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DETERMINATION OF LOCAL BACTERIA SYNTHESIZING ACC DEAMINASE ON PLANT GROWTH INDICATORS UNDER NICKEL AND CADMIUM STRESS CONDITIONS

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SUMMARY

Assessing the ability of 26 bacteria isolated from heavy metal-contaminated soils to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase validates their vital role in reducing heavy metal stress conditions. Eight of the 26 bacterial isolates showed positive results for ACC deaminase production. Isolate #11 had the highest enzyme activity by producing α-ketobutyrate (102 µM/mg protein/h). Additionally, ACC deaminase-producing, root-colonizing, non-pathogenic bacteria with a variety of advantageous properties were choices, including *Bacillus licheniformis 10* (#10), *Pseudomonas aeruginosa 18* (#18), *Enterobacter ludwigii 11Uz* (#11), and *Enterobacter cloacae Uz_5* (#5). Treating wheat cultivar 'Chillaki' seeds with suspension #11 revealed a remarkable improvement in seed germination and growth strength (22%) under metal stress conditions. Plants grown under severe metal stress bore suspension #11 treatment, and the results showed a considerable improvement in plant growth metrics and total chlorophyll content compared with the control treatment. Additionally, in wheat seeds, the proline, catalase, and SOD activity rose by treating them with *Enterobacter ludwigii 11Uz* suspension. The results supported using ACC deaminase-producing *Enterobacter ludwigii 11Uz* (#11) for stress reduction by demonstrating that it can protect wheat plants from heavy metal stress via its antioxidant system.

Keywords: Local bacteria, wheat seeds, metal stress conditions, ACC deaminase, *Enterobacter ludwigii,* resistance, proline, SOD, CAT, germination ratio, growth strength

Key findings: ACC deaminase synthesizing bacteria with plant-growth stimulating properties showed the highest resistance to Ni and Cd cations. Select bacteria successfully investigated the morphometric characteristics and chlorophyll content of wheat plants grown under Ni and Cd stress conditions. Bacteria were notable for mitigating Ni and Cd stress conditions.

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INTRODUCTION

In the present era, one of the most critical problems for today's society is the heavy metal pollution caused by rapid urbanization and industrialization. Past studies described the distribution and type of heavy metals in soils of the Almalyk mining and smelting industrial area (Kodirov and Shukurov, 2009; Shukurov *et al*., 2014). Since heavy metals are non-decomposing into nontoxic substances, the complete removal of such metals is an effective solution to combat them (Bian *et al*., 2021; He *et al*., 2022). Therefore, in the ecosystem, metal pollution leads to contamination of food sources and eventually reaches the human food chain.

Considerable hazardous effects interlinked with heavy metals and metalloids, including Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb, and other minor metals. Cadmium belongs to group I carcinogens and is the most toxic environmental pollutant for human and animal life. Its high solubility in water increases its mobility in the soil ecosystem. Through the cycle of nutrients, it passes from the soil to plants and causes severe problems in human health (Liu *et al*., 2024) . Nickel (Ni) reduces seed germination, root and shoot growth, biomass accumulation, and total yield. In addition, Ni toxicity causes chlorosis and necrosis, inhibiting various physiological processes (photosynthesis, transpiration) and causing oxidation in plants. Examples of harmful effects of Ni on human health include allergic dermatitis, reproductive toxicity, and respiratory distress.

The grave pollution of agricultural fields constantly increased with heavy metals due to the overuse of mineral fertilizers, increasing crop cultivation intensity, and expanding cultivated areas. In such a situation, soil microorganisms, with ACC deaminase enzyme activity, break down ACC into α-ketobutyrate and ammonia, helping reduce ethylene production, which inhibits plant growth. As a result, it becomes easier for plants to adapt to stressful conditions and survive better with enhanced growth and productivity (Kang *et al*., 2019).

Furthermore, the phytohormone auxin can trigger the transcription of the ACC synthase enzyme, produced by soil microorganisms after pairing with the indole-3-acetic acid (auxin) produced by plant tissues. Therefore, using microorganisms that generate auxin phytohormones and ACC deaminase enzyme activity is crucial for reducing the quantity of ethylene and promoting plant growth during the bioremediation of heavy metal-contaminated soils (Nascimento *et al*., 2019). The findings of Kang *et al*. (2019) enunciated that tomato plants' resistance to salt stress received enhancement from the *Leclercia adecarboxylata (MO1)* strain, which generates ACC deaminase and indole-3-acetic acid.

Wheat plant is an essential food product in Uzbekistan. Toxic pollutants, such as heavy metals, can significantly affect plant and human health throughout the entire food chain. Wheat seeds with accumulated heavy metals can harm the cardiovascular and respiratory systems, and pollutants, such as Pb and Cd, can hurt the nervous system and kidney functions (An *et al*., 2020; Yang *et al*., 2021). In the presented investigations, the ACC deaminase enzyme activities' qualitative and quantitative assessment ensures the adaptation of plants to toxic conditions under heavy metal influences with different concentrations of Ni^{2+} and Cd^{2+}. Therefore, the promising study aimed to investigate the mechanisms of selected bacteria to prevent heavy metal stress conditions and increase plant productivity.

MATERIALS AND METHODS

Description of the study sites

This study proceeded in Southwest Uzbekistan, Samarkand Region (Pastdargom district - 39°41′11.0″N 66°48′19.8″E) and Kashkadarya Region (Dehkanobod district - 38°20′36.1″N 66°26′45.3″E). These areas have a typical hot climate, with an average annual temperature of roughly 28.4 °C and 200 mm of

precipitation. Based on the USDA soil taxonomy, the soil at this study's sites is in the serozem category. They have 14.23 g kg-1 of soil organic matter (SOM), 2.31 g kg-1 of total nitrogen (TN), 0.74 g kg-1 of total phosphorus (TP), 36.67 mg kg-1 of available phosphorus (AP), 0.42 g kg-1 of available potassium (AK), and a pH of 5.81.

