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Article

Bioremediation of oil-contaminated soil containing elevated concentrations of sodium chloride and heavy metals

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Abstract. The study of the effect of heavy metals and sodium chloride on the oxidative activity of oil destructor strains of the genus *Acinetobacter* showed that the bacteria retain the ability to biodegrade oil at concentrations of lead and zinc ions up to 1000 and 125 mg/l, respectively, and sodium chloride up to 3% by weight. The presence of oil (5% by volume) and sodium chloride (3% by weight) did not affect the synthesis of indolyl-3-acetic acid by microorganisms, the addition of lead and zinc salts to the nutrient medium stimulated the production of this phytohormone. In a model experiment, the possibility of using *Acinetobacter* strains, as well as associations of these bacteria and barley plants, to restore soils contaminated with oil (50 g/kg), also including (together with other pollutants): lead (200 mg/kg), zinc (300 mg/kg), and sodium chloride (5 g/kg). The introduction of *Acinetobacter* strains into contaminated soil increased the mass of barley shoots by 20.8–38.9% compared with untreated plants. When the soil was contaminated with oil alone, due to bacterization, the root mass increased by 11.7–23.1%, and when contaminated with oil and heavy metals – by 23.2–33.5%. In the presence of lead, zinc and sodium chloride, the efficiency of hydrocarbon biodegradation turned out to be higher than in the variant where the soil was contaminated only with oil. During 70 days of the experiment, with the combined use of plants and bacteria, the oil content in the soil decreased from 50.0 g/kg to 8.0–10.5 g/kg of soil.

Keywords: oil, zinc, lead, salinization, *Acinetobacter*, barley, biodegradation, plant-microbial interaction

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Научная статья

Биоремедиация нефтезагрязненной почвы, содержащей повышенные концентрации хлорида натрия и тяжелых металлов

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Аннотация. Изучение влияния тяжелых металлов и хлорида натрия на окислительную активность штаммов-нефтедеструкторов рода *Acinetobacter* показало, что бактерии сохраняют способность к биодеградации нефти при концентрации ионов свинца и цинка до 1000 и 125 мг/л соответственно, хлорида натрия – до 3% масс. Присутствие нефти (5% об.) и хлорида натрия (3% масс.) не оказывало влияния на синтез микроорганизмами индолил-3-уксусной кислоты, добавление в питательную среду солей свинца и цинка стимулировало выработку данного фитогормона. В модельном эксперименте была исследована возможность применения штаммов *Acinetobacter*, а также ассоциаций этих бактерий и растений ячменя для восстановления почв, загрязненных нефтью (50 г/кг), в том числе совместно с другими поллютантами: свинцом (200 мг/кг), цинком (300 мг/кг), хлоридом натрия (5 г/кг). Внесение штаммов *Acinetobacter* в загрязненную почву увеличивало массу побегов ячменя на 20.8–38.9% по сравнению с необработанными растениями. При контаминации почвы только нефтью за счет бактериализации масса корней увеличилась на 11.7–23.1%, нефтью и тяжелыми металлами – на 23.2–33.5% соответственно. В присутствии свинца, цинка и хлорида натрия эффективность биодеградации углеводов оказалась выше, чем в варианте, где почва была загрязнена только нефтью. За 70 суток эксперимента при совместном использовании растений и бактерий содержание нефти в почве снизилось с 50.0 г/кг до 8.0–10.5 г/кг почвы.

Ключевые слова: нефть, цинк, свинец, засоление, *Acinetobacter*, ячмень, биодеструкция, растительно-микробное взаимодействие

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Introduction

In most cases, environmental pollution is of a complex multichemical nature. When pollutants are combined, the toxic effect on wildlife is usually increased. Thus, the simultaneous presence of oil and other pollutants (heavy metals (HM), chlorides, etc.) in the soil is a serious environmental problem, especially relevant for agricultural lands adjacent to oil production areas (Khudur et al., 2018; Suleymanov et al., 2018; Tafeeva et al., 2016).

In Russia, the problem of HM pollution is quite acute. Most often, excess of safety standards for zinc, lead and cadmium is detected (Pishchik et al., 2016). Despite the fact that zinc is an essential element, an excess content in the environment can interfere with the normal physiology and contribute to human disease (El-Agawany and Kaamouh, 2023; Golovin et al., 2021).

