EFFECT OF BACTERIA ON GROWTH AND DEVELOPMENT OF WHEAT IN SOILS CONTAMINATED WITH Cu AND Pb CATIONS

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Abstract. *This study demonstrates the impact of microorganisms, including bacterial strains Bacillus pumilus, Bacillus atropheus, Bacillus licheniformis, Enterobacter ludwigii 11, Enterobacter cloacea 5 and their associations B. pumilus+B. licheniformis+B. atropheus; E. ludwigii+E. cloacea+B. pumilus on the growth and development of wheat under conditions of soil contamination with copper and lead ions. Exposure to heavy metals as plant growth parameters was determined by the length of their stems and roots, as well as dry and wet biomass compared to the control. It was found that when using a bacterial consortium consisting of three resistant strains, plant growth performance was significantly improved compared to the result obtained when using a single strain. In particular, in soil samples containing Cu cation at a concentration of 118 mg/kg of soil, it was observed that the length of the stem and root of the plant treated with the consortium of bacteria Enterobacter ludwigii 11+Enterobacter cloacae 5+Bacillus pumilis used in the experiments stimulated by 46.6% and 53.8%, respectively, compared to the control group. Based on the results obtained, it was shown that the studied bacteria have a synergistic potential in providing bioremediation of soils contaminated with Cu^{2+} and Pb^{2+} and stimulating plant growth.*

Keywords: *bacteria, Cu (II), Pb(II), heavy metal, soil, bioremediation, plant, root, stem.*

INTRODUCTION. In recent years, due to the rapid development of industry and agriculture, soil pollution with heavy metals has become more and more significant, which poses a great threat to humans and the environment [1, 2]. Examples of metals known to be significantly toxic to humans and the environment include chromium (Cr^{6+}), nickel (Ni^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), cadmi (Cd^{2+}) and mercury (Hg^{2+}) includes [3, 4]. Since heavy metals are not biodegradable, they are very persistent and their biological half-life is very long, leading to toxicity in the ecosystem. Therefore, it is very important to remove these heavy metals from the wastewater and soil system. Modern technologies for removing polluting heavy metals include sedimentation, cementation, ion exchange, and reverse osmosis, but these processes create new wastes such as combustion residues and do not solve the problem. In recent years, microbial bioremediation of heavy metals is replacing physicochemical methods [5-7]. Agricultural soils are contaminated with many heavy metals, which is a serious problem because they inhibit plant growth and cause many

toxicity symptoms in plants [8-10]. By eliminating the harmful effects of these heavy metals, it is possible to increase the yield and productivity of crops [11, 12].

The purpose of this work is to investigate the vegetative experiments carried out in laboratory conditions in the "plant + heavy metal + bacteria" model system. Cu^{2+} and Pb^{2+} aimed at researching the effect of various concentrations of ions on the growth and development of wheat plants.

MATERIALS AND METHODS. Characterization of the soil and preparation for the experiment The experimental soil was collected from the top layer (0-20 cm) of an agricultural field that was not contaminated with any heavy metals, Tashkent city, 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 experimental soil sample was characterized by physicochemical characteristics, including: pH 6.8, total organic carbon 15.7 g/kg, total nitrogen 1.3 g/kg 1.32 smol/kg CEC. Before planting, Cu(II) and Pb(II) solutions with 3 different concentrations were added to sterilized soils (Cu(II) - 35.4, 59.0, 118.0 mg/kg, Pb(II) - 28 .7; 48.0; 95.9). Solutions of Cu(II) and Pb(II) were prepared in distilled water and added to soil in containers and mixed thoroughly (Figure 1).

1-figure. Preparation of soil samples for experiments

Distilled water was added to the control soil samples in a volume equal to the volume of the solution added to the experimental samples.

Preparation of bacterial suspensions

In experiments, strains of *Enterobacter ludwigii, Enterobacter cloacea, Bacillus pumilis, Bacillus licheniformis, Bacillus atropheus and B. pumilus+B resistant to Cu(II) and Pb(II) cations. licheniformis+B. atrophy; E. ludwigii+E. cloacea+B.* Pumilus and its associations were selected according to their properties that stimulate the growth and development of the wheat plant. Microorganisms selected for research were grown in the following nutrient media: peptone broth (PB), g/l: L- glucose –20; K_2HPO_4 – 0,5; $MgSO_4x7H_2O$ – 0,5; NaCl – 0,5; pepton – 10. Bacteria planted in PB were grown at 28°C for 48 hours. In the research, 7 random forms of bacterial strains and their combinations were used (Enterobacter ludwigii, Enterobacter cloacea, Bacillus pumilis, Bacillus licheniformis, Bacillus atropheus, B. pumilus+B. licheniformis+B. atropheus; E. ludwigii+E. cloacea+B. . pumilus). of bacteria in soil samples 10^6 CFU ml^{-1} 15 ml of the suspension containing 5.0 ml of each culture suspension was added to the variants in which the combination of three bacteria was added [7]. Controls were supplemented with 15 ml of sterilized PB [13].