Bacterial isolates in soil samples

The collection of 24 heavy metal-contaminated soil samples came from Samarkand, Pastdargom District, and Kashkadarya, Dehqonabad District, Uzbekistan, and remained in sterile paper bags at 40 °C until further additional study. The soil samples came from around areas with large factories producing chemical fertilizers with heavy metal contaminations found in the Samarkand and Kashkadarya regions. Brief random 5 cm diameter soil samples, taken from 15 to 30 cm depth, continued to mix as one composite sample. Collected soil samples in sterilized paper bags totaled 24, then packed in an icebox and delivered to the laboratory. The samples' serial dilution up to 10^7 times had each diluted soil sample independently inoculated on a meat peptone agar (MPA) medium in the Petri dishes. Then, the samples sustained incubation for 24 h at 37 °C. Every distinct colony in Petri dishes became a pure, sub-cultured one given a unique name.

Determination of ACC deaminase activity

Qualitative analysis

For this purpose, culturing 1 µl of pure bacterial suspension in Petri dishes had Dworkin-Foster (DF) agar supplemented with 5.0 g/l 1-aminocyclopropane-1-carboxylic acid (ACC) as the nitrogen source. Then, the samples reached incubation at 30 °C, using the DF agar without ACC as a control and monitoring for colony formation for 10 days (Gupta and Pandey, 2019). Bacterial isolates that formed colonies on DF agar supplemented with ACC were positive for ACC deaminase activity.

Quantitative analysis

The culturing of ACC deaminase-positive bacteria in DF broth supplemented with 3 mM ACC bore incubation on a shaker (48 h at 37 °C, 150 min/rotation). Incubated samples reached centrifugation in the cold (10,000 rpm for 10 min), with the supernatant measured in a spectrophotometer at 540 nm. The amount of α-ketobutyrate produced by the bacterial isolate after ACC degradation depended on the α-ketobutyrate standard graph (Pandey *et al*., 2013).

Bacterial cells count

Counting the microorganisms commenced in the liquid nutritional medium with high biomass at various Ni and Cd cations concentrations. For the first 24 h, these bacteria culturing ensued in peptone broth at 28 °C. Bacterial suspensions diluted up to 107 continued culturing on peptone agar with concentrations at 5, 10, and 30 times higher than the Permissible standard (PS) of $NiSO_4 \times 7H_2O$ and $CdCl_2$, i.e., 95.7 mg/l and 191.41 mg/l; 574.23 mg/l and 4.1 mg/l; and 8.2 mg/l and 24.6 mg/l and, respectively, and without addition (for the control variant). Culture growing for 24 h comprised a temperature of 28 °C. Using the McCready table as a guide helped determine the number of microorganisms.

Determining the total number of colony-forming units (CFU) continued by counting the number of microorganisms formed on the surface of Petri dishes. Following the formula by Zvyagintsev (1991) helped determine the number of microorganisms in 1 milliliter of suspension.

$$a = b \times c \times g,$$

Where:
α = represents the number of cells in milliliters of suspension;
b = is the average number of colonies per plate;
c = is the dilution at which planting happened, and

g = is the number of drops in milliliters of suspension.

Bacterial identification

Following morphological identification, bacteria with powerful resistance to nickel and copper, non-pathogenic, and positive for ACC deaminase production underwent biochemical analysis (using the Biochemical Reagent Kit from Hi-Media). Via the Marmur approach, the DNA extraction of specific bacteria transpired. Polymerase Chain Reaction (PCR) proceeded on isolated DNA using universal primers 27 F (AGAGTTTGATCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) for the 16S rRNA gene (Singh *et al*., 2019). Drawing a phylogenetic tree using the 16S rRNA gene sequence accessible in the NCBI database ascertained the degree of similarity between various bacteria.

Bacterial isolates ability to promote plant development

Bacterial isolates' ability to promote plant growth included indole 3-acetic acid (IAA) formation, nitrogen fixation, phosphate solubilizing activity, exopolysaccharide forming activity, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. These tested in vitro for root colonization ability (Badawy *et al*., 2022).

Bacteria effect on seed germination and growth

Growing selected bacterial isolates for three days continued in broth with different concentrations of Ni and Cd. Wheat cultivar 'Chillaki' seeds were samples for the research. One hundred wheat seeds, sorted and treated with hydrogen peroxide (H_2O_2), received washing three times in sterilized water. The seed soaking began with the bacterial supernatant grown in different concentrations of heavy metal and only distilled water for the control. Then, positioning the seeds for growing in the thermostat had the temperature set to 26 °C. Counting the number of seeds that sprouted on the third day of cultivation helped determine the germination energy of seeds. The growth capacity of the seeds was evident on the seventh day. In this case, the determination of the number of seeds that formed buds equal to the size of the seed or longer ensued (Bakhshandeh *et al*., 2021).

Determination of osmoprotector-proline quantity

Fresh wheat leaves (500 mg) bore crushing with aqueous 3% sulfosalicylic acid (10 ml) to produce a homogenate for centrifugation. Adding cold acetic acid (2 mL) and acid-hydrin solution (2 mL) to 2 mL of the supernatant advanced the mixture to reach incubation in a hot water bath (1 h) and then cooled immediately. Toluene (4 mL) was added to the reaction mixture, followed by stirring, and then incubated in the dark for 20 min. Light refraction visualization of the toluene phase used a spectrophotometer at the wavelength of 520 nm (Badawy *et al*., 2022).

