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DEGRADATION, REHABILITATION, AND CONSERVATION OF SOILS

Influence of Bioremediation on the Biological Activity of Leached Chernozem Contaminated with Oil and Lead

G. F. Rafikova[a], E. V. Kuzina[a], and T. Yu. Korshunova[a, *]

[a] *Ufa Institute of Biology, Ufa Federal Research Center, Russian Academy of Sciences, Ufa, 450054 Russia*

**e-mail: korshunovaty@mail.ru*

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Abstract—The effect of contamination with oil, lead, and their combinations and the influence of bioremediation with the use of microorganisms on the biological activity of leached chernozem (Luvic Chernozem) was comprehensively analyzed in a model experiment. The studied soil was sampled in Ufa district of the Republic of Bashkortostan and artificially contaminated with the listed pollutants. The soil was treated with hydrocarbon-oxidizing bacterial strains resistant to high concentrations of lead ions. All types of pollutants increased the soil phytotoxicity, while applied microorganisms reduced it, which was manifested in a rise in the seed germination index by 1.2–19.2% as compared to untreated variants. The addition of lead into the oil-contaminated soil reduced the degree of decomposition of hydrocarbons by 4.4–11.2%. Bacterization of contaminated soils enhanced the degradation of hydrocarbons by 6.2–33.8%. The total number of microorganisms increased in soils with oil and with oil and lead. Actinomycetes were most sensitive to the presence of xenobiotics. By the end of the experiment, the enzymatic activity of the oil-contaminated soil decreased. The presence of lead caused a slight rise in the catalase and invertase activities in the first half of the experiment. The combined contamination significantly suppressed the activity of catalase and urease. Bioaugmentation exerted a favorable effect on the restoration of the soil enzymatic activity. The applied bacterial strains contributed to a decrease in phytotoxicity and to an increase in the enzymatic activity of the soil, which makes them promising agents for soil bioremediation.

Keywords: oil, lead, hydrocarbon-oxidizing bacteria, phytotoxicity, enzymatic activity

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INTRODUCTION

In recent decades, special attention has been paid to the problems of anthropogenic pollution of soils and remediation of contaminated soils. Among the large number of pollutants, heavy metals (HMs) should be singled out: soil contamination with HMs is a global problem because of their long half-life and persistence in the environment. This leads to the accumulation of HMs in soils to the levels, when they begin to exert a toxic effect on living organisms and soil biological activity [9, 13, 79, 80]. The appearance of HMs in soils may be related to weathering of the parent rock, but the main factors of the soil contamination with HMs are the aerial emissions of metallurgical enterprises and motor vehicles, hydrogenic pollution with untreated wastewater and sewage sludge, the use of pesticides and fertilizers, the improper disposal of industrial waste, etc. [43, 56].

Another priority pollutant is oil. Emergency spills may occur during its extraction, transportation, storage, and processing. The input of hydrocarbons to a soil exerts a significant negative impact on the soil physicochemical and biological properties, as well as on the soil micro- and macrobiota [8, 9, 12, 15, 77]. In addition, there are cases of combined pollution, when HMs and oil are simultaneously present in the soil, which makes difficult its remediation [34, 50].

Bioremediation with the use of the metabolic potential of biological objects is a promising method of ecosystem rehabilitation, because this efficient and inexpensive method enables the removal of pollutants with minimal damage to the environment [57]. Numerous experiments have already been performed to determine the remediation impact on the biological activity of different types of soils contaminated with oil and HMs [16, 18, 24, 49], in the Republic of Bashkortostan, in particular [4, 14, 15]. Bioaugmentation is one of the variants of bioremediation; it implies the treatment of disturbed areas by particular microorganisms or their consortia with the required enzymatic properties and adaptive potential. Hydrocarbon-oxidizing bacterial strains resistant to increased concentrations of HMs in the environment are of particular interest for purification of soils contaminated with both oil and HMs [26, 42, 50, 59]. However, there are few studies of the biological activity of soils purified from oil and HMs by applied microorganisms [7].

The aim of this work is to study the effect of oil and various doses of lead on the enzymatic activity and

microbiological parameters of leached chernozem, as well as to evaluate the efficiency of the application of oil-destructing bacteria for its recovery.