Planting a plant and preparing growing conditions

Healthy seeds of the same size were surface sterilized with 1% sodium hypochlorite solution for ten minutes and rewashed 3-4 times with sterilized distilled water. The seeds were left in plain water until the formation of a niche. Germinated seeds were planted in soil in plastic containers (diameter 17 cm, depth 10 cm) disinfected with 5 grams of ethanol, and a suspension of resistant cultures was added.

The sample system used is given below:

T1. Control
T2. 35,4 mg/kg soil Cu(II) + seed
T3. 35,4 mg/kg soil Cu(II) + seed + *Enterobacter ludwigii*
T4. 35,4 mg/kg soil Cu(II) + seed + *Enterobakter cloacea*
T5. 35,4 mg/kg soil Cu(II) + seed + *Bacillus pumilus*
T6. 59,0 mg/kg soil Cu(II) + seed
T7. 59,0 mg/kg soil Cu(II) + seed + *Enterobacter ludwigii*
T8. 59,0 mg/kg soil Cu(II) + seed + *Enterobacter cloacea*
T9. 59,0 mg/kg soil Cu(II) + seed + *Bacillus pumilus*
T10. 118,0 pdk soil Cu(II) + seed
T11. 118,0 pdk soil Cu(II) + seed + *Enterobacter ludwigii*
T12. 118,0 mg/kg soil Cu(II) + seed + *Enterobacter cloacea*
T13. 118,0 mg/kg soil Cu(II) + seed + *Bacillus pumilus*
T14. 35,4 mg/kg soil Cu(II) + seed + *E.ludwigii+E.cloacea+B.pumilus*
T15. 59,0 mg/kg soil Cu(II) + seed + *E.ludwigii+E.cloacea+B.pumilus*
T16. 118,0mg/kg soil Cu(II) + seed + *E.ludwigii+E.cloacea+B.pumilus*
T17. Control
T18. 28,7, mg/kg Pb + seed
T19. 28,7, mg/kg soil Pb(II) + seed + A8 *Bacillus pumilus*
T20. 28,7 mg/kg soil Pb(II) + seed + *Bacillus licheniformis*
T21. 28,7 mg/kg soil Pb(II) + seed + *Bacillus atropheus*
T22. 48,0 mg/kg soil Pb(II) + seed
T23. 48,0 mg/kg soil Pb(II) + seed + *Bacillus pumilus*
T24. 48,0 mg/kg soil Pb(II) + seed + *Bacillus licheniformis*
T25. 48,0 mg/kg soil Pb(II) + seed + *Bacillus atropheus*
T26. 95,9 pdk soil Pb(II) + seed
T27. 95,9 pdk soil Pb(II) + seed + *Bacillus pumilus*
T28. 95,9 mg/kg soil Pb(II) + seed + *Bacillus licheniformis*
T29. 95,9 mg/kg soil Pb(II) + seed + *Bacillus atropheus*
T30. 28,7 mg/kg soil Pb(II) + seed + *B.pumilus+B.licheniformis+B.atropheus*
T31. 48,0mg/kg soil Pb(II) + seed + *B.pumilus+B.licheniformis+B.atropheus*
T32. 95,9mg/kg soil Pb(II) + seed + *B.pumilus+B.licheniformis+B.atropheus*

3 replicates of each sample were prepared. During plant growth, the relative humidity of the soil is 53-57%, the storage temperature is 27/21oC during the day/night, the light intensity is 100-200 μmol m^{-2} s^{-1}, CO_2 value 300-410 mkmol mol^{-1} and all plants in pots were watered with deionized distilled water every 2 days.

Determination of plant growth parameters and chlorophyll content

After 30 days, the wheat plants in each pot were harvested and the plant roots were washed with distilled water until no soil particles remained. Stem and root length, wet biomass of wheat plants was measured using Vernier calipers and an electronic analytical balance (EP214C 224S-CW, Switzerland) was determined [14].