Determination of antioxidant enzymes activity

For the protein content determination, half a gram of fresh wheat leaf incurred grinding in 10 mL of 50 mM KH_2PO_4 buffer (pH 7-8) and centrifuging at 10.000 g for 15 min. The catalase activity test (CAT) process used H_2O_2 as a substrate and potassium phosphate as a buffer and measured in a spectrophotometer at the wavelength of 240 nm. Combining the 0.4 ml of riboflavin (60 µM), 1.2 ml of methionine (130 mM), 0.6 ml of NBT (750 mM), 3.4 ml of phosphate buffer (0.2 M pH-7-8), and 0.3 ml of EDTA (10 mM) solution to 0.1 ml of supernatant to measure the superoxide dismutase activity (SOD). Using this approach, adding riboflavin will also make $O_{2,}$ and its inclusion initiates the reaction. The prepared samples' incubation took 15 min at 30 °C under 15 W fluorescent lamps. The control samples remained stored in a dark place. Subsequently, its measurement ensued at the wavelength of 560 nm in a spectrophotometer, with the results expressed as µmol/min/mg of protein. The results calculation used the means after running the analysis in triplicate.

RESULTS

Bacterial isolates and ACC deaminase activity

Twenty-six pure bacterial cultures were isolated from 24 soil samples and cultivated on a DFA medium supplemented with ACC to examine the ASA degradation ability. The selected bacterial isolates continued growing for 10 days on a DFA medium enriched with ACC, the substrate of the ACC deaminase enzyme, for the bacterial qualitative analysis. Bacteria cultivation in a DFA medium without ACC served as a control. The ACC deaminase enzyme activity in the bacteria was evident by observing growth in the nutritional media. Based on the qualitative analysis, the ACC deaminase activity confirmation was notably present in eight of the 26 bacterial isolates.

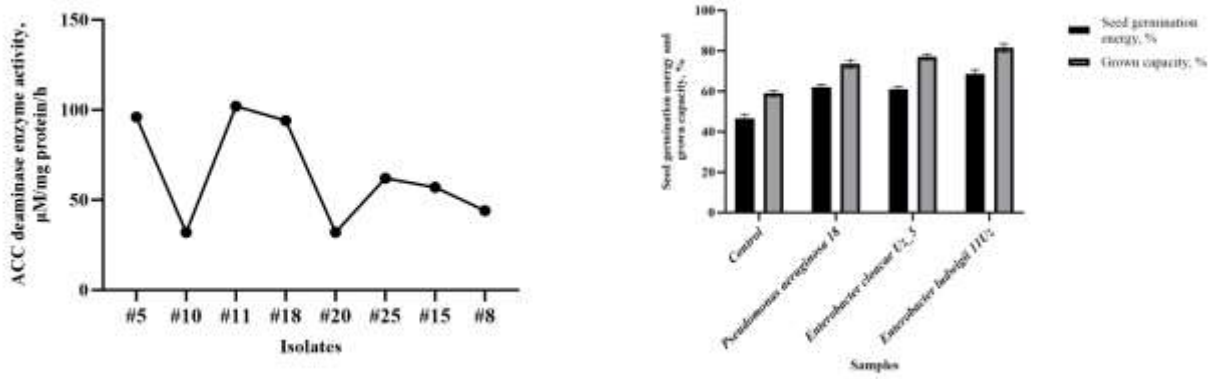
The study conducted a bacterial quantitative analysis of ACC deaminase enzyme activity using M. Murali's method. Based on the results, isolates #5, #11, and #18 have more ACC deaminase activity than other isolates (Figure 1B). Specifically, it was apparent that the formation of 96 µM/mg of protein/hour of α-ketobutyrate occurred in isolate #5 and 102 µM/mg of protein/hour of α-ketobutyrate in isolate #11.

Bacterial resistance assessment

The presented research used the microbiological dilution method to examine the titer of microorganisms cultured in nutrient media with varying ratios of nickel and cadmium. Initially, these bacteria culturing materialized for 24 h at 28 °C in Bj-11_0 liquid nutrition medium and peptone broth. Then, dilutions up to 10^7 times before planting them in MPA supplemented and not supplemented (for control) with 5, 10, and 30 times higher concentrations of Ni^{2+} and Cd^{2+} cations than PS. Cultures grew at 28 °C for 24 h. Bacterial isolates with powerful resistance to Ni^{2+} and Cd^{2+} cations were prominent in the bacterial isolates #5, #11, #10, and #18, selecting these for further studies for authentication. Therefore, these bacterial cultures can grow and develop in polluted soils with high Ni^{2+} and Cd^{2+} cations concentrations.

ACC deaminase impact on plant growth

Selected ACC deaminase-positive bacteria underwent qualitative and quantitative assessments of their ability to promote plant development. Based on the qualitative analysis, only four isolates (#5, #11, #10, and #18) out of eight were choices for additional study because of their durable resistance to Cd^{2+} and Ni^{2+} cations.



A **B**

Figure 1. Quantitative analysis of ACC deaminase enzyme activity of bacteria (A); Effect of bacteria on seed germination energy and grown capacity (B).

Table 1. Patterns of the most potent plant growth-promoting bacterial isolate identification of bacteria.

Isolates	Phosphate mobilization, μg mL^{-1}μg mL^{-1}	Root Colonization, Ability, log10 CFU/g	IAA, mL−1μg mL^{-1}	Exopolysaccharide, mg/l	Nitrogen fixation
#5	10.53±0.25	6.34±0.15	5.32±0.13	13± 0.16	+
#11	9.73±0.06	5.53±0.28	5.14±0.22	20±0.22	+
#18	9.12±0.31	4.92±0.33	4.34±0.37	14± 0.43	+
#10	8.53±0.02	4.32±0.17	3.78±0.41	16± 0.11	-

*Note: +: Positive result, -: Negative result.

