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BIOREMEDIATION OF NI (II) CATION-CONTAMINATED SOILS BY BACTERIA

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Abstract. The interaction of plant growth-promoting bacteria with plants and their ability to clean up contaminated soil has attracted more attention in recent years. In this study, three rhizobacteria strains (*Enterobacter ludwigi, Enterobacter cloacae, and Pseudomonas aeruginosa*) were examined to determine their individual and combined synergistic effects on the remediation of Ni -contaminated soils. Wheat was used as a test plant. Wheat seedlings were sown in soils containing 57.42, 95.7, and 191.4 mg/kg of nickel and were then given a 30-day treatment with a mixture of the rhizosphere bacteria *Enterobacter ludwigi, Enterobacter cloacae*, and *Pseudomonas aeruginosa*. Next, plant height, biomass in the stems and roots, and chlorophyll content were measured. It was discovered that plant growth characteristics were greatly improved when a consortium of three bacterial strains was used as opposed to the outcome when only one strain was used. The outcomes demonstrated the potential for synergistic bioremediation of Ni-contaminated soils and enhancement of plant development among the studied rhizobacteria. The findings of this study offer solid evidence in using an inexpensive, highly efficient microorganism-based bioremediation for soils contaminated with nickel.

Key words: bacteria, nickel, chlorophyll, bioremediation, pollution.

INTRODUCTION

One of the most important problems for today's society is heavy metal (HM) pollution caused by urbanization and industrialization. [1–3]. Effects of metal pollution on agricultural fields/soils lead to contamination of food sources [4, 5]. Cr, Co, Cu, Zn, Hg, Mn, Pb, Ni, Cd, Sn and other HM and metalloids have a great toxic effect. Increasing cropping intensity, bringing more areas under cultivation, and the development of farming practices with the latest technology, such as the use of fertilizers, have led to HM contamination of agricultural fields [6, 7]. Concentrations of metals or metalloids in agricultural soils are increasing rapidly, affecting plant growth, food security and soil microflora [8, 9]. HMs that are toxic to the environment can directly affect the integral part of plants and change their biochemical, metabolic and physiological processes [10, 11]. However, some of the HMs such as Mn, Cu, Co, Cr and Zn are important for the completion of metabolic activities in small amounts. Pollution with HMs is one of the main problems that affects not only human health, but also other biota living in polluted areas [12, 13]. The major components of pollutants are anthropogenic sources, although various pollutants can occur naturally in soil as mineral components and can be highly toxic [14–16]. Soil pollution is usually not clearly measured or visible, but is often a hidden problem. Thus, their complex nature and varying rates of degradation make soil studies difficult and expensive to determine the true extent of pollutant exposure. An accurate model for predicting Ni accumulation in plants will facilitate the assessment of soil quality and the potential risk of metals. However, due to soil heterogeneity, it is difficult to create such models on a large regional scale. The application of a multi-surface speciation model (MSM) to predict Ni accumulation in wheat at the field scale may serve as a useful tool in regional soil risk assessment and thus in the development of soil conservation measures [17]. Soil pollution is the third most important soil function in Europe and Eurasia, fourth in North Africa, fifth in Asia, seventh in the Pacific

Northwest, eighth in North America, and ninth in sub-Saharan Africa and Latin America. But due to their permanent and stable nature, these metals cannot be completely eliminated. These HMs and metalloids are bio-accumulators and gradually enter plants, animals and humans through the food chain or water and air. [3, 18]. However, some studies have shown that some microorganisms with strong biodegradability of HMs can reduce the toxicity of HMs and create a suitable soil environment for growing food crops [19, 20].

Therefore, this research aims to study the synergistic effect of *Enterobacter ludwigi, Enterobacter cloacae, and Pseudomonas aeruginosa* to promote plant growth in contaminated soils with Ni(II) cations.