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 metal behavior in soils. Duncan's test was used to analyze statistical significance between treatments at a probability level of $P < 0.05$. Also, all figures were generated by GraphPad Prism 8.

OBTAINED RESULTS AND DISCUSSION

In preliminary studies, more than 50 isolates isolated from soil samples contaminated with heavy metals around the chemical enterprises of Samarkand and Kashkadarya region were identified as resistant species with high viability to Cu and Pb ions [15].

In these studies, Cu and Pb cations were much higher than the permissible limit (REM), i.e. 35.4 mg/kg, respectively; 59.0 mg/kg; 118 mg/kg soil and 28.7 mg/kg; 48.0 mg/kg; Heavy metal-resistant bacteria *Enterobacter ludwigii 11, Enterobacter cloacae 5, Bacillus pumilis, Bacillus licheniformis, Bacillus atropheus* local strains and their consortia were investigated compared to the control varieties. In particular, according to the data presented in Table 1, Enterobacter ludwigii strain 11 Cu cations of 59.0 mg/kg of soil and 118.0 mg/kg of soil compared to the control of wheat plant stem and root length. respectively 17.6% and 20% and 8.8% and

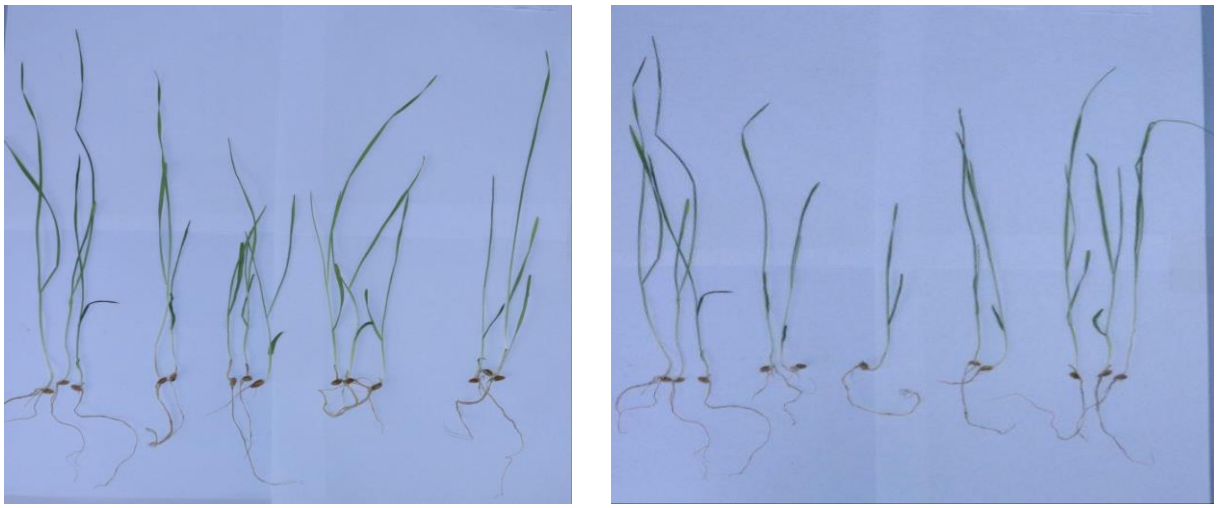
1-table

Stem and root length of wheat grown in different concentrations of Cu cation

Cultures	Stem length, cm				Root length, cm			
	Cu(II), mg/kg soil							
	0	35,4	59,0	118,0	0	35,4	59,0	118,0
Control	23±1,1	19±0,9	17±0,7	15±0,6	21,5±1,0	19±0,9	17±0,7	13±0,5
Enterobacter ludwigii 11	25±1,2	21±1,1	20±0,8	18±0,7	22±1,1	21±1,1	18,5±0,8	16±0,6
Enterobacter cloacae 5	27±1,3	24±1,2	19±0,9	17±0,7	23±1,1	22±1,1	19±0,9	17±0,7
Bacillus pumilis	28±1,4	25±1,3	21±1,1	19±0,9	25±1,2	24±1,2	21±1,1	18±0,8
E. ludwigii 11+ E. cloacae 5+ B. pumilis	31±1,6	28±1,5	25±1,2	22±1,1	27±1,3	25±1,2	23±1,1	20±1,0

It was observed that it was stimulated by 23% (Table 1, Figure 2). It should be noted that the plant properties treated with *Enterobacter ludwigii 11 + Enterobacter cloacae 5 + Bacillus pumilis* consortium of the bacteria used in the experiments showed a significant positive effect on plant properties treated with a single bacterial suspension. That is, as a result of the treatment of wheat plants with a consortium of bacteria, it was found that the stem and root length of the plant was stimulated by 46.6% and 53.8%, respectively, in the soil samples of Cu cation 118 mg/kg. It

is known that copper (Cu) is an important trace element for plants, because it participates in several oxidation-reduction reactions and structural structure of the Fe-Cu cluster.



A B

T1 T3 T4 T5 T14 T1 T7 T8 T9 T15

Figure 2. The general view of the stem and root of wheat grown in different concentrations of Cu(II) Cu concentration of 35.4 mg/kg soil; B) 59.0 mg/kg soil concentration of Cu.

Although Cu is required at very low concentrations, toxic levels cause physiological and biochemical disturbances that slow plant growth. The normal range of Cu concentration in higher plants is 2-20 mg/kg, Cu toxicity may occur at concentrations above the limit of this range [16, 17].

In subsequent experiments, the growth and development of wheat plants grown in different concentrations of Pb cation were affected by local strains of Bacillus pumilis, Bacillus licheniformis, Bacillus atropheus and their consortium B. pumilis +B, which are resistant to this metal. licheniformis +B. atropheus effect was studied. According to the data presented in Table 2, the amount of Pb cations selected in the research is 28.7 mg/kg of soil; Treatment of wheat plants grown at concentrations of 48.0 mg/kg soil and 95.9 mg/kg soil with these metal-resistant strains of bacteria and their consortium has a positive effect on the development of stems and roots of plants (2 -table, Fig. 3).

In particular, the treatment of wheat plants with Bacillus pumilis strain at concentrations of 48.0 mg/kg soil and 95.9 mg/kg soil of Pb cation increased the stem and root length of the plant by 26.4% under stress conditions, respectively. and increased by 14.3% and 9.6% and 15.38%. Pb^{2+} B. pumilis+B. licheniformis+B. atropheus consortium treatment was observed to promote stem length by 50% compared to the control.

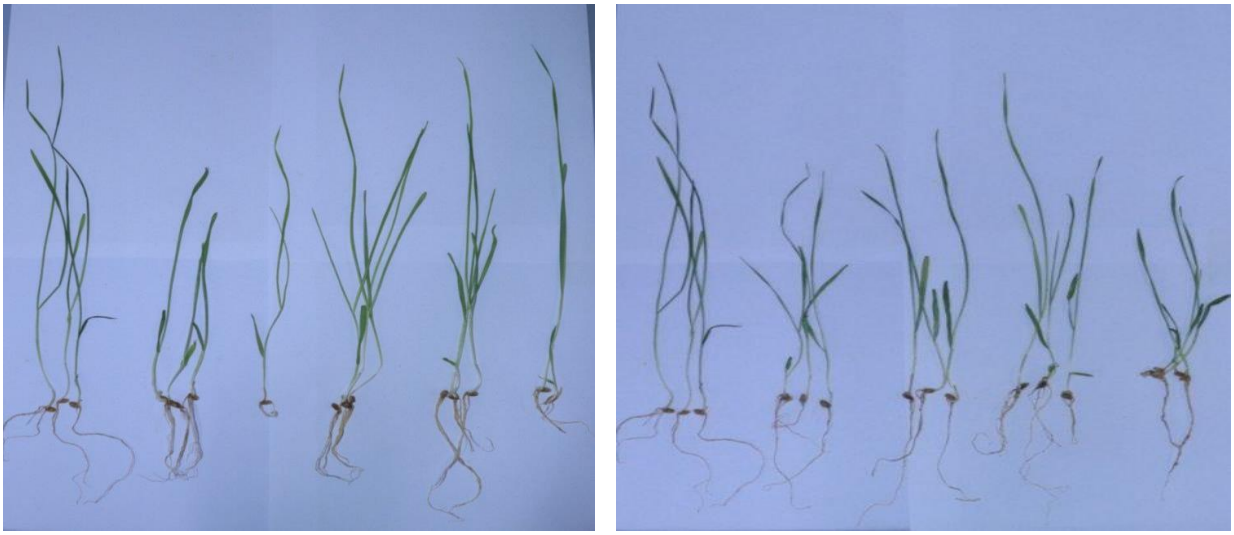
2-table

Stem and root length of wheat grown in different concentrations of Pb(II).

Cultures	Stem length, cm				Root length, cm			
	Pb(II), mg/kg soil							
	0	28,7	48,0	95,9	0	28,7	48,0	95,9
Control	21,5±1,1	19,1±1,0	17±0,7	14±0,6	19±0,9	18,5±0,8	15,5±0,6	13±0,5

Bacillus pumilis	23±1,2	22±1,1	21,5±1,1	16±0,6	21±1,1	20±0,9	17±0,7	15±0,6
Bacillus licheniformis	25±1,3	24±1,2	18±0,8	17±0,7	23±1,2	22±1,1	18±0,8	16±0,7
Bacillus atropheus	27±1,4	25±1,3	21±1,1	18±0,8	26±1,3	23±1,2	20±1,0	19±0,9
B. pumilis +B. licheniformis +B. atropheus	30±1,5	27±1,4	24±1,2	21±1,1	28±1,4	26±1,4	24,5±1,2	21±1,1

It is known from the literature that lead (Pb) is a potential example of a heavy metal, it is not an exchangeable element and does not play any role in the process of cell metabolism, but it is easily absorbed and is stored in various parts of the plant. will be planned. A high concentration of heavy metals such as lead slows down the growth of plants, has a negative effect on photosynthesis (chlorosis), roots



A B

T17 T18 T19 T30 T20 T21 T17 T24 T25 T31 T23

Figure 3. General view of wheat stem and root grown in different concentrations of Pb(II). 28.7mg/kg soil concentration of Pb; B) 48.0 mg/kg soil concentration of Pb

Lead has the ability to inhibit photosynthesis, disrupt mineral nutrition and water balance, change hormonal status, affect the structure and permeability of membranes [18, 19]. Alternatively, after uptake of heavy metals, plants increase various plant responses to combat heavy metal or metalloid stress, including the synthesis of the plant hormone auxin. Recent studies have shown the potential of auxins to counteract these stresses in plants, mainly by reducing their uptake, promoting chelation and vacuolar sequestration in plant tissues, and counteracting stress-induced oxidative damage. [20]. Therefore, in our studies, stimulation of wheat growth parameters under copper and lead stress by local bacterial strains confirms the above-mentioned points.

The significant effect of bacteria in reducing the harmful toxic effects of Cu and Pb can be seen from changes in plant growth parameters and biomass. The dry biomass of wheat stem and root also decreased proportionally with increasing Cu and Pb concentrations. According to the results obtained Cu^{2+} When the suspension of Enterobacter ludwigii 11 culture is introduced into the soil in the absence of ions, if the dry biomass of the plant stem is 0.15 g, Cu^{2+} 0.04 g of ion at a soil concentration of 118.0 mg/kg and Pb^{2+} The dry biomass of the stem treated with Bacillus pumilis culture in the soil sample that did not retain ions was 0.21 g, Pb^{2+} It was observed that it was 0.06 g at the soil concentration of 95.9 mg/kg.

CONCLUSION

Heavy metals are one of the main environmental factors affecting plants and microorganisms. In this article, the interaction of soil contamination with heavy metals (Cu, Pb) on the "plant-microorganism" system was considered. One of the most promising methods is the use of effective soil microorganisms to increase stress tolerance, as plant-associated microbes reduce metal accumulation and its toxic effects. The ability of metal-resistant Enterobacter ludwigii, Enterobacter cloacae, Bacillus pumilis, Bacillus licheniformis, Bacillus atropheus local strains in bioremediation of soils contaminated with Cu and Pb cations and their team (consortium) was tested. With increasing Cu(II) and Pb(II) concentrations in the soil, plant growth parameters, including plant stem and root length, wet and dry weight, were significantly decreased. However, it was observed that the inoculation of plants with a suspension of bacteria highly resistant to Cu and Pb cations leads to stimulation of plant growth parameters compared to the control. Metals, in turn, cause oxidative stress through the generation of free radicals; they have a negative effect on biochemical and physiological processes, disrupt photosynthetic and respiratory reactions, which subsequently leads to a general decrease in plant growth and development. The results of the study showed that the use of a consortium of several microorganisms against the effect of metal concentration on oxidative stress is more effective than the use of single microorganisms.

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