A **B** **C**

Figure 2. Isolate #11 - a) general view of colony, b) colony, and c) microscopic view of cells.

Three bacterial isolates demonstrated a high level of efficacy when tested for their capacity to stimulate plant development (Table 1). The study discovered that in isolates #5, #11, and #18, the root colonization ability was 6.34±0.15, 5.53±0.28, and 4.92±0.33 log^{10}CFU/gr, and the amount of IAA synthesis was 5.32±0.13, 5.14±0.22, and 4.34±0.37 μg/mL^{-1}, respectively. Furthermore, it was also evident that these bacterial isolates revealed elevated levels of exopolysaccharide production and phosphate mobilization. Selected ACC deaminase-positive bacterial isolates (#5, #10, #11, #18) were initially set apart based on morphological (Figure 2) and biochemical characteristics (Table 2).

The molecular identification confirmed the findings based on the morphological, microscopic, and biochemical documentation. According to the results, isolates #5, #11, and #18 emerged as *Enterobacter cloacae, Enterobacter ludwigii,* and *Pseudomonas aeruginosa,* respectively. The research submitted the 16S rRNA nucleotide sequence of genetically identified strains to the National Center for Biotechnology Information (NCBI, USA) database and registered *Pseudomonas aeruginosa 18* (OQ932917.1); *Enterobacter ludwigii 11UZ* (OQ932957.1), and *Enterobacter cloacae UZ_5* (OQ932923.1). A phylogenetic tree construction of the 16S rRNA gene of the isolates sequenced used MEGA-64 (Figure 3).

Bacteria to produce ACC deaminase

Based on measuring wheat seed germination energy and growth capacity, the selected bacteria considerably enhanced the seed germination energy and growth potential compared with the control treatment (Figure 1B). For instance, while employing the *Pseudomonas aeruginosa 18* strain, the seed germination energy and growth capacity rose by 14% and 18%. In the *Enterobacter cloacae Uz_5* strain, it increased by 17% and 18%, and

Table 2. Morphologic-cultural and physiological-biochemical characteristics of bacteria resistant to high concentrations of heavy metal cations.

Descriptions	#5 isolate	#11 isolate	#18 isolate
Shape of Colony	Circular	Circular	Circular
The color	Transparent	Cream colored	Green- yellow
Margin	Entire	Entire	Entire
Surface	Smooth	Smooth	Smooth
Elevation	Convex	Umbonate	Flat
Gram staining	-	-	-
Mobility	+	+	+
Cell shape	Short rod	Short rod	Short rod
Optimum pH	7,0-7,2	7,0-7,5	4,5-6,0
Optimum °C	27 °C–35 °C	28 °C–32 °C	30 °C–37°C
Hydrolysis of starch	-	-	-
Hydrolysis of casein	-	+	-
Hydrolysis of tyrosine	-	-	-
Hydrolysis of gelatin	-	+	+
Acid production from D-glucose	+	-	+
Sucrose	+	+	+
D-mannose	+	+	+
Rhamnose	-	-	-
Acid production from lactose	-	-	-
Oxygen Relationship	Facultative Anaerobic	Facultative Anaerobic	Aerobic
Catalase	+	+	+
Oxidase	-	-	+
Citrate disposal	+	+	+

Note: +: Positive result, -: Negative result.

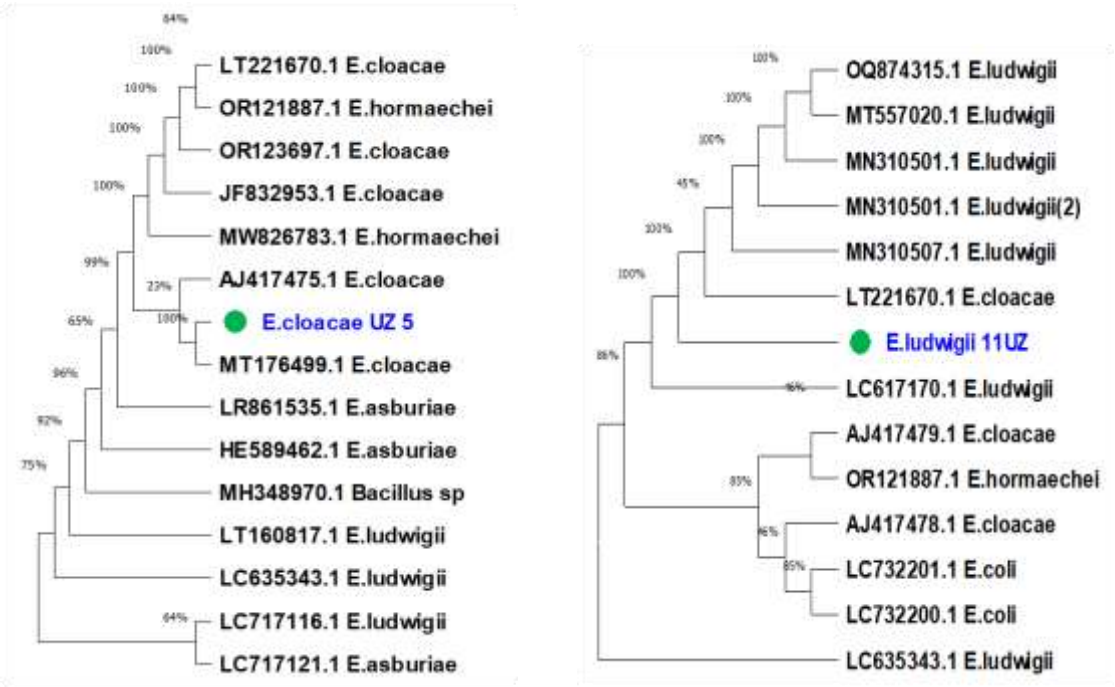


Figure 3. a) Phylogenetic tree of Enterobacter cloacae Uz_5, and b) Enterobacter ludwigii 11UZ.

in the *Enterobacter ludwigii 11Uz,* the growth energy and growing capacity enhanced by 22%, respectively, compared with the control treatment. The results further revealed that among the three selected bacterial strains, the strain *Enterobacter ludwigii 11Uz* was remarkable, with significantly higher potential in seed growth parameters than other strains and a selection for further studies.