OBJECTS AND METHODS

Research objects. We studied the top 20-cm-thick layer of leached chernozem (Luvic Chernozem) from Ufa district of the Republic of Bashkortostan in a model experiment. The soil was characterized by the following parameters: pH_{KCl} 6.3, N_{total} 0.61%, the humus content 6.8%, and available (0.2 N KCl extract) P_2O_5 and K_2O 94.5 and 101.7 mg/kg soil, respectively. Coarse roots and other plant residues were preliminarily removed from the soil samples, which were then air-dried, sifted through a sieve (mesh size was 1 cm), and placed in pots (3 kg each). The water-air regime was optimized by drainage; the soil water content was maintained at the level of 60% of the total water capacity during the experiment, and regular soil loosening was performed. We applied oil (density, 852 kg/m^3; viscosity, 28 mPa s; the contents of paraffins 3.3 wt %, resins 8.5 wt %, and asphaltenes 5–9 wt %) at the rate of 50 g/kg soil. This variant of the experiment corresponds to the range of oil concentrations, which is considered a stress zone for the soil microbial system characterized by an increase in catabolic activity and in the number of hydrocarbon-oxidizing microorganisms (HCOMs) [11]. Bioremediation with the use of active hydrocarbon-oxidizing strains of microorganisms is usually very efficient at this level of soil contamination [3, 6]. Higher concentrations of petroleum hydrocarbons may inhibit bacterial growth, which results in a low intensity of biodegradation and even in the death of hydrocarbon-oxidizing bacteria [54]. We also applied lead (Pb^{2+}) in the form of chemically pure salt dissolved in water ($Pb(CH_3COO)_2 \cdot 3H_2O$) (Reakhim, Russia) at the rates of 450, 900, and 1800 mg Pb^{2+}/kg, which corresponded to 15, 30, and 60 MPC (at present, the maximum permissible concentration (MPC) of lead in soil in the Russian Federation is 30 mg/kg), and a liquid culture of bacteria at the rate of 2×10^6 CFU/g. A corresponding amount of distilled water was added to the variants without application of solutions of the lead salt and/or inoculum. The experiment was performed in triplicate. The variant without lead salt, oil, and bacteria (background variant) was used as a control. Soils were sampled on the third, 45th, and 95th days of the experiment. The samples were air dried, sieved through a 2-mm sieve for homogeneous mixture, and stored in plastic bags at 4°C for the analyses.

Strains of microorganisms. Strains of bacteria from the collection of microorganisms of the Ufa Institute of Biology, Ufa Federal Research Center, Russian Academy of Sciences—*Thalassospira xiamenensis* UOM 2 (UOM 2), *Enterobacter* sp. UOM 3 (UOM 3), *Pseudomonas songnenensis* UOM 4 (UOM 4), and a microbial composition (**MC**) of all of these strains—were applied to the contaminated soil.

Strain *Enterobacter* sp. UOM 3 is close to typical strains of the species *E. asburiae* and *E. ludwigii* [23]. Representatives of these species have been described as typical endophytic and rhizospheric bacteria and recommended for their application in agriculture [68, 83]. Numerous studies of recent years, ours in particular, have shown the efficiency of *Enterobacter* strains for oil decomposition in soils because of their high viability hydrocarbon-oxidizing capacity [23, 27, 35, 38, 47, 73].

These microorganisms proved to be not antagonists towards one another; they were tolerant to both oil and lead and could synthesize the phytohormone indolyl-3-acetic acid [23, 31]. Bacteria were cultivated in a thermostatically controlled shaker on meat-peptone broth at 180 rpm and a temperature of 28°C during 72 h [19]. Liquid cultures of each strain were mixed at the ratio of 1 : 1 : 1 to create a microbial composition. It was determined that the number of cells of each bacterial strain cultured on the Raymond medium with oil (5%) [67] and lead acetic acid (Pb^{2+} content was 2.5 mg/mL) reached at least 1×10^6 CFU/mL within five days of incubation. This indicates that the strains are resistant to increased concentrations of Pb in the medium and can use petroleum hydrocarbons as a source of carbon.

We isolated microorganisms by a conventional method of inoculation on nutrient agarized media: nutrient agar (peptone, 10 g/L; yeast extract, 5 g/L; NaCl, 5 g/L; glucose, 1 g/L; agar. 15 g/L; and 1000 mL of distilled water) for heterotrophic microorganisms; Ashby medium for oligonitrophils and nitrogen fixers; Hutchinson medium for cellulolytic microorganisms; starch–ammonia agar for actinomycetes; Tsukamura medium for HCOMs (100 μL of sterile diesel fuel was applied to the medium surface as a carbon source in each Petri dish); and acidified Czapek's medium (pH 4.5) for micromycetes [19, 21].