Soil pollution with hydrocarbons can be accompanied by soil salinization caused by the influx of easily soluble salts from mineralized waters (Camacho-Monteaegre et al., 2021; Suleimanov et al., 2018). At the same time, a high content of toxic ions may have a negative effect on soil microorganisms, inhibits the activity of soil enzymes, and leads to stunted plant growth and development and lower crop yields (Fedorova et al., 2015; Kuzina et al., 2023).

There are various approaches to cleaning the natural environment. The main conclusions obtained by researchers in the study of soil detoxification processes are as follows. Plants are significantly less efficient than microorganisms at degrading and utilizing organic pollutants (Anokhina et al., 2018; Kuzina et al., 2021). Unlike petroleum products, HM are non-degradable and persist in the environment, but they are redistributed between different natural objects (Babayev et al., 2015; Khalid et al., 2017). Metal-resistant rhizosphere bacteria can have a certain impact on a plant's intake of HM (Anokhina et al., 2018), including by accumulating these compounds (Buzoleva and Krivosheeva, 2013). The efficiency of biological treatment of saline soils can be improved by introducing degrader bacteria that are high salt environments (Yastrebova and Plotnikova, 2007). Microbial remediation faces the problem of preserving introduced strains in an open ecosystem. Since the rhizosphere, as a natural environment, helps maintain a high population of microorganisms, the most effective option for restoring contaminated soils appears to be the use of plant-microbial complexes (Domracheva et al., 2022).

Numerous scientific papers discuss the impact of environmental pollution on the composition of the soil microbiota. Usually environmental pollution, leads to a decrease in beneficial rhizospheric bacteria and an increase in phytopathogenic microorganisms (Jiao et al., 2019; Li et al., 2022; Sazykina et al., 2022). Evidently, in such situations, the introduction of plant growth promoting bacteria (PGPB) is justified, since it allows plants not only to cope more easily with stress caused by soil contamination,

but also to participate more effectively in the process of its remediation. It is known that artificial microbial-plant associations are well adapted to adverse conditions, however, some factors (humidity, physico-chemical properties of the soil, composition and level of pollution) can negatively affect the effectiveness of the interaction of introduced PGPB and plants (Anokhina et al., 2018).

The aim of this work was to study the possibility of using strains of the genus *Acinetobacter*, as well as associations of these bacteria and barley for the restoration of soil with complex contamination. Oil was the main pollutant, while lead, zinc, and sodium chloride were associated pollutants.

Material and methods

The study involved strains from the collection of microorganisms of the Ufa Institute of Biology of the Ufa Federal Research Center of the Russian Academy of Sciences *Acinetobacter calcoaceticus* UOM 22 and UOM 29 and *A. courvalinii* UOM 35, isolated from soil samples from the territory of the Republic of Bashkortostan. These strains are characterized by the ability to biodegrade oil at 24 and 8 °C, synthesize indole-3-acetic acid (IAA), and are resistant to NaCl and HM (Korshunova et al., 2024).

To study the effect of sodium chloride and HM ions on oil degradation, bacteria were cultured in Raymond's liquid medium (Raymond, 1961) on a thermostatically controlled shaker (160 rpm, temperature +28 °C). Oil was introduced into a sterile nutrient medium in an amount of 5% vol. Sodium chloride, lead acetate and zinc sulfate were added according to the resistance of the strains to NaCl, Pb²⁺, and Zn²⁺ (Korshunova et al., 2024): 3% w/v sodium chloride, 1000 mg/L, 125 mg/L lead and zinc ions, respectively. After five days of cultivation, the paraffin-naphthene fraction of oil was extracted with hexane and analyzed on a gas chromatograph (Crystal Lux 4000, Russia) with a flame ionization detector and a Zebron™ ZB-1XT capillary column (30 m × 0.53 mm × 2.65 μm; initial column temperature +100 °C, heating rate 5 °C/min, final temperature +270 °C, carrier gas – helium). The degree of oil biodegradation (%) was calculated on the basis of chromatographic data using the internal normalization method in accordance with the instructions for the device (Borzenkov et al., 2006).

The IAA content in the culture fluid of the strains in the presence of oil, HM and sodium chloride was determined chromatographically (Starikov and Chetverikov, 2020). Bacteria were grown for 5 days at 28 °C on a medium with glucose (Dzerzhinskaya, 2008) with the addition of appropriate substances: oil 5% vol., Pb²⁺ 1000 mg/L, Zn²⁺ 125 mg/L, NaCl 3% mass.