***Enterobacter ludwigii 11Uz* effect on plant growth**

From the results, regardless of the different levels of heavy metal stress, the vegetative growth parameters were significantly higher in wheat plants treated with bacterial strain *Enterobacter ludwigii 11Uz* than the control plants without the bacterial addition. Plant height and fresh and dry biomass of the wheat plants treated with *Enterobacter ludwigii 11Uz* improved from 11.3 cm, 0.04 g, and 0.11 g to 18 cm, 0.09 g, and 0.25 g, and from 13 cm, 0.15 g, 0.05 g to 18 cm, 0.2 g, 0.07 g, respectively (Figure 4AB).

Total chlorophyll content

Total chlorophyll content was significantly superior in wheat plants treated with *Enterobacter ludwigii 11Uz* compared with untreated plants, regardless of the level of heavy metal stress (Figure 4F). Plants grown in soils containing high concentrations of CdCl2 and $NiSO_4 \times 7H_2O$ showed total chlorophyll content of 24.3 and 21.5 mg/l after application of bacterial suspension. Meanwhile, control plants without bacterial suspension showed the lowest total chlorophyll content of 15.6 and 14.7 mg/l. According to the results, it was noteworthy that the chlorophyll content increased by 35.8% and 31.6% in the wheat plants treated with bacteria grown in the soils compared to the respective control plants.

Bacteria's role in proline formation

Wheat plants placed in the control and bacterial suspension-supplemented soil showed an increase in the synthesis of a non-enzymatic antioxidant - proline, in response to increasing heavy metal concentrations (Figure 4E). Wheat plants cultivated at 57.42 and 191.4 mg/kg of $NiSO_4 \times 7H_2O$ soil occurred with increased proline levels, from 13.6 to 21.33 µmol/g when treated with *Enterobacter ludwigii 11Uz*, compared with control plants where proline level increased from 3.17 to 6.23 µmol/g grown without bacteria.

Similarly, proline synthesis under Cd^{2+} stress was higher in wheat plants treated with *Enterobacter ludwigii 11Uz* than in control plants. Specifically, plants grown in soil containing 2.4 mg/kg of $CdCl_2$ showed 14.43 µmol/g, while plants cultivated in soil containing 4.2 and 8.4 mg/g of $CdCl_2$ produced 17.98 and 23.22 µmol/g proline, respectively. In wheat plants planted as a control in soils without bacterial inoculation, the proline contents were 2.93, 2.7, and 2.3 times lower than values obtained with bacterial treatments.

Bacteria's role in synthesis of antioxidant enzymes

The synthesis of CAT and SOD was considerably better in the wheat leaves when plant cultivation was under Ni and Cd stress as opposed to non-stressed conditions (Figure 4CD). Concurrently, wheat plants grown under normal and stressful environments showed a considerable increase in CAT and SOD enzyme activity upon treatment with *Enterobacter ludwigii 11Uz*. The results further revealed that in cultivated plants in soils with 4.1 mg/kg of $CdCl_2$ added *Enterobacter ludwigii 11Uz* strain, the CAT content increased from 8.2% to 10.5%; SOD activity increased from 18.4% to 25.5%. Similarly, CAT and SOD enzyme activities increased by 1.12 and 0.76 times in bacteriologically treated plants grown with 191.4 mg/kg $NiSO_4 \times 7H_2O$ compared with the control plants. Plants treated with the *Enterobacter ludwigii 11Uz* strain, cultivated in soil containing 8.2 mg/kg $CdCl_2$, exhibited the highest enzyme activity of 154 (CAT) and 15.4 (SOD) U $min^{-1}mg^{-1}$ protein. Applying bacterial strain *Enterobacter ludwigii 11Uz* has manifested to protect wheat plants considerably from heavy metal stress conditions.

