j.ijbiomac.2022.03.047>.
- Agnihotri P, Banerjee S, Maitra M, Mitra AK. Isolation, characterization and identification of an As(V)-resistant plant growth promoting bacteria for potential use in bioremediation. *Asia-Pacific J Sci Tech.* 2021;26:1-11. <https://doi.org/10.14456/apst.2021.29>.
- Agarwal M, Rathore RS, Chauhan AA. Rapid and High Throughput MIC Determination Method to Screen Uranium Resistant Microorganisms. *Methods Protoc.* 2020;3:21. <http://dx.doi.org/10.3390/mps3010021>.
- Ahmad F, Ahmad I, Khan M. Indole Acetic Acid Production by the Indigenous Isolates of *Azotobacter* and Fluorescent *Pseudomonas* in the Presence and Absence of Tryptophan. *Turkish J Biol.* 2005;29:29–34.
- Sardari RRR, Kulcinskaja E, Ron EY, Björnsdóttir S, Friðjónsson ÓH, Hreggviðsson GÓ *et al.* Evaluation of the production of exopolysaccharides by two strains of the thermophilic bacterium *Rhodothermus marinus*. *Carbohydr Pol.* 2017;156:1–8. <https://doi.org/10.1016/j.carbpol.2016.08.062>.
- Zainab N, Amna Din BU, Javed MT, Afridi MS, Mukhtar T, Kamran MA, *et al.* Deciphering metal toxicity responses of flax (*Linum usitatissimum* L.) with exopolysaccharide and ACC-deaminase producing bacteria in industrially contaminated soils. *Plant Physiol Biochem.* 2020;152:90-99. <https://doi.org/10.1016/j.plaphy.2020.04.039>.
- Alves ARA, Yin Q, Oliveira, Rui S, Silva EF, Novo LAB. Plant growth-promoting bacteria in phytoremediation of metal-polluted soils: Current knowledge and future directions. *Sci Total Environ.* 2022;838:156435. <https://doi.org/10.1016/j.scitotenv.2022.156435>.
- Chakraborty S, Das Sh, Banerjee S, Mukherjee S, Ganguli A, Mondal S. Heavy metals bio-removal potential of the isolated *Klebsiella* sp TIU20 strain which improves growth of economic crop plant (*Vigna radiata* L.) under heavy metals stress by exhibiting plant growth promoting and protecting traits. *Bio-cat Agricul Biotechnol.* 2021;38:102204. <https://doi.org/10.1016/j.bcab.2021.102204>.
- Mowafy AM, Agha MS, Haroun SA, Abbas MA, Elbalkini M. Insights in nodule-inhabiting plant growth promoting bacteria and their ability to stimulate *Vicia faba* growth. *Egypt J Bas Appl Sci.* 2022;9:51–64. <https://doi.org/10.1080/2314808X.2021.2019418>.
- Liu M, Hu Z, Fan Y, Hua B, Yang W, Pang Sh *et al.* Effects of leguminous green manure–crop rotation on soil enzyme activity and stoichiometry. *J Plant Ecol.* 2024;17(6):rtae065. <https://doi.org/10.1093/jpe/rtae065>.
- Ghori NH, Ghori T, Hayat MQ, Imadi SR, Gul A, Altay V, *et al.* Heavy metal stress and responses in plants. *Int J Environ Sci Technol.* 2019;16:1807–1828. <https://doi.org/10.1007/s13762-019-02215-8>.
- Jayakumar M, Surendran U, Raja P, Kumar A, Senapathi V. A review of heavy metals accumulation pathways, sources and management in soils. *Arabian J Geosci.* 2021;14:2156. <https://doi.org/10.1007/s12517-021-08543-9>.
- Wang X, Sun Y, Zhang L, Mei Y. Spatial variation and influence factor analysis of soil heavy metal As based on geo Detector. *Stoch Environ Res Risk Assess.* 2021;35:2021–2030.
- Gong WJ, Niu ZF, Wang XR, Zhao HP. How the soil microbial communities and activities respond to long-term heavy metal contamination in electroplating contaminated site. *Microorganisms.* 2021;9(2):362. <https://doi.org/10.3390/microorganisms9020362>.
- Mohite B, Koli S, Narkhede C, Patil S, Patil S. Prospective of Microbial Exopolysaccharide for Heavy Metal Exclusion. *Appl Biochem Biotechnol.* 2017;183:582–600.
- Mathivanan K, Chandirika JU, Mathimani T, Rajaram R, Anandurai G, Yin H. Production and functionality of exopolysaccharides in bacteria exposed to a toxic metal environment. *Ecotox Environ Saf.* 2021;208:111567. <https://doi.org/10.1016/j.ecoenv.2020.111567>.