The ability of cultures to synthesize biosurfactants was assessed by emulsifying activity (E_{24}) in relation to n-hexadecane (Mukhamatdyarova et al., 2024).

A model experiment on cleaning soil contaminated with oil and associated pollutants was conducted in laboratory conditions. Podzolized chernozem (Luvic Phaeozems) (upper horizon (0–2 cm)) was used, collected in the Baltachevsky District of the Republic of Bashkortostan with the following characteristics: humus content – 9.5%, pH_{KCl} – 5.9, N_{total} – 0.5%, P_{total} – 0.2%, K_{total} – 1.6%.

The experiment took place in two stages. At the first stage, the effect of the introduction of strains of the genus *Acinetobacter* on the reduction of oil content in the soil in the presence of excessive amounts of NaCl and HM was evaluated. 1 kg of a mixture of soil and sand (10% mass.) contaminated with oil from the Mamontovskoye field (Nefteyugansk district, Khanty–Mansi Autonomous Okrug – Yugra) in an amount of 50 g/kg was placed in plastic containers. Oil characteristics: density – 0.885 g/cm³, sulfur content – 1.2%, paraffins – 3.3%, resins – 7.9%, asphaltenes – 3.1%. Next, NaCl (5 g/kg) or HM salts were added. Soil contamination with lead (in the form of acetate) and zinc (in the form of sulfate) was carried out at doses exceeding the approximate permissible concentrations (UEC) (SanPiN 1.2.3685-21)¹: 200 and 300 mg/kg, respectively. Chemical analysis of the soil before the start of the experiment showed that the content of lead and zinc in it did not exceed the UEC and was at the level of 12.1 and 26.2 mg/kg, respectively.

The experiment was carried out in three repetitions. Soil treatment with microorganisms was carried out twice: at the beginning of the experiment and on the 21st day – at the rate of 10⁶ CFU/g of soil. For this purpose, a liquid culture of bacteria grown in meat-peptone broth (Dzerzhinskaya, 2008) was used for three days at 28 °C. For comparison, oil-contaminated soil was used, including those with additives of sodium chloride, lead acetate and zinc sulfate, but without bacterization.

¹ SanPiN 1.2.3685-2. Safety standards and requirements for ensuring the safety and (or) harmlessness of environmental factors for humans.

Every seven days, the soil was mixed and watered to 60% of the total moisture capacity. The number of the main ecological and trophic groups of microorganisms was taken into account by sowing a soil suspension on agarized nutrient media: ammonifiers on meat–peptone agar, micromycetes on Chapek medium, nitrogen-fixing on Ashby medium, hydrocarbon–oxidizing on Raymond medium, oligotrophs on low-nutrient agar, amylolytic on starch–ammonium agar, phosphate-dissolving on the Pikovskaya's agar, cellulose-decomposing – on the Hutchinson agar (Dzerzhinskaya, 2008; Pikovskaya, 1948). The total duration of the first stage of the experiment was 42 days.

The objective of the second stage of the experiment was to study the effectiveness of post-treatment of contaminated soil using combinations of the same bacterial strains with barley plants. Plants of spring barley (*Hordeum vulgare* L.) of the Chelyabinsk 99 variety were used. The choice of the latter as a phytoremediant is due to our previous studies (Kuzina et al., 2021), in which it has proven itself well as a component of microbial–plant complexes for cleaning oil-contaminated soil. To carry out the second stage with soil from containers (see the first stage) was filled with plastic cups, into which 6 pieces of 3-day-old barley seedlings were then planted. 7 days after that, the soil in the glasses was treated with a liquid culture of the same bacteria that were used at the first stage of the experiment (at the rate of 10^6 CFU/g of soil). The plants were grown at room temperature (+22...+24 °C) under controlled lighting (photon flux density $240 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, a 14 h photoperiod), and soil moisture was maintained at 60% of the total moisture capacity. The duration of the second stage of the experiment was 28 days.

After the experiment was completed, the dry mass of the shoots and roots of the plants was determined. For this purpose, each plant was divided into shoots and roots. The samples were dried in foil at 70°C for 48 hours in a dry-heat oven and weighed on an analytical balance.

The effectiveness of the microbial–plant associations used in this work was assessed by the loss of petroleum hydrocarbons in the soil and by the amount of HM accumulated by plants.