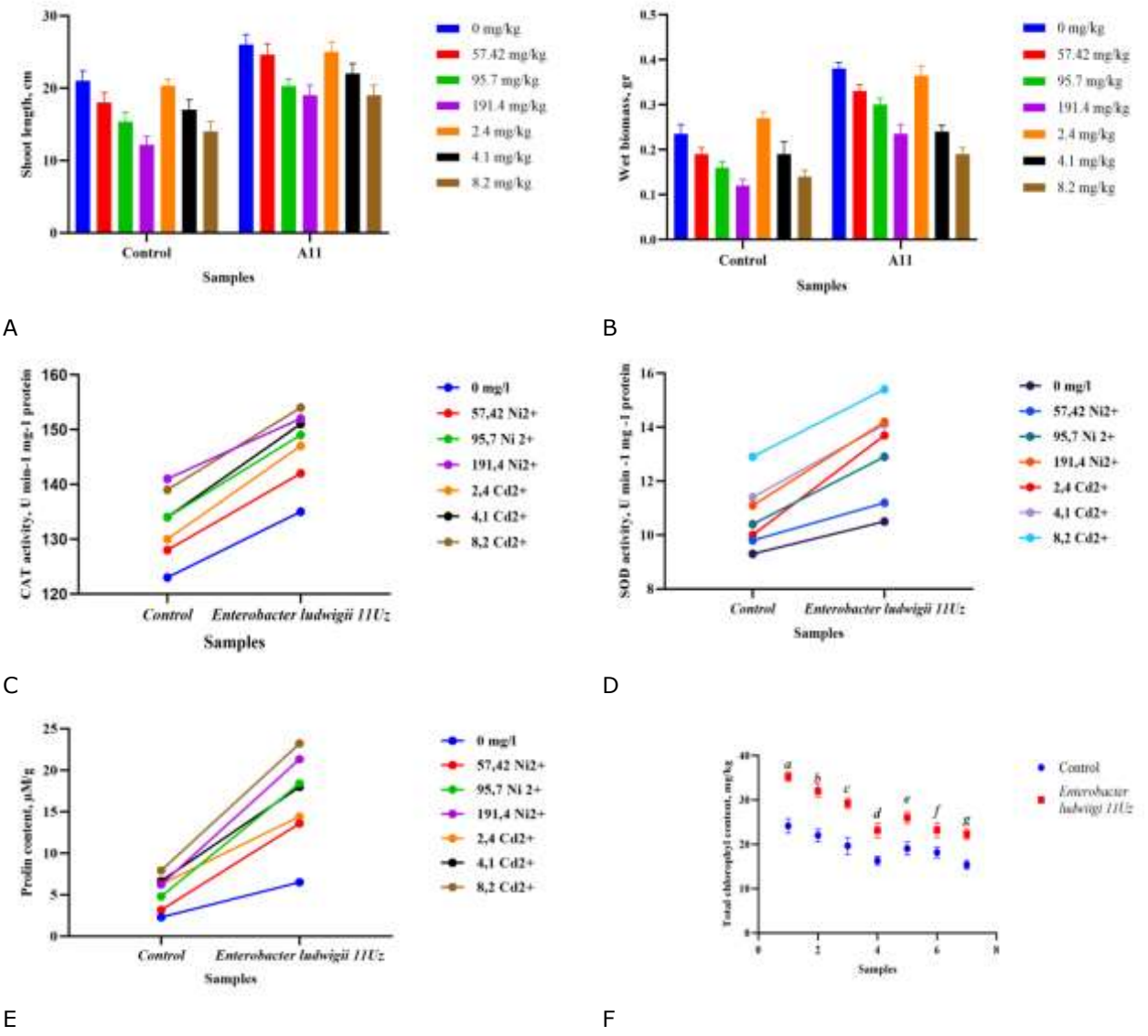


Figure 4. Morphometric and biochemical characteristics of plants grown in different concentrations of Ni and Cd. Shoot length (A); Wet biomass (B); CAT activity (C); SOD activity (D); Total proline content (E); and Total chlorophyll content of wheat plant (F): a-0 mg/kg; b- $NiSO_4 \times 7H_2O$ 57.42 mg/kg; c- $NiSO_4 \times 7H_2O$ 95.7 mg/kg; d- $NiSO_4 \times 7H_2O$ 191.4 mg/kg; e- 2.4 mg/kg $CdCl_2$; f- 4.2 mg/kg $CdCl_2$; and g-8.4 mg/kg $CdCl_2$

DISCUSSION

Plant growth-promoting soil bacteria with ACC deaminase enzyme activity are essential for plants to overcome abiotic stresses and ensure their better productivity (Nadeem *et al*., 2014; Tiwari and Yadav, 2019). Through the synthesis of enzymes, phytohormones, exopolysaccharides, amino acids, mineralizing organic phosphate, and modifying nutrient permeability, these enhanced the bioavailability of nutrients in the rhizosphere and hence stimulate plant development (Fouda and Sofy, 2022; Rahayu *et al*., 2023; Zubair *et al*., 2024). Plants can produce and accumulate solutes, including glycine, betaine, and proline, and lower ethylene levels to reduce the formation of reactive oxygen species (ROS). As

a result, using soil bacteria was visibly a crucial sustainable agriculture technique for lowering plants' osmotic and oxidative stresses (Mowafy *et al*., 2022; Usmonkulova *et al*., 2022, 2023).

HM-tolerant soil bacteria became selections for their capacities to stimulate plant growth and their resistance to elevated levels of Cd and Ni. One has observed that specific strains of *Pseudomonas aeruginosa, Enterobacter ludwigii,* and *Enterobacter cloacae* can solubilize poorly soluble phosphate salts. The soil contains massive mineral phosphorus as insoluble phosphates, which are unusable to plants (Pradhan and Sukla, 2006). Similar to the bacteria under investigation, documentation reported *Micrococcus luteus* and *Enterobacter cloacae* generate auxins and gibberellins and can solubilize phosphorus (Jha *et al*., 2014).

Cd and Ni stress conditions correlate with long-term storage of heavy metals in soil and water, significantly detrimental to agricultural productivity and human health (Ali *et al*., 2019). The accumulation of HMs in agriculture causes relevant damage to plant systems, including plant growth impairment (Khan *et al*., 2020), leaf chlorosis (Mohamed, 2011), and enzyme inhibition (El-Sheshtawy *et al*., 2021). In addition, Cd and Ni stress conditions also enhanced ROS generation and induced oxidative resistance in plants (Li *et al*. 2020). The ROS also adversely affects the DNA, chlorophyll, proteins, and membrane functions. Plants activate antioxidant systems, particularly non-enzymatic molecules (proline and carotenoids) and enzymatic substances (CAT and SOD), to repair and reduce the damage caused by ROS (Maksoud *et al*., 2022; El-Mahdy *et al*., 2021).