## توصيف وتحديد بعض السلالات البكتيرية المحلية لتراكم المعادن الثقيلة من التربة الملوثة

عزيزة عثمانكولوف<sup>1</sup>، جولتشيخرا قديروف<sup>1</sup>، ناصر شكوروف<sup>2</sup>، تولكين شوناخونوف<sup>1</sup>، سماتوف نور الدين<sup>3</sup>

<sup>1</sup> معهد الميكروبيولوجيا التابع لأكاديمية العلوم، طشقند، أوزبكستان.

<sup>2</sup> معهد الجيولوجيا والجيوفيزيائية، جامعة العلوم الجيولوجية، طشقند، أوزبكستان.

<sup>3</sup> معهد بحوث البيئة وتقنيات الحفاظ على الطبيعة.

### الخلاصة

أفادت دراسات عديدة بوجود كميات مرتفعة من الكاديوم والنيكل في التربة، مما أدى إلى انخفاض نمو النباتات، وخاصة في الكتلة الحيوية ومحتوى الكلوروفيل وسمات التمثيل الضوئي نظرًا لطبيعتها الخطرة. لذلك، من الضروري تعزيز مقاومة النباتات لإجهاد المعادن الثقيلة والحد من سمية الكاديوم والنيكل. في هذه الدراسة، تم عزل عدة سلالات بكتيرية من التربة الملوثة بالمعادن الثقيلة للتحقيق في دورها المحتمل في تسهيل العواقب الضارة لإجهاد المعادن الثقيلة. تم تقييم قدرة العزلات على زيادة إنتاج النباتات تحت إجهاد المعادن الثقيلة والتركيز المثبط الأدنى بعد تحديد العزلات على مستوى الأنواع. كشف التحديد الميكروبيولوجي التقليدي والجيني الجزيئي أن العزلات رقم 5 و18 و11 صُنفت على أنها *Enterobacter cloacae* Uz\_5 و *Pseudomonas aeruginosa* 18 و *Enterobacter ludwigii* 11Uz على التوالي. أنتجت *P. aeruginosa* 18 الأوكسين بمعدل 4 و4.8 و5.3 مرة أكثر من العينة القياسية. في المقابل، أنتجت *E. ludwigii* 11Uz و *Bacillus licheniformis* 10 و *Bacillus simplex* 8 الأوكسين بما يعادل العينة القياسية عند كميات مختلفة من كاتيون الكاديوم (2.4 و4.1 و8.2 ملغم/لتر). خلال اليوم الرابع عشر من النمو، عند تركيز النيكل 191.4 ملغم/لتر، أنتجت *Enterobacter ludwigii* 11 81 ملغم/لتر من السكريات متعددة السكريد خارج الخلية (EPS)، أي 1.2 مرة أعلى من العينة القياسية. أنتجت *Bacillus atrophaeus* 4 و *Enterobacter ludwigii* 11Uz 25-28 ملغم/لتر و6.12 ملغم/لتر من EPS في اليومين السابع والرابع عشر من الزراعة، على التوالي، عند تركيزات كاتيون  $Cd^{2+}$  8.2 و24.6 ملغم/لتر. تُظهر هذه الكائنات الدقيقة إمكانات واعدة للمعالجة الحيوية للتربة الملوثة بالمعادن الثقيلة من خلال تقليل سمية  $Ni^{2+}$  و  $Cd^{2+}$  وزيادة إنتاج الهرمونات النباتية والسكريات متعددة السكريد خارج الخلية في ظروف إجهاد المعادن الثقيلة.

**الكلمات المفتاحية:** الكاديوم، النيكل، التركيز المثبط الأدنى، الهرمون النباتي، السكريات متعددة السكريد خارج الخلية.