The content of oil products in the soil at the end of the first (42 days) and second (70 days) stages of the experiment was determined gravimetrically according to PND F 16.1.41–04².

The content of HM in plants was determined using an atomic absorption spectrometer with electrothermal atomization (Kvant-Z-ETA, Russia) according to the guidelines³.

Statistical processing was performed using standard MS Excel 2019 programs. Data are presented as mean \pm standard error of the mean. To assess the reliability of differences, Student's t-test was used ($p \leq 0.05$).

Results and discussion

The *Acinetobacter* bacterial strains used in the experiment were previously tested for resistance to increased HM content, as well as resistance to NaCl (Korshunova et al., 2024). In the course of this work, it was found that all three strains retain the ability to decompose oil hydrocarbons in the presence of lead and zinc salts, as well as sodium chloride (Table 1).

The concentration of sodium chloride in the liquid medium of 3% w/v had a negative effect on the biodegradation of oil. The ability to biodegrade oil was preserved to the greatest extent in the *A. courvalinii* UOM 35 strain.

In the presence of lead ions (1000 mg/l) and zinc (125 mg/l), the ability of bacteria to decompose oil decreased, but remained at a fairly high level (Table 1). At the same time, zinc sulfate was more toxic to microorganisms than lead acetate. This is because the zinc sulfate has a more pronounced dissociation of the aqueous solution, therefore, the release of components with an inhibitory effect is more intense. A similar effect of metal salts on bacteria of the genus *Bacillus* was reported by Buzoleva and Krivosheeva (2013). Lead, to a lesser extent than other HM (Zn^{2+} , Cu^{2+} , Co^{2+} , Cd^{2+}), inhibits the growth of microorganisms and is most actively adsorbed (adsorbed) by them.

The presence of oil, TM, and sodium chloride in the concentrations used did not have an inhibitory effect on the production of IAA by bacteria (Table 2). On the contrary, in the UOM 29 strain, zinc stimulated the ability to form this phytohormone, the production of which increased 4.7 times. In the presence of lead, its amount in the culture fluid of strains UOM 29 and UOM 35 also increased by 3.5 and 5.4 times, respectively.

² PND F 16.1.41–04. Quantitative chemical analysis of soils. The method of measuring the mass concentration of petroleum products in soil samples by gravimetric method.

Methodological guidelines for the determination of heavy metals in agricultural soils and crop production, 1992. Central Information Scientific and Analytical Association, Moscow, 61 p.

Table 1. Degradation of oil in a liquid medium in the presence of TM and sodium chloride, %; * – according to T.Yu. Korshunova et al. (2024).

Strain	Oil 5% vol.	Oil + Pb ²⁺ 1000 mg/L	Oil + Zn ²⁺ 125 mg/L	Oil + NaCl 3% w/v
UOM 22	93.0*	81.0	69.5	41.8
UOM 29	95.6*	91.0	82.1	59.6
UOM 35	95.9*	86.5	84.3	77.5

In contrast to the data presented above, in the laboratory experiment on soil purification from combined contamination, the rate of biodegradation of oil hydrocarbons in the presence of lead, zinc and sodium chloride was higher than in the variants where the soil was contaminated only with oil (Table 3). It is known that microorganisms, interacting with HM ions, may accumulate them to maintain the normal enzyme function. Alongside the accumulation of HM, the process of their detoxification takes place. As a result of redox reactions, metals change from an ionic form to a metallic form, form complexes with organic and inorganic compounds, while their toxicity decreases (Angulo-Bejarano et al., 2021; Klimova and Barysheva, 2017).

In addition, we found that the UOM 22, UOM 29, and UOM 35 strains are characterized by significant emulsifying activity ($E_{24} = 54\text{--}60\%$). It is possible that in the presence of metal cations, the production of biosurfactants increases, which helps the introducers to survive in an environment contaminated with TM. The produced biosurfactants also protect the native microbiota from toxic compounds, which contributes to its recovery and, therefore, may be one of the mechanisms underlying accelerated oil degradation.

According to M.V. Nosova et al. (2023), in the epicenter of soil pollution by oil emulsions, the total salt concentration is 0.35–1.57%. In our experiment, artificial salinization of the soil was created using sodium chloride at a concentration of 0.5%, corresponding to average salinity (Kallas and Maron, 2018).