In the presented study, growing wheat plants continued in soil supplemented with Cd (8.2 mg/kg) and Ni (191.4 mg/kg) (without and with bacteria). It was apparent that a significant reduction in plant growth appeared under bacterial untreated Cd and Ni stress conditions. These results align with past research that showed how hazardous Cd and Ni are to several plant species (El-Mahdy *et al*., 2021; Mohamed *et al*., 2021). The results showed that microbial inoculation of seeds improved the developmental traits of plants in heavy metal stress conditions. Microbes can boost plant growth and biomass by changing the type of metals found in the soil and lowering the amount of metals that accumulate in plants. Certain soil bacteria can synthesize ACC-deaminase, an enzyme that promotes the uptake of essential nutrients, such as N, K, and P, and enhances plant growth under abiotic stress conditions (Al-Haithloul *et al*., 2022).

CONCLUSIONS

The results revealed that the bacterial strain *Enterobacter ludwigii 11Uz* produced the ACC deaminase and strengthened wheat's tolerance against heavy metal stress conditions. Plant growth metrics also indicated improvement by bacterial efficacy in enhancing heavy metal tolerance and by antioxidant mechanisms, such as enzymatic (SOD and CAT) and non-enzymatic (proline). Soil bacteria, such as *Enterobacter ludwigii 11Uz,* can enhance plant biomass and growth and counteract the harmful effects of metal wastes. It was due to their ability to synthesize active auxin, exopolysaccharide, ACC deaminase, and secondary metabolites, converting HMs into stable complexes. The ASK deaminase breaks down 1-aminocyclopropane-1-carboxylate into ammonia, reduces the level of ethylene in plants, and increases their resistance to stress. The EPS synthesized by bacteria strongly binds to metals and forms organic metal complexes, increasing the resistance of plants to toxic metals. As a result, the heavy metals content may decline in plants because the immobilization of heavy metals by microorganisms reduces the mobility of metals and the absorption of heavy metals by plants and their translocation to roots and stems. Therefore, the bacterial strain *Enterobacter ludwigii 11Uz* can be beneficial as the best bio-inoculant for wheat seeds to mitigate heavy metal stress conditions in the future.

REFERENCES

Al-Haithloul HAS, Khan MI, Musa A, Ghoneim MM, Aysh A, Khan I, Azab E, Gobouri AA, Sofy

MR, El-Sherbiny M, Soliman MH (2022). Phytotoxic effects of *Acacia saligna* dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. *Peer J.* 1:495–526.

Ali H, Khan E, Ilahi I (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *J. Chem.* 6730305.

An FQ, Li HH, Zhao QQ, Li BH, Lv J (2020). Study on isolation, identification and lead biosorption capability of a lead-tolerant penicillium sp. Pb-g from contaminated soil. *Appl. Ecol. Environ. Res*. 18(4): 5743–5757.

Badawy IH, Hmed AA, Sofy MR, Al-Mokadem AZ (2022). Alleviation of cadmium and nickel toxicity and phyto-stimulation of tomato plant, l. by endophytic *Micrococcus luteus* and *Enterobacter cloacae*. *Plants* 11(15): 2018. https://doi.org/10.3390/ plants11152018.

Bakhshandeh E, Abdellaoui R, Boughalleb F (2021). Modeling the effects of salt stress and temperature on seed germination of cucumber using halo thermal time concept. *Theor. Exp. Plant Physiol*. 33: 79–93.

Bian Z, Sun L, Tian K, Liu B, Zhang X, Mao Z, Huang B, Wu Y (2021). Estimation of heavy metals in tailings and soils using hyperspectral technology: A case study in a tin-polymetallic mining area. *Bull. Environ. Contam. Toxicol.* 107: 1022–1031. https://doi.org/10.1007/s00128-021-03311-7.

El-Mahdy OM, Mohamed HI, Mogazy AM (2021). Biosorption effect of *Aspergillus niger* and *Penicillium chrysosporium* for cd-and pb-contaminated soil and their physiological effects on *Vicia faba* L. *Environ. Sci. Pollut. Res.* 7: 1–24.

El-Sheshtawy HS, Sofy MR, Ghareeb DA, Yacout GA, Eldemellawy MA, Ibrahim BM (2021). Eco-friendly polyurethane acrylate (pua)/natural filler-based composite as an antifouling product for marine coating. *Appl. Microbiol. Biotechnol.* 105: 7023–7034.

Fouda HM, Sofy MR (2022). Effect of biological synthesis of nanoparticles from *Penicillium chrysogenum* as well as traditional salt and chemical nanoparticles of zinc on canola plant oil productivity and metabolic activity. *Egypt J. Chem.* 65:1–2.

Gupta S, Pandey S (2019). ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. *Front Microbiol.* 10: 1506.

He Z, Xu Y, Yang X, Shi J, Wang X, Jin Z, Zhang D, Pan X (2022). Passivation of heavy metals in copper–nickel tailings by in-situ bio-mineralization: A pilot trial and mechanistic analysis. *Sci. Total. Environ.* 838: 156504.

Jha Y, Subramanian RB, Jethwa R, Patel N (2014). Identification of plant growth promoting rhizobacteria from *Suaeda nudiflora* plant and its effect on maize. *Indian J. Plant Prot.* 42: 422–429.

Kang SM, Shahzad R, Bilal S, Khan AL, Park YG, Lee KE, Lee IJ (2019). Indole3-acetic-acid and ACC deaminase-producing *Leclercia adecarboxylata* MO1 improve *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. *BMC Microbiol*. 19(1): 80.

Khan ZS, Rizwan M, Hafeez M, Ali S, Adrees M, Qayyum MF, Khalid S, Ur Rehman MZ, Sarwar MA (2020). Effects of silicon nanoparticles on growth and physiology of wheat in cadmium contaminated soil under different soil moisture levels. *Environ. Sci. Pollut. Res.* 27: 4958–4968.