MATERIALS AND METHODS

Characterization of the soil uncontaminated with heavy metals and preparation for the experiment. The experimental soil was obtained from the top 0–20 cm of an uncontaminated agricultural field near Tashkent, Uzbekistan (N31°32′2′′, E104° 41′41′′). The collected soil was passed through a 3 mm sieve and sterilized in a high-capacity autoclave sterilizer (JIBIMED LS-75HV, CHINA) at 121°C for 20 minutes. The physicochemical properties of the experimental soil sample included pH 6.5, total organic carbon 15.7 g/kg, total nitrogen 1.3 g/kg, and 1.32 smol/kg CEC. Prior to planting, sterilized soil was treated with 3 different concentrations of $NiSO_4 * 7H_2O$ solutions (57.42; 95.7, and 191.4 mg/kg). Solutions of nickel were prepared in distilled water and added to the soil in the containers and mixed well. Distilled water was added to the control soil samples in the volume equal to the volume of the solution added to the other samples.

Preparation of bacterial suspensions. In our previous studies, more than 50 new isolates isolated from the soils of Samarkand and Kashkadarya regions contaminated with heavy metals were found to be highly viable and resistant to Ni ions. Then, *Enterobacter cloacae, Enterobacter ludwigii* and *Pseudomonas aeruginosa* strains were selected for their plant growth and development promoting properties and designated as A5, A11 and A18, respectively. The following nutrients were used to growth the microorganisms chosen for research: peptone broth (PB) g/l: L-glucose -20, K_2HPO_4 - 0.5, $MgSO_4x7H_2O$ - 0.5, NaCl - 0.5, and peptone - 10. Four separately bacterial strains and their combination were used in the experiment (A5, A11, A18, A5&A11&A18). For 48 hours, bacteria were cultured at 28 ° C in peptone broth. 15 ml of a suspension containing 10^6 CFU ml-1 of one bacterium was added to the rhizosphere of plants. 5.0 ml of each culture suspension was added to the variants with three bacterial combinations. We added 15 mL of sterile peptone broth as a control [21 ,22].

Planting and preparation of growing conditions. Healthy seeds of the same size are surface sterilized with 1 % sodium hypochlorite solution for ten minutes. It is then washed with sterilized distilled water. The seeds are left in plain water until they form a seed. Germinated seeds are planted in the soil in plastic containers (diameter 17 cm, depth 10 cm) disinfected with five grams of alcohol, and a suspension of active cultures is added. Examples are given below:

T1. Control
T2. 57.42 mg/kg Ni + seed
T3. 57.42 mg/kg Ni soil + seed + A8
T4. 57.42 mg/kg Ni soil + seed + A10
T5. 57.42 mg/kg Ni soil + seed + A11
T6. 95.7 mg/kg Ni soil + seed
T7. 95.7 mg/kg Ni soil + seed + A8
T8. 95.7 mg/kg Ni soil + seed + A10
T9. 95.7 mg/kg Ni soil + seed + A11
T10. 191.4 mg/kg Ni soil + seed
T11. 191.4 mg/kg Ni soil + seed + A8
T12. 191.4 mg/kg Ni soil + seed + A10
T13. 191.4 mg/kg Ni soil + seed + A11

T14. 57.42 mg/kg Ni soil + seed + A8&A10&A11

T15. 95.7 mg/kg Ni soil + seed + A8&A10&A11

T16. 191.4 mg/kg Ni soil + seed + A8&A10&A11

Each sample was prepared in 3 replicates. During plant growth, the soil relative humidity is 53-57 %, the storage temperature is 27/21 ^{0}C during the day/night, the light intensity is 100-200 µmol m-2 s-1, and the ambient CO_2 level is 300-410 µmol mol- 1 is ensured. All plants in pots were watered with deionized distilled water every 2 days.

Measurement of plant growth indicators and chlorophyll concentration. After 74 days, wheat plants in each pot were harvested. Plant roots were washed with distilled water until soil particles disappears. Then their stem and root length and wet biomass were measured using an electronic analytical balance (EP214C 224S-CW, Switzerland). Root and stem dry weight samples were dried in a drying oven at 70° C for six days, and then their mass was determined [23].

Chlorophyll content was determined by SPAD-502 Plus chlorophyll content analyzer by measuring three locations of each plant leaf (Zhejiang Top Cloud-Agri Technology Co. Ltd, China).

Statistical analysis. All experimental data were analyzed by statistical package (SPSS 22.0) with one-way analysis of variance (ANOVA) to evaluate wheat growth performance and metals behavior in soils. Duncan's test was used to analyze statistical significance between treatments at a probability level of $P < 0.5$.

RESULTS AND DISCUSSION

Effect of nickel on plant growth parameters

Plants grown in soils with medium and high Ni concentrations compared to the control showed yellowing of leaves, biomass weight, and length were lower. Plant properties with three (A5&A11&A18) bacterial suspensions were significantly improved compared to plants with one bacterial suspension.

The shoot`s length was specifically 32.3 cm in the variants with the inclusion of the A5&A11&A18 bacterial suspension and 29 cm in the sample with only one A5 bacterial suspension. The control without the addition of bacteria suspension was found to have the lowest plant height (20 cm) and lowest amount of biomass (table 1, 2). The also demonstrated a significant impact of Ni stress on plant height. A significant difference was observed in plant shoot length at different nickel concentrations (highest 20 cm at 0 mg/kg soil and lowest 11.3 cm at 191.4 mg/kg soil). The significant effect of bacteria in reducing the harmful toxic effects of nickel can be known from changes in plant growth parameters and biomass.

Table 1 - Shoot and root length of wheat grown in different concentrations of Ni

Shoot length, cm					Root length, cm			
Ni mg/kg	0	57.42	95.7	191.4	0	57.42	95.7	191
Control	20	17	14.4	11.3	7.5	5.2	4.3	2.5
A5	29	26.1	25.2	17	8.4	7.8	6.5	4.5
A11	25.6	23.6	19.5	18	10.5	9	7.1	6.6
A18	22	19.3	19	14.3	8.5	6.5	6	5.5
A5&A11& A18	32.3	28	25	18	11.7	9.6	7.2	5.8

The length of the wheat shoot and root in the sample with a concentration of 191.4 mg/kg of nickel was 11.3 and 2.5 cm, respectively, in the absence of bacterial suspension, and 19.5 and 6.6 cm in the sample with the A11 bacterial suspension (figure 1). The plant shoot and root lengths were 18 and 5.8 cm when the A5&A11&A18 bacterial suspension was applied at a concentration of 191.4 mg/kg of nickel.

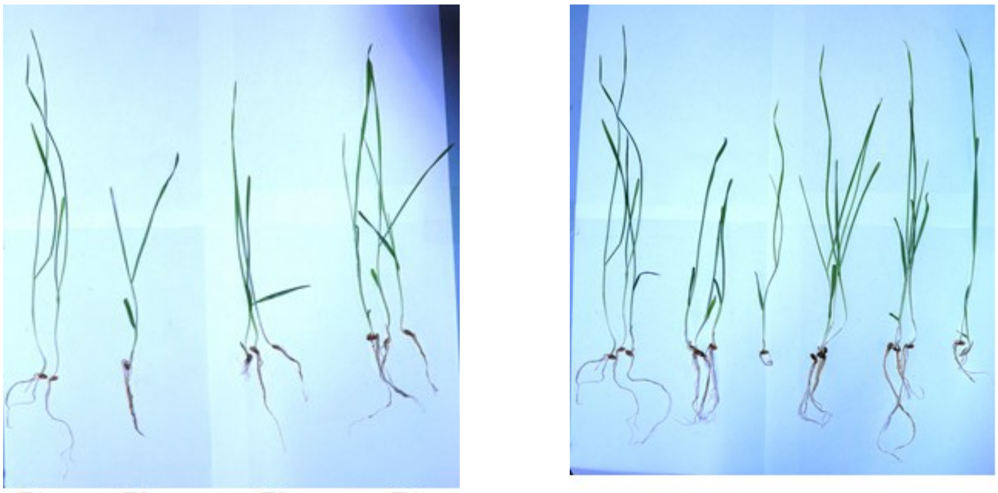


Figure 1 - General view of wheat shoot and root grown in different concentrations of Ni 57.42 mg/kg Ni concentration B) 95.7 mg/kg Ni concentration

From these results, it can be concluded that it is appropriate to use several bacterial communities to reduce the harmful effects of nickel on plant growth parameters. The interaction between Ni and bacteria had a significant effect on plant height as well as biomass production. Biomass of plants in the samples with bacterial suspension containing 3 bacteria recorded a higher result compared to the variants with one bacterial suspension.