An increased salt concentration leads to an increase in osmotic pressure, creates obstacles for the metabolic activity of microorganisms, but at the same time can stimulate growth of halotolerant bacteria (Yastrebova and Plotnikova, 2007). The studied degrader strains demonstrated that soil salinization (0.5%) does not preclude their oil-oxidizing activity. Barley plants placed in soil contaminated with NaCl showed significant growth inhibition, they died at the seedling stage, so the dry mass of shoots and roots and the content of oil products in variants with such soil were not measured.

The most significant consequences of the action of HM on plants are the inactivation of photosynthesis enzymes, disruption of the transport of assimilates and mineral nutrition, change in water and hormonal status, inhibition of growth and a decrease in productivity (Golovin et al., 2021; Patel et al., 2021). Evidently, an increase in the concentration of HM in the soil leads to an increase in their level in plant organisms. The results of the experiment showed that in the presence of *Acinetobacter* bacteria, the accumulation of HM in barley plants was less active (Table 4). In variants with the introduction of bacteria, the total lead content in barley shoots and roots was reduced to 0.7 to 0.75 of the level in the control. Similar results were published by I.O. Plekhanova et al. (2023) who established that the use of bacteria of the genus *Pseudomonas* reduces the accumulation of HM in the vegetative mass and roots of spring wheat plants.

It is most probable that the observed phenomenon is explained by the fact that the synthesis of IAA by rhizobacteria leads to an increase in the release of plant exudates (carbon compounds and lectins) into the rhizosphere. As a result, bacteria multiply intensively, and in turn accumulate HM, binding them to chelate complexes inaccessible to plants (Domracheva et al., 2022; Pishik et al., 2016).

The presence of oil negatively affected the development of both aboveground and underground organs of barley. In all experimental variants, the appearance of leaf chlorosis is recorded. Since the content of HM ions in the soil exceeded the UEC of these elements, non-inoculated plants grown in the presence of zinc significantly decreased in vegetative mass compared to the pure control (16.9%).

Table 2. The effect of oil, HM, and sodium chloride on the synthesis of indolyl-3-acetic acid by microbial strains; * – according to T.Yu. Korshunova et al. (2024).

Strain	IAA, ng/ml				
	Control	Oil 5% vol.	Pb ²⁺ 1000 mg/L	Zn ²⁺ 125 mg/L	NaCl 3% w/v
UOM 22	1416 ± 73*	1320 ± 75	1335 ± 73	1373 ± 75	1384 ± 74
UOM 29	521 ± 27*	534 ± 32	1822 ± 97	2455 ± 143	544 ± 37
UOM 35	658 ± 36*	680 ± 43	3552 ± 189	645 ± 46	687 ± 41

At the same time, in the variants where lead was present (with and without bacterization), the plants did not show a decrease in shoot weight (Table 5). Possibly the absence of lead phytotoxicity is due to the presence of root system's barrier functions (Lebedeva and Arzamasova, 2010). The toxicity of zinc to plants could be because its compounds, unlike lead salts, are more mobile, have less affinity for organic matter, greater solubility and, therefore, penetrate more easily into the root system of plants (Plekhanova et al., 2019).

The introduction of *Acinetobacter* strains into oil-contaminated soil (in the absence of HM) increased the mass of barley shoots by 26.3–30.7% compared to untreated plants (Table 5). In the experiments where HM were present along with oil, the use of bacteria also improved the condition of plants in soil with both zinc and lead.

The protective effect of bacterization can be explained by the immobilization of free zinc ions in the plant rhizosphere (Anokhina et al., 2018). As a result of this process, inoculated barley plants probably absorbed less zinc, therefore they had a more developed aboveground part compared to variant without the introduction of bacteria exposed to the same concentration of metal.

The formation of the barley root system was approximately the same in the soil contaminated only with oil and in those variants where, in addition to oil, HM salts were added. Inoculation of plants stimulated the growth of the root system. At the same time, contamination of the soil with only oil with the addition of bacteria led to an increase in the dry mass of roots by 11.7–23.1%, and in the presence of lead and zinc, this indicator increased by 23.2–32.3% and 24.1–33.5%, respectively. Thus, we are observing an example of an artificially created plant-microbial system that functions effectively under abiotic stress caused by combined soil pollution. Bacteria placed in the barley rhizosphere stimulate root formation through the production of IAA, which, among other things, induces plants to synthesize specific substances that promote the growth of beneficial microorganisms (Domracheva et al., 2022).