Kodirov O, Shukurov N (2009). Heavy metal distribution in soils near the Almalyk mining and smelting industrial area, Uzbekistan. *Ac. Geol. Sin.* 83(5): 845–1030. https://doi.org/10.1111/j.1755-6724.2009.00127.x.

Li H, Liu X, Wassie M, Chen L (2020). Selenium supplementation alleviates cadmium-induced damages in tall fescue through modulating antioxidant system, photosynthesis efficiency, and gene expression. *Environ. Sci. Pollut. Res.* 27: 9490–9502.

Liu M, Hu Z, Fan Y, Hua B, Yang W, Pang Sh, Mao R, Zhang Y, Bai K, Fadda C, Santis PD, Bergamini N, Usmankulova A, Buriyev SS, Zhang X (2024). Effects of leguminous green manure–crop rotation on soil enzyme activity and stoichiometry**.** *J Plant Ecol.* 17(6):rtae065. https://doi.org/10.1093/ jpe/rtae065

Maksoud MA, Bekhit M, El-Sherif DM, Sofy AR, Sofy MR (2022). Gamma radiation-induced synthesis of a novel chitosan/silver/mn-mg ferrite nanocomposite and its impact on cadmium accumulation and translocation in brassica plant growth. *Int. J. Biol. Macromol.* 194:306–316.

Mohamed HI (2011). Molecular and biochemical studies on the effect of gamma rays on lead

toxicity in cowpea (*Vigna sinensis*) plants. *Biol. Trace Elem. Res.* 144:1205–1218.

Mohamed HI, Abd-Elsalam KA, Tmam AM, Sofy MR (2021). Silver-based nanomaterials for plant diseases management: Today and future perspectives. *Elsevier* 495: 21-526, https://doi.org/10.1016/B978-0-12-823528-7.00031-7.

Mowafy AM, Agha M, Haroun S, Abbas M, Elbalkini M (2022). Insights in nodule-inhabiting plant growth promoting bacteria and their ability to stimulate *Vicia faba* growth. *Egypt J. Basic Appl. Sci.* 9: 51–64.

Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.* 32: 429–448.

Nascimento FX, Tavares MJ, Franck J, Ali S, Glick BR, Rossi MJ (2019). ACC deaminase plays a major role in Pseudomonas fluorescens YsS6 ability to promote the nodulation of Alpha- and Betaproteobacteria rhizobial strains. *Arch. Microbiol.* 201: 817–822.

Pandey S, Ghosh PK, Ghosh S, Kumar TD, Maiti TK (2013). Role of heavy metal resistant Ochrobactrum sp. and Bacillus spp. strains in bioremediation of a rice cultivar and their PGPR-like activities. *J. Microbiol.* 51: 11–17.

Pradhan N, Sukla L (2006). Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *Afr. J. Biotechnol.* 5: 850–854.

Rahayu T, Suparti, Asngad A, Widyayanti S, Kristamtini, Sidiq Y (2023). Endophytic bacteria from banana plant improves the growth and yield of black rice plant. *SABRAO J. Breed. Genet*. 55(3): 951-964. http://doi.org/10.54910/sabrao2023.55.3.29.

Shukurov N, Kodirov O, Peitzsch M, Kersten M, Pen-Mouratov S, Steinberger Y (2014). Coupling geochemical, mineralogical and microbiological approaches to assess the health of contaminated soil around the Almalyk mining and smelter complex, Uzbekistan. *J. Sci. Tot. Environ.* 476-477: 447–459. https://doi.org/10.1016/j.scitotenv.2014.01.031.

Singh SB, Gowtham HG, Murali M, Hariprasad P, Lakshmeesha TR, Murthy KN, Amruthesh KN, Niranjana SR (2019). Plant growth promoting ability of ACC deaminase producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). *Biocatal. Agric. Biotechnol.* 18: 101089. https://doi.org/10.1016/j.

Tiwari YK, Yadav SK (2019). Temperature-induced changes in anti-oxidative stress metabolism in maize*. Israel J Plant Sci.* 66: 203–212.

Usmonkulova A, Kadirova G, Juliev M, Samatov N, Sadullaeva M (2023). Bibliometric analysis of articles published in the Scopus database in 2019-2021 pertaining to the problem of heavy metals. *J. Sur. Fish. Sci.* 10(1): 209–227. https://doi.org/10.17762/sfs.v10i1.465.

Usmonkulova A, Shonakhunov T, Kadirova G (2022). Activity of nitrogen-fixing cyanobacteria under salinity and heavy metals stress. *J. Pharm. Neg. Resul*. 13(3):355–363. https://doi.org/10.47750/pnr.2022.13.03.055.

Yang YY, Zhang JX, Xiao X, Du M, Luan HJ, Yu QX, Liang Y (2021). Speciation and potential ecological risk of heavy metals in soils from overlapped areas of farmland and coal resources in Northern Xuzhou, China. *Bull. Environ. Contam. Toxicol.* 107(6): 1053–1058. doi: 10.1007/s00128-021-03148-0.

Zubair F, Riaz N, Khalid I, Amjad N, Usman M, Noorka IR, Khan ZI, Ahmad K, Ashfaq A, Ashraf S, Iqbal A, Javed S (2024). Antibacterial effect of *Cuminum cyminum* against selected bacterial strains. *SABRAO J. Breed. Genet*. 56(2): 787-793. http://doi.org/10.54910/sabrao2024.56.2.29.

Zvyagintsev DG (1991). Methods of Soil Microbiology and Biochemistry. Moscow State University Publishing House, Moscow, 304: 5-211-01675-0.