Table 2 - Biomass of wheat grown in different concentrations of Ni

wet/dry biomass of shoot, gr					wet/dry biomass of root, gr			
Ni mg/kg	0	57.42	95.7	191.4	0	57.42	95.7	191.4
Control	0,17/ 0,06	0,15/ 0,05	0,12/ 0,04	0,11/ 0,04	0,04/ 0,01	0,03/ 0,01	0,02/ 0,01	0,01/ 0,007
A5	0,41/ 0,15	0,38/ 0,14	0,35/ 0,12	0,26/ 0,09	0,06/ 0,02	0,05/ 0,02	0,04/ 0,014	0,03/ 0,01
A11	0,39/ 0,14	0,34/ 0,13	0,31/ 0,11	0,25/ 0,09	0,07/ 0,025	0,06/ 0,02	0,05/ 0,018	0,04/ 0,014
A18	0,31/ 0,11	0,26/ 0,09	0,23/ 0,08	0,18/ 0,06	0,05/ 0,018	0,04/ 0,013	0,03/ 0,01	0,02/ 0,008
A5&A11&A18	0,45/ 0,16	0,42/ 0,15	0,32/ 0,11	0,27/ 0,1	0,09/ 0,03	0,08/ 0,028	0,07/ 0,02	0,05/ 0,017

The suspension of rhizobacteria also increased plant growth and biomass production in Ni-added soils (figure 2). When A8&A10&A11 strains suspension was applied to soils without Ni, the wet weight of shoot and root was significantly higher (0.49;0.09g). When the strains were applied separately to soils with different concentrations of nickel, the highest efficiency was shown by the A5 strain (57.42 mg/kg Ni in shoot-0.38g root-0.05g). At a nickel concentration of 191.4 mg/kg, the lowest weight shoots and roots was recorded in the sample using A18 strain suspension (table 2).

The dry biomass of wheat stem and root also decreased proportionally with increasing Ni concentration. At 0 mg/kg of Ni, the dry biomass of the shoot was 0.14 g when the suspension of culture strain A11 was inoculated and it was 0.09 g at the concentration of Ni at 191.4 mg/kg. The dry biomass of the root of the control variant increased by 0.01 mg/kg at the concentration of 191.4 mg/kg of nickel and when the A11 culture was added, the dry biomass of the root increased 3 times.

Effect of Ni (II) oxidative stress on wheat chlorophyll content

The chlorophyll content of plants inoculated with three bacterial suspensions was significantly higher than that of plants in all other samples (37.2). The control variant showed the lowest value (25.3) significantly.

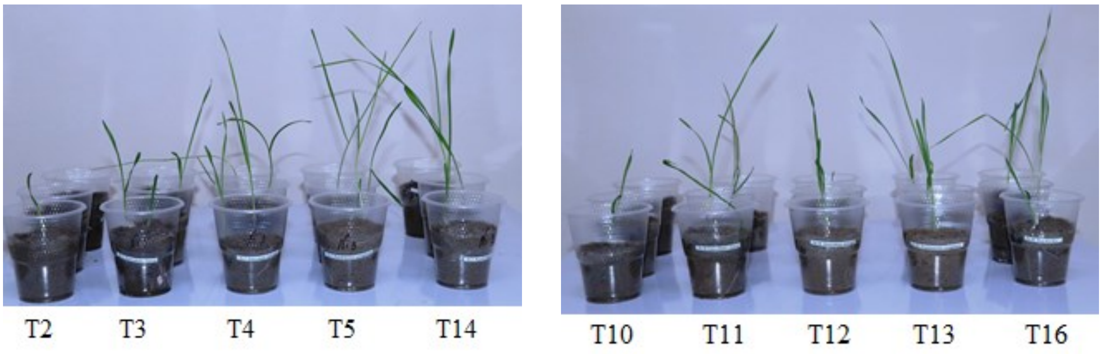
Table 3 - The relationship of different concentrations of Ni (mg/kg soil) and different bacterial strains to wheat chlorophyll content

Chlorophyll content mg/L				
Ni mg/kg	0	57,42	95,7	191,4
Control	25.3 ±0.21	21.1 ±0.27	18.3 ± 0.22	15.6±0.17
A8	28.7 ±0.23	23.7±0.37	19.6±0.5	17.3±0.26
A10	31.3±0.49	28.0 ±0.31	24.8±0.42	20.4±0.43
A11	34.5 ± 0.2	31.0±0.29	28.4±0.25	24.3±0.19
A8&A10&A11	37.2 ± 0.13	33.5± 0.27	29.2 ± 0.24	25.4 ±0.14

According to the results, chlorophyll content had an inverse relationship with Ni concentration, that is, the highest (25.3) chlorophyll content in plants was at 0 mg/kg Ni concentration and the lowest (15.6) value was recorded at 191.4mg/kg Ni concentration.

At various Ni concentrations, there are considerable differences in the amount of chlorophyll. Under the impact of A5&A11&A18 bacterial suspension from plants, the maximum average chlorophyll content (37.2) at the concentration of 0 mg/kg Ni and the minimum chlorophyll content (15.6) were determined in the no bacterial suspension variant at the 191.4 mg/kg concentration of Ni (table 3).

According to a number of studies, rhizobacteria are crucial for the removal of pollutants and toxins from the soil as well as for fostering plant growth in the face of diverse abiotic challenges [23–25]. Even though a suspension of a single strain increased plant biomass output, three strains were more effective. According to several studies, Klebsiella pneumoniae and Klebsiella sp. produce large quantities of phytohormones that promote plant growth, including IAA and ACC deaminase, which may be the reason for the high biomass yield [26]. Some authors have argued that the use of several bacterial strains is more effective than a single strain in the bioremediation of heavy metals [27–29]. The ability of Klebsiella sp and Enterobacter sp to tolerate high Cd concentrations favors plant growth in soils contaminated with heavy metals [24, 30, 31]. Therefore, this study aimed to investigate the synergistic effects of rhizobacteria in the remediation of Cd-contaminated soils.



A B

Figure 2 - General view of a wheat plant grown in different concentrations of nickel 57.42 mg/kg Ni concentration; B) 191.4 mg/kg Ni concentration

Concentrations of metals or metalloids in agricultural soils are increasing rapidly, affecting plant growth, food security, and soil microflora. Environmentally toxic heavy metals can directly affect plants and alter their biochemical, metabolic, and physiological processes. However, some heavy metals such as Cu, Mn, Co, Zn and Cr are important for metabolic activity, but in very small amounts [18]. The use of green plants and rhizobacteria promoting their growth is considered to be more effective in solving heavy metal pollution compared to several methods with different levels of efficiency and cost [32]. Microorganisms are important in soil-water-plant-pollution interactions. Their resistance to heavy metals, the toxicity of metals and the transformation of metal species into less toxic and soluble forms for plants, stimulating the growth of plants leads to an increase in plant biomass even under stress conditions. Soil microorganisms also play an important role in nitrogen fixation and carbon cycling in terrestrial ecosystems by decomposing plant and animal remains [33].

CONCLUSION

In the study, 3 communities of rhizobacteria and the individual ability of each of them were investigated in the bioremediation of soil contaminated with Ni (II) cations. As Ni concentration in the soil increases, plant growth parameters, including plant stem and root length, their wet and dry weight, and chlorophyll content decrease significantly. However, when plants were inoculated with rhizobacteria suspension, the measured growth parameters were observed to increase. As a result of individual and combined use of rhizobacteria, significant differences were found in plant growth parameters and chlorophyll content. The study showed that the application of several microorganism suspensions against the effect of metal concentration on oxidative stress had a higher efficiency in plant growth and development compared to the application of a single microorganism suspension. The study's findings suggest that soils contaminated with nickel can be bioremediated utilizing microorganisms that can withstand high nickel concentrations and lessen and soften the toxicity of heavy metals.

High resistance microorganisms to heavy metals aid in the growth and development of plants by allowing them to adapt to stressful situations in heavy metal -contaminated soil. In the future, useful soil microorganisms with high resistance to heavy metals and their synergistic activity can be used in the bioremediation process of heavy metal soils.

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ТҮЙІН

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БАКТЕРИЯЛАРДЫ ПАЙДАЛАНА ОТЫРЫП NI (II) ЛАСТАНҒАН ТОПЫРАҚТАРДЫҢ БИОРЕМЕДИАЦИЯСЫ

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Өсімдіктердің өсуін ынталандыратын бактериялардың өсімдіктермен өзара әрекеттесуі және олардың ластанған топырақты тазарту қабілетіне соңғы жылдары көбірек назар аударылуда. Бұл зерттеуде ризобактериялардың үш штаммы (*Enterobacter ludwigi, Enterobacter cloacae* және *Pseudomonas aeruginosa*) олардың Ni-мен ластанған топырақтарды қалпына келтіруге жеке және біріктірілген синергиялық әсерін анықтау жүргізілді. Бидай сынақ зауыты ретінде пайдаланылды. Бидай көшеттері құрамында 57,42; 95,7 және 191,4 мг/кг никель бар топыраққа егілді, содан кейін *Enterobacter ludwigii, Enterobacter cloacae және Pseruginosa aeruginosa* ризосфералық бактериялардың қоспасымен 30 күндік өңдеуден өтті. Әрі қарай өсімдіктің биіктігі, сабақтар мен тамырлардағы биомасса және хлорофилл мөлшері өлшенді. Тек бір штамм пайдаланған кездегі нәтижеге қарағанда үш бактериялық штаммнан тұратын консорциум пайдаланылған кезде өсімдік өсу сипаттамалары айтарлықтай жақсарғаны анықталды. Нәтижелер Ni-мен ластанған топырақтың синергетикалық биоремедиациясының әлеуетін және зерттелген ризобактериялар арасында өсімдіктердің дамуының жақсарғанын көрсетті. Бұл зерттеудің нәтижелері никельмен ластанған топырақтар үшін арзан, жоғары тиімді микроорганизмдер негізіндегі биоремедиацияны қолданудың нақты дәлелдерін ұсынады.

Түйінді сөздер: бактериялар, никель, хлорофилл, биоремедиация, ластану.

РЕЗЮМЕ

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БИОРЕМЕДИАЦИЯ ПОЧВ, ЗАГРЯЗНЕННЫХ КАТИОНАМИ НИКЕЛЯ С ИСПОЛЬЗОВАНИЕМ БАКТЕРИЙ

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Взаимодействие бактерий, стимулирующих рост растений, с растениями и их способность очищать загрязненную почву в последние годы привлекают все больше внимания. В этом исследовании были изучены три штамма ризобактерий (Enterobacter ludwigii, Enterobacter cloacae и Pseudomonas aeruginosa) для определения их индивидуального и комбинированного синергетического воздействия на ремедиацию почв, загрязненных никелем. В качестве опытного растения использовалась пшеница. Проростки пшеницы высевали в почву, содержащую 57,42; 95,7 и 191,4 мг/кг никеля, а затем подвергали 30-дневной обработке смесью ризосферных бактерий Enterobacter ludwigi, Enterobacter cloacae и Pseudomonas aeruginosa. Затем измеряли высоту растений, биомассу в стеблях и корнях и содержание хлорофилла. Было обнаружено, что характеристики роста растений значительно улучшались, когда использовался консорциум из трех бактериальных штаммов, в отличие от результата, когда использовался только один штамм. Результаты продемонстрировали потенциал синергетической биоремедиации почв, загрязненных никелем, и улучшения развития растений среди изученных ризобактерий. Результаты этого исследования предлагают убедительные доказательства использования недорогой, высокоэффективной биоремедиации на основе микроорганизмов для почв, загрязненных никелем.

Ключевые слова: бактерии, никель, хлорофилл, биоремедиация, загрязнение.

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