One of the pressing issues in environmental research is the study of the soil microbiome and its impact on the processes of transformation of substances in the ecosystem (Porkhuntsova et al., 2015; Prasad et al., 2021). Any type of soil pollution leads to the restructuring of indigenous microbial complexes. This is manifested in a decrease in the diversity of species composition, a decrease in the number of certain groups of microorganisms, outbreaks of phytopathogens and toxin-forming agents.

The presence of oil in the soil can either stimulate the reproduction and development of microorganisms, or inhibit them. In our experiment, oil and TM pollution caused a decrease in the number of cellulose-decomposing bacteria only; the number of representatives of other physiological groups increased (Table 6). The population density of hydrocarbon-oxidizing microorganisms and micromycetes increased by more than an order of magnitude. In soil contaminated with lead and sodium chloride, the number of micromycetes was the highest. The number of amylolytic microorganisms and oligotrophs increased more noticeably in the variants with soil polluted with zinc. The introduction of oil-degrading bacteria had a greater effect on the number of hydrocarbon-oxidizing microorganisms, which seems quite logical. In general, the number of bacteria in the rhizosphere of barley plants in the presence of lead and zinc ions was higher than in the variants without HM.

Table 3. Oil content in the soil, g/kg; «–» – no data available, because the barley plants died at the germination stage.

Experiment variants	1st stage	2nd stage
Without bacteria	30.7 ± 1.3	22.4 ± 1.2
UOM 22	23.8 ± 0.9	10.4 ± 0.5
UOM 29	23.6 ± 1.1	10.5 ± 0.6
UOM 35	22.8 ± 0.8	8.8 ± 0.4
Without bacteria + Pb ²⁺	24.1 ± 1.1	17.4 ± 0.9
UOM 22 + Pb ²⁺	17.4 ± 0.8	8.9 ± 0.5
UOM 29 + Pb ²⁺	16.8 ± 0.8	8.3 ± 0.4
UOM 35 + Pb ²⁺	16.8 ± 0.9	8.0 ± 0.5
Without bacteria + Zn ²⁺	25.8 ± 1.3	19.4 ± 1.0
UOM 22 + Zn ²⁺	17.3 ± 0.7	8.6 ± 0.4
UOM 29 + Zn ²⁺	16.0 ± 0.8	8.6 ± 0.5
UOM 35 + Zn ²⁺	16.1 ± 0.7	8.1 ± 0.4
Without bacteria + NaCl	25.2 ± 1.1	–
UOM 22 + NaCl	16.9 ± 0.7	–
UOM 29 + NaCl	16.7 ± 0.8	–
UOM 35 + NaCl	16.9 ± 0.9	–

Table 4. The content of lead and zinc in barley (shoot + root), mg/kg.

Experiment variants	Pb ²⁺	Zn ²⁺
Without bacteria	51.3 ± 2.6	123.1 ± 6.9
UOM 22	36.2 ± 1.5	115.3 ± 5.4
UOM 29	36.6 ± 1.9	109.4 ± 5.0
UOM 35	38.4 ± 2.1	110.8 ± 6.3

Table 5. Dry weight of shoots and roots of barley in oil- and HM-contaminated soil, mg.

Experiment variants	Root weight	Shoot weight
Without bacteria	10.21 ± 0.54	21.67 ± 1.07
UOM 22	11.40 ± 0.48	27.50 ± 1.45
UOM 29	12.00 ± 0.63	28.33 ± 1.32
UOM 35	12.57 ± 0.57	27.37 ± 1.14
Without bacteria + Pb ²⁺	10.67 ± 0.53	22.70 ± 1.02
UOM 22 + Pb ²⁺	13.15 ± 0.71	28.19 ± 1.54
UOM 29 + Pb ²⁺	14.12 ± 0.58	29.97 ± 1.53
UOM 35 + Pb ²⁺	13.57 ± 0.62	27.42 ± 1.53
Without bacteria + Zn ²⁺	10.00 ± 0.47	18.00 ± 0.93
UOM 22 + Zn ²⁺	12.41 ± 0.52	23.67 ± 1.29
UOM 29 + Zn ²⁺	13.13 ± 0.62	25.00 ± 1.35
UOM 35 + Zn ²⁺	13.35 ± 0.73	23.45 ± 1.31

Conclusions

1. The oil-decomposing strains *Acinetobacter calcoaceticus* UOM 22, *A. calcoaceticus* UOM 29 and *A. courvalinii* UOM 35 retain the ability to decompose oil in the presence of lead and zinc salts, as well as sodium chloride. The negative effect of zinc on the oxidative properties of strains is significantly higher than that of lead. The presence of NaCl in the amount of 3% w/v reduces the decomposition of oil by 18.4–51.2%. In the model experiment on soil bioremediation, the rate of hydrocarbon biodegradation in the presence of additional pollutants (heavy metals and NaCl) was higher than in the variants where only oil was present.