Phytotoxicity of the samples of leached chernozem was assessed by biotests on seed germination and growth of radish (*Raphanus sativus* L.) of Pink-red variety with white tip sterilized in a 1% solution of potassium permanganate. Water extracts from the soil were prepared by shaking weighed soil samples with distilled water (with t the weight to volume ratio of 1 : 10) for 1 h followed by filtering red [66]. Filter paper was placed in Petri dishes, 5 mL of soil water extract was applied to it, and 20 radish seeds were put. Seeds treated with distilled water were used as a control. Seed germination was evaluated according to [75]. Petri dishes were incubated for 72 hours at 22°C. When root appeared, the seeds were considered sprouted. The percentage of seed germination, root elongation, and germination index were calculated according to [66]:

Seed germination (%) = (number of germinated seeds in the sample extract/number of germinated seeds in the control extract) × 100.

Root elongation (%) = (mean root elongation in the sample/mean root elongation in the control extract) × 100.

Germination index (%) = (seed germination (%) × root elongation (%))/100.

The soil water-retention capacity was determined according to *GOST* 26713-85.

We used the following methods to analyze the activity of soil enzymes: by Galstyan for catalase and invertase activity and by Shcherbakov and Raikhinshtein for urease activity [22].

The soil reaction (pH) was evaluated by the potentiometric method according to *GOST* 27979-88.

The content of petroleum products in the soil was determined gravimetrically as described in [58].

The data were statistically processed using standard MS Excel programs. Mean dada ± standard error were presented in figures and tables. The reliability of differences was assessed by the Student *t*-criterion.

RESULTS AND DISCUSSION

Phytotoxicity. The germination index of tested seeds on the 95th day of the experiment was 91.4% for the background soil and 48.4% for the soil with oil (Table 1). The low germination index was related to the high concentration of toxic components in the pollutant, which exerted an inhibitory effect on seed germination and root growth [39]. In addition, oil pollution reduced water availability due to the formation of an oily film on the surface of seeds, which prevented their germination and restricted further plant growth [30, 61].

The use of HCOMs exerted a favorable effect on the germination of radish seeds in the oil-contaminated soil. This could be related to an enhanced decomposition of hydrocarbons in the soil with a corresponding decrease in the amount of toxic oil components. The germination index of seeds reached its maximum (67.6%) after the application of the strain *Thalassospira xiamenensis* UOM 2.

Soil contamination with lead caused a decrease in the germination index of radish seeds as compared to the control. The germination index decreased with an increase in the lead content. High lead content (1800 mg/kg) significantly inhibited seed germination. This is one of the proven symptoms of the toxicity of this HM related to its effect on the enzymatic activity of plant cells, damage of membranes, etc. [52]. Lead caused intensive development of microscopic fungi and infection of radish seedlings, which negatively affected the growth and development of plants.

In the variants with combined oil and lead contamination, seed germination was also lower than in the control: the germination index varied within 39.2–54.4%. However, it was higher under the combined influence of pollutants than in the case of contamination with lead only. This is in agreement with an earlier study [53], in which a decrease in phytotoxicity under the impact of combined contamination with HM and organic substances was explained by the binding of lead ions with oil components with a decrease in lead bioavailability.

Application of bacteria to the soil with combined contamination improved radish germination. In the case of the minimum lead content (450 mg/kg), the germination index was maximal, when strains UOM 3 and UOM 4 were applied: 61.6 and 62.1%, respectively. With an increase in the lead contents to 900 and 1800 mg/kg, the germination index was the highest, when the soil was treated with the strain UOM 2 and MC. This could be related to the biological decomposition of toxic oil components and possible deactivation of lead cations due to their binding by microorganisms. The ability to bind metal ions by extracellular biopolymers was previously described for representatives of the families Pseudomonadaceae and Enterobacteriaceae [46]. In addition, the capacity of pseudomonades to absorb HMs on the cell walls and inside the cells was revealed [74].

The germination index is considered to be an indicator of the environment phytotoxicity (<50%), moderate phytotoxicity (50–80%), or its absence (>80%) [66]. It may be concluded that soils contaminated with oil at the concentration of 5% and with lead at the concentration >450 mg/kg are phytotoxic. The application of bacteria to the soil with oil and combined (oil and HM) pollution decreases its phytotoxicity and transforms it to a moderately toxic state.

Degradation of petroleum hydrocarbons. The survival of introduced bacteria is an important parameter of the efficiency of the biological preparation.

To assess the survival of the studied strains in the soil, we preliminarily studied samples of sterile soil for excluding the development of an indigenous hydrocarbon-oxidizing microbiota. On the 14th day after the application of the studied strains of microorganisms, the number of HCOM was 10^6–10^7 CFU/g, whereas they were not detected in the oil-contaminated sterile soil.

The amount of HCOMs after 45 days was greater in the treated samples than in the untreated samples, which indirectly characterized the adaptation and survival of the applied HCOMs.

According to our data, the number of HCOMs remained sufficient for their destructive activity (Table 2). Biodegradation is usually considered the main mechanism for removing pollutants from soil [36], although other mechanisms (for example, volatilization) may be involved in this process.

In the oil-contaminated soil, the degradation of hydrocarbons was more intensive than in the soil with combined contamination. The application of bacterial preparations increased the decomposition of hydrocarbons by 6.2–30.8% as compared to the untreated

Table 1. The effect of bioremediation on phytotoxicity of leached chernozem contaminated with oil and lead (on the 95th day of the experiment)

Variant	Parameters of phytotoxicity, %		
	percentage of seed germination	root elongation	germination factor
BS (control)	93.3	98.0	91.4
	Oil contamination		
OS	73.3	66.0	48.4
OS + UOM 2	93.3	72.5	67.6
OS + UOM 3	76.7	72.0	55.2
OS + UOM 4	83.3	68.0	56.6
OS + MC	80.0	62.0	49.6
	Lead contamination		
BS + Pb^{2+} 450 mg/kg of soil	70.0	73.3	51.3
BS + Pb^{2+} 900 mg/kg of soil	66.7	67.3	44.9
BS + Pb^{2+} 1800 mg/kg of soil	46.7	26.5	12.4
	Contamination with oil and lead		
	Pb^{2+} at the rate of 450 mg/kg of soil		
OS + Pb^{2+}	80.0	68.0	54.4
OS + Pb^{2+} + UOM 2	83.3	69.0	57.5
OS + Pb^{2+} + UOM 3	83.3	74.0	61.6
OS + Pb^{2+} + UOM 4	76.7	81.0	62.1
OS + Pb^{2+} + MC	80.0	73.0	58.4
	Pb^{2+} at the rate of 900 mg/kg of soil		
OS + Pb^{2+}	76.7	58.0	44.4
OS + Pb^{2+} + UOM 2	76.7	71.0	54.5
OS + Pb^{2+} + UOM 3	80.0	62.5	50.0
OS + Pb^{2+} + UOM 4	80.0	64.0	51.2
OS + Pb^{2+} + MC	73.3	73.0	53.5
	Pb^{2+} at the rate of 1800 mg/kg of soil		
OS + Pb^{2+}	70.0	56.0	39.2
OS + Pb^{2+} + UOM 2	76.7	70.0	53.7
OS + Pb^{2+} + UOM 3	70.0	60.3	42.2
OS + Pb^{2+} + UOM 4	73.3	70.0	51.3
OS + Pb^{2+} + MC	80.0	68.1	54.5

Here and in the following tables: BS is background soil, and OS is oil-contaminated soil.

variant. By the end of the experiment, biodegradation in the oil-contaminated soil reached maximum in the variant with the introduction of microorganisms UOM 2 and UOM 4 (83.8 and 79.8%, respectively).

In the presence of lead at all the studied concentrations, the decomposition of hydrocarbons decreased by 4.4–11.2%. The application of HCOMs exerted a favorable effect on the processes of oil degradation in the soil with combined contamination.. The most efficient cultures were UOM 3 and UOM 4 for the soil with oil and lead acetate concentration of 450 mg/kg and UOM 2 and MC for the soil with oil and lead acetate concentration of 900 and 1800 mg/kg. A direct dependence of the degree of biodegradation of hydrocarbons on the lead concentration in the soil with combined contamination was absent.

Soil pH. Oil, lead, and their combined effect caused some acidification of the soil by the middle of the

Table 2. Dynamics of the number of hydrocarbon-oxidizing microorganisms, the degree of biodegradation of hydrocarbons, and changes in pH in leached chernozem during bioremediation

Variant	Titer of HCOM, × 10^5 CFU/g			Biodegradation of hydrocarbons, %		pH_{KCl}		
	3 days	45 days	95 days	45 days	95 days	3 days	45 days	95 days
BS (control)	0.1 ± 0.004	0.1± 0.005	0.2 ± 0.008	–	–	6.40	6.36	6.42
Oil contamination								
OS	2.0 ± 0.09	330.0 ± 15.1	100.0 ± 4.7	33.2	53.0	6.35	5.97	6.19
OS + UOM 2	9.0 ± 0.36	1040.0 ± 45.0	360.0 ± 17.6	53.2	83.8	6.39	6.03	6.21
OS + UOM 3	3.0 ± 0.10	460.0 ± 22.6	54.0 ± 2.4	44.8	65.8	6.38	5.78	6.10
OS + UOM 4	3.0 ± 0.11	510.0 ± 24.3	71.0 ± 3.2	46.8	79.8	6.37	5.95	6.16
OS + MC	3.0 ± 0.19	320.0 ± 15.6	40.0 ± 1.8	39.2	59.2	6.41	6.17	6.37
Lead contamination								
BS + Pb^{2+} 450 mg/kg	–	–	–	–	–	6.20	5.94	6.00
BS + Pb^{2+} 900 mg/kg	–	–	–	–	–	6.08	5.90	5.91
BS + Pb^{2+} 1800 mg/kg	–	–	–	–	–	6.00	5.86	5.88
Contamination with oil and lead								
Pb^{2+} at the rate of 450 mg/kg of soil								
OS + Pb^{2+}	1.0 ± 0.06	52.0 ± 2.6	24.0 ± 1.1	28.4	48.6	5.80	5.65	6.15
OS + Pb^{2+} + UOM 2	2.0 ± 0.08	130.0 ± 6.4	53.0 ± 2.2	31.4	59.6	5.82	6.07	6.24
OS + Pb^{2+} + UOM 3	3.0 ± 0.08	620.0 ± 30.5	100.0 ± 4.7	37.6	70.4	5.79	6.12	6.25
OS + Pb^{2+} + UOM 4	3.0 ± 0.13	535.0 ± 25.8	120.0 ± 5.6	35.4	70.0	5.78	6.10	6.28
OS + Pb^{2+} + MC	2.0 ± 0.09	210.0 ± 10.6	71.0 ± 3.2	33.2	68.8	5.81	6.18	6.34
Pb^{2+} at the rate of 900 mg/kg of soil								
OS + Pb^{2+}	6.0 ± 0.32	250.0 ± 12.2	44.0 ± 2.3	26.6	44.6	6.28	6.00	6.10
OS + Pb^{2+} + UOM 2	7.0 ± 0.34	540.0 ± 24.1	350.0 ± 16.2	36.6	78.4	5.72	6.12	6.28
OS + Pb^{2+} + UOM 3	4.0 ± 0.20	320.0 ± 15.2	61.0 ± 3.0	36.8	60.8	5.81	6.19	6.30
OS + Pb^{2+} + UOM 4	8.0 ± 0.42	450.0 ± 23.1	82.0 ± 3.8	40.2	62.8	5.75	6.17	6.31
OS + Pb^{2+} + MC	3.0 ± 0.07	480.0 ± 26.5	150.0 ± 7.2	41.2	72.6	5.79	6.21	6.39
Pb^{2+} at the rate of 1800 mg/kg of soil								
OS + Pb^{2+}	2.0 ± 0.11	43.0 ± 2.0	10.0 ± 2.1	24.2	41.8	6.27	5.99	6.11
OS + Pb^{2+} + UOM 2	7.0 ± 0.34	300.0 ± 14.3	150.0 ± 6.1	36.4	68.8	6.03	6.60	6.57
OS + Pb^{2+} + UOM 3	10.0 ± 0.47	110.0 ± 5.2	65.0 ± 3.2	39.8	57.6	6.00	6.67	6.62
OS + Pb^{2+} + UOM 4	11.0 ± 0.72	180.0 ± 8.6	50.0 ± 2.3	35.4	59.4	5.94	6.55	6.56
OS + Pb^{2+} + MC	5.0 ± 0.20	240.0 ± 11.1	100.0 ± 4.6	38.2	59.6	5.96	6.70	6.71

Dash signifies not determined.

experiment (to pH 5.65–6.0), which probably enhanced the adverse effect of the pollutants (Table 2). The use of lead in the form of acetic acid salt could cause an acid soil reaction. In oil-contaminated variants, this could be related to the decomposition of hydrocarbons and the formation of acid compounds and free cations, which corresponded to data of other researchers [28]. Soil pH is an important parameter, because it often exerts a strong effect on the solubility of metals in soil and soil solution. A decrease in pH by a unit causes about twofold increase in the concentration of metals [63].

However, by the end of the experiment, pH shifted to the alkaline range and became close to that in the background soil. This tendency to alkalization was obviously explained by the high acid-base buffer capacity of chernozems [10]. In general, the soil reaction was favorable for the development of microorgan-

isms and the decomposition of organic substances, including oil, since the pH from 6 to 8 is considered optimal for their growth [60].

The number of ecological-trophic groups of microorganisms. The number of microorganisms is an important parameter of soil biological activity and may be an indicator of soil status. Oil and combined contamination of soil caused an increase in the number of heterotrophic microorganisms, which was probably related to the role of oil as an additional carbon source (Table 3). It is obvious that the contribution of HCOMs to the rise in the number of heterotrophs was the greatest. Bioaugmentation of oil-contaminated soil also caused an increase in the number of heterotrophic microorganisms, probably due to the intensive development of the used hydrocarbon-oxidizing microbiota, which was confirmed by published data [32]. Contamination with lead at various rates did not affect the number of heterotrophic microorganisms in the soil.

Oil and lead in the soil (individually and in combination) inhibited the development of actinomycetes: their number decreased by 1–2 orders of magnitude as compared to the control. On one hand, this decrease in the polluted and remediated soil could indicate a strong sensitivity actinomycetes to pollutants. It was found [1] that an increased content of mobile forms of HMs in soils caused structural and functional changes in the communities of actinomycetes: a decrease in their number and species diversity. On the other hand, actinomycetes are rather sensitive to changes in the medium acidity [2], so their smaller number could be related to soil acidification. It is possible that the actinomycete complex was initially dominated by species adapted to a narrower pH range. Thus, the negative effect of soil pollution on the development of actinomycetes could be caused by a direct toxic effect of pollutants and by an indirect effect of changes in the medium conditions after oil and lead were added to the soil.

The number of microscopic fungi slightly increased in the first day after oil and lead application, which could be related to soil acidification. In addition, micromycetes are capable of using a wide range of organic compounds, including oil [20, 76], so its low concentrations do not exert a pronounced toxic effect on these microorganisms. The application of bacteria caused a decrease in the number of fungi, which could be related to their competition with HCOMs for food.

The number of oligonitrophilic, nitrogen-fixing, and cellulose-decomposing microorganisms reflected their negative response to pollutants in soil. The application of HCOMs sometimes exerted a positive effect on the development of these groups of microorganisms. For example, their number became close to the control by the end of the experiment as a result of the application of strain UOM 2 to the soil contaminated with oil and with oil and lead at the rate of 900 mg/kg. Thus, actinomycetes were highly sensitive to the oil and lead pollution. An increase in the number of heterotrophic microorganisms in variants with oil, combined pollution, and bioremediation was obviously related to active development of hydrocarbon-oxidizing microbiota.

Enzymatic activity. Soil enzymes are catalysts of important metabolic processes, including the decomposition of organic pollutants [25, 70]. Enzymes are considered to be sensitive to pollution and are easily determined without expensive and complex tools, so they are often used as indicators to assess various kinds of soil pollution [29]. Recent studies have shown that microorganisms can improve the enzymatic activity of soil [44, 62] and that the resistance of bacteria to HMs is related to increased activity of antioxidant enzymes and urease [33, 40, 69]. It is known that HMs may be precipitated by bacterial urease and thus removed from soils [51].

Catalase activity. Catalase is very sensitive to contamination with HMs and oil [37, 48], so its activity may be used to analyze the substrate toxicity. It is known that this bacterial enzyme causes degradation of reactive oxygen species, the formation of which is induced by HMs, oil, and some other xenobiotics [82].

In the oil-contaminated soil, the catalase activity (**CA**) initially increased, but then began to decrease and became lower than in the control (Fig. 1a). This generally corresponded to data on the inhibition of catalase activity by hydrocarbons [64]. When strain UOM 3 and MC were applied to the oil-contaminated soil, the CA remained constant (3 mL O_2/g min), starting from the second half of the experiment, which could indicate the beginning of stabilization of soil conditions.

In the variants with lead, the CA was higher than that in the control. It became maximum (6.1 mL O_2/g min) on the 45th day of the experiment in the variant with the lead rate of 1800 mg/kg soil (Fig. 1d). This confirmed that the CA could increase parallel to the concentration of HMs [81]. The activation of this enzyme in the presence of lead acetate was probably caused by a reaction between Pb^{2+} and the functional groups of catalase.

Similarly to oil, the combined contamination caused an increase in the CA at the initial stage; later, the activity of the enzyme decreased (Figs. 1b, 1c).

The CA of the soil contaminated with oil and lead (450 mg/kg) was higher after bioaugmentation in the middle and at the end of the experiment as compared to the untreated variant, the highest values being achieved by HCOM 4 and MC. The application of bacteria to the soil contaminated with oil and lead in high concentration (1800 mg/kg) exerted a positive effect on the enzyme activity. There was even an increase in the CA in variants with the strain UOM 4 and MC added to the soil contaminated with oil and lead in high concentration by the end of the experiment, which indirectly attested to the normalization of oxidation-reduction processes.

Table 3. Dynamics of the number of various ecological-trophic groups of microorganisms in leached chernozem during bioremediation

Variant	Heterotrophic microorganisms, $\times 10^6$ CFU/g		Micromycetes, $\times 10^4$ CFU/g		Actinomycetes, $\times 10^5$ CFU/g		Oligonitrophiles and nitrogen fixers, $\times 10^5$ CFU/g		Cellulose decomposers, $\times 10^3$ CFU/g	
	3 days	95 days	3 days	95 days	3 days	95 days	3 days	95 days	3 days	95 days
BS (control)	1.0 ± 0.1	5.0 ± 0.2	5.0 ± 0.31	1.5 ± 0.08	90.0 ± 4.60	80.0 ± 3.62	5.0 ± 0.31	3.2 ± 0.11	8.0 ± 0.32	10.0 ± 0.38
Oil contamination										
OS	2.0 ± 0.1	100.0 ± 4.8	2.5 ± 0.12	0.3 ± 0.01	11.0 ± 0.58	1.0 ± 0.04	1.2 ± 0.09	0,8 ± 0.03	2.0 ± 0.08	0.2 ± 0.01
OS + UOM 2	12.0 ± 0.5	310.0 ± 14.2	0.5 ± 0.02	0.1 ± 0.01	5.0 ± 0.24	1.0 ± 0.03	3.7 ± 0.12	3.1 ± 0.21	6.0 ± 0.22	10.0 ± 0.51
OS + UOM 3	8.0 ± 0.3	120.0 ± 5.1	0.7 ± 0.02	0.2 ± 0.01	3.5 ± 0.14	0.5 ± 0.02	4.2 ± 0.32	1.9 ± 0.10	3.0 ± 0.14	1.0 ± 0.06
OS + UOM 4	9.0 ± 0.4	145.0 ± 5.7	1.0 ± 0.04	0.3 ± 0.02	0.5 ± 0.03	0.1 ± 0.01	3.3 ± 0.21	0.3 ± 0.02	5.0 ± 0.35	4.0 ± 0.33
OS + MC	5.0 ± 0.2	150.0 ± 6.1	0.8 ± 0.02	0.1 ± 0.01	1.1 ± 0.03	0.5 ± 0.02	0.2 ± 0.01	0.1 ± 0.01	5.0 ± 0.22	1.0 ± 0.03
Lead contamination										
BS + Pb^{2+} 450 mg/kg	6.0 ± 0.2	2.0 ± 0.1	6.0 ± 0.05	0.2 ± 0.01	1.0 ± 0.03	0.1 ± 0.01	1.0 ± 0.06	0.1 ± 0.01	1.0 ± 0.05	1.0 ± 0.05
BS + Pb^{2+} 900 mg/kg	8.0 ± 0.3	8.0 ± 0.3	10.0 ± 0.44	0.2 ± 0.01	6.5 ± 0.38	0.2 ± 0.01	1.0 ± 0.05	0.1 ± 0.01	0.5 ± 0.03	0.4 ± 0.01
BS + Pb^{2+} 1800 mg/kg	2.0 ± 0.1	1.0 ± 0.1	8.0 ± 0.05	0.3 ± 0.02	1.0 ± 0.04	6.0 ± 0.30	1.0 ± 0.05	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Contamination with oil and lead										
Pb^{2+} at the rate of 450 mg/kg of soil										
OS + Pb^{2+}	22.0 ± 1.0	65.0 ± 1.2	8.0 ± 0.23	1.0 ± 0.07	13.0 ± 0.62	5.0 ± 0.25	20.0 ± 1.01	2.0 ± 0.10	1.1 ± 0.06	0.7 ± 0.03
OS + Pb^{2+} + UOM 2	40.0 ± 2.5	75.0 ± 1.4	1.0 ± 0.08	0.6 ± 0.03	4.5 ± 0.23	1.0 ± 0.08	15.0 ± 0.76	0.5 ± 0.02	2.0 ± 0.12	3.0 ± 0.03
OS + Pb^{2+} + UOM 3	44.0 ± 2.3	210.0 ± 15.6	2.5 ± 0.11	0.3 ± 0.02	0.5 ± 0.02	0.1 ± 0.01	12.5 ± 0.64	0.2 ± 0.01	3.0 ± 0.20	3.0 ± 0.20
OS + Pb^{2+} + UOM 4	46.0 ± 2.2	230.0 ± 10.0	3.0 ± 0.20	0.5 ± 0.02	0.5 ± 0.02	0.1 ± 0.01	20.0 ± 1.27	0.4 ± 0.02	3.0 ± 0.14	1.0 ± 0.04
OS + Pb^{2+} + MC	43.0 ± 2.5	90.0 ± 3.5	2.0 ± 0.10	0.7 ± 0.03	1.3 ± 0.03	0.5 ± 0.02	10.0 ± 0.63	1.0 ± 0.04	2.0 ± 0.16	5.0 ± 0.12
Pb^{2+} at the rate of 900 mg/kg of soil										
OS + Pb^{2+}	13.0 ± 0.6	100.0 ± 4.5	10.0 ± 0.30	0.1 ± 0.01	1.1 ± 0.05	0.5 ± 0.03	11.0 ± 0.53	1.0 ± 0.05	0.5 ± 0.03	0.7 ± 0.03
OS + Pb^{2+} + UOM 2	27.0 ± 1.2	165.0 ± 7.3	2.0 ± 0.11	0.3 ± 0.01	1.3 ± 0.05	0.3 ± 0.02	16.0 ± 0.72	3.1 ± 0.14	0.5 ± 0.02	5.2 ± 0.22
OS + Pb^{2+} + UOM 3	24.0 ± 1.3	55.0 ± 2.6	3.0 ± 0.23	0.5 ± 0.02	1.2 ± 0.06	0.1 ± 0.01	13.0 ± 0.77	0.4 ± 0.01	0.2 ± 0.01	1.0 ± 0.05
OS + Pb^{2+} + UOM 4	32.0 ± 1.5	50.0 ± 2.1	2.0 ± 0.09	0.2 ± 0.01	0.7 ± 0.03	0.1 ± 0.01	18.0 ± 0.94	7.2 ± 0.37	0.7 ± 0.02	11.0 ± 0.51
OS + Pb^{2+} + MC	26.0 ± 1.2	180.0 ± 5.4	4.0 ± 0.18	0.5 ± 0.03	1.0 ± 0.06	0.5 ± 0.02	20.0 ± 1.17	8.1 ± 0.35	0.5 ± 0.02	5.0 ± 0.37
Pb^{2+} at the rate of 1800 mg/kg of soil										
OS + Pb^{2+}	5.0 ± 0.2	20.0 ± 1.2	12.0 ± 0.16	0.3 ± 0.02	1.0 ± 0.04	0.5 ± 0.03	2.0 ± 0.10	0.7 ± 0.04	0.1 ± 0.01	0.1 ± 0.01
OS + Pb^{2+} + UOM 2	14.0 ± 1.2	90.0 ± 4.3	3.0 ± 0.11	0.2 ± 0.01	1.3 ± 0.03	0.2 ± 0.01	3.0 ± 0.10	0.5 ± 0.03	0.1 ± 0.01	2.0 ± 0.18
OS + Pb^{2+} + UOM 3	6.0 ± 0.2	45.0 ± 2.1	3.0 ± 0.10	0.1 ± 0.01	0.8 ± 0.04	0.1 ± 0.01	5.0 ± 0.25	1.1 ± 0.06	0.2 ± 0.01	1.0 ± 0.04
OS + Pb^{2+} + UOM 4	8.0 ± 0.4	60.0 ± 2.9	2.0 ± 0.05	0.5 ± 0.03	1.1 ± 0.05	0.6 ± 0.03	2.0 ± 0.13	0.8 ± 0.04	0.1 ± 0.01	2.0 ± 0.01
OS + Pb^{2+} + MC	15.0 ± 0.7	80.0 ± 4.1	0.5 ± 0.03	0.4 ± 0.02	1.8 ± 0.08	0.3 ± 0.02	1.0 ± 0.06	0.3 ± 0.01	0.3 ± 0.02	1.0 ± 0.05