2. The introduction of oil, sodium chloride and heavy metals did not have an inhibitory effect on the process of IAA production by *Acinetobacter* strains. In the presence of lead, the synthesis of this phytohormone increased in strains UOM 29 and UOM 35, and in the presence of zinc – in strain UOM 29.

3. In the presence of *Acinetobacter* bacteria, the accumulation of TM by barley plants decreased. Apparently, the introduced bacteria, actively multiplying in the rhizosphere of the host plant, are able to regulate the level of toxic ions by bioaccumulation.

4. The increased concentration of zinc in the soil (1.5 UEC) had a more pronounced toxic effect on barley plants than lead contamination (1.5 UEC). The authors offer the following explanation: zinc compounds are more mobile, have less affinity for organic matter, greater solubility and, therefore, penetrate more easily into the root system of the plant. The introduction of *Acinetobacter* strains improved the condition of plants in oil-contaminated soil, as well as where oil and heavy metals were present.

5. Inoculation of plants enhanced the growth of shoots and the root system of plants. The ability of *Acinetobacter* bacteria to increase plant root formation is associated with their production of exogenous IAA. It is known that IAA stimulates plants to secrete exudates, contributing to an increase in the content of rhizospheric microorganisms.

6. Combined pollution caused a decrease in the number of cellulose-decomposing bacteria. At the same time, the content of other physiological groups increased: the number of micromycetes was greatest in soil contaminated with lead and sodium chloride, the content of amylolytic microorganisms and oligotrophs increased in variants with soil contaminated with zinc.

Table 6. The number of different physiological groups of microorganisms in the barley rhizosphere, CFU/g.

Experiment variants	Ammonifiers, $\times 10^8$	Oligotrophs, $\times 10^8$	Micro-mycetes, $\times 10^5$	Hydro-carbon-oxidizing bacteria, $\times 10^6$	Nitrogen-fixing microorganisms, $\times 10^8$	Amylolytic microorganisms, $\times 10^7$	Phosphate dissolving bacteria, $\times 10^6$	Cellulose-decomposing bacteria, $\times 10^2$
Control	0.11	0.11	0.40	0.014	0.13	0.15	0.16	45.3
Without bacteria	0.22	0.25	5.2	0.51	0.43	0.49	0.74	4.3
UOM 22	0.51	2.5	2.0	2.2	1.3	0.80	4.5	3.0
UOM 29	1.1	3.2	1.4	1.7	0.92	0.51	3.8	2.9
UOM 35	0.55	1.2	1.9	2.0	1.2	0.79	7.0	3.6
Without bacteria + Pb ²⁺	1.3	1.8	37.4	0.50	1.1	1.1	2.0	4.1
UOM 22 + Pb ²⁺	1.4	2.8	72.1	3.4	1.2	1.7	6.3	3.6
UOM 29 + Pb ²⁺	1.3	1.8	36.0	4.2	2.7	2.0	6.4	2.7
UOM 35 + Pb ²⁺	2.3	2.3	42.4	4.1	2.1	1.3	4.8	3.8
Without bacteria + Zn ²⁺	0.76	3.6	5.0	0.36	6.4	3.1	1.9	4.2
UOM 22 + Zn ²⁺	1.3	2.0	3.2	14.1	2.5	6.4	3.8	3.1
UOM 29 + Zn ²⁺	1.2	3.8	1.3	18.2	5.0	7.8	2.8	4.2
UOM 35 + Zn ²⁺	1.7	4.5	2.3	17.3	1.5	2.0	3.6	5.4
Without bacteria + NaCl	1.1	1.1	34.3	1.1	2.3	2.0	4.5	1.4
UOM 22 + NaCl	1.7	1.7	54.2	2.2	2.5	3.1	5.2	3.8
UOM 29 + NaCl	1.8	1.8	48.3	4.6	3.8	2.8	4.3	2.9
UOM 35 + NaCl	1.5	1.5	30.2	5.2	2.0	2.2	4.6	5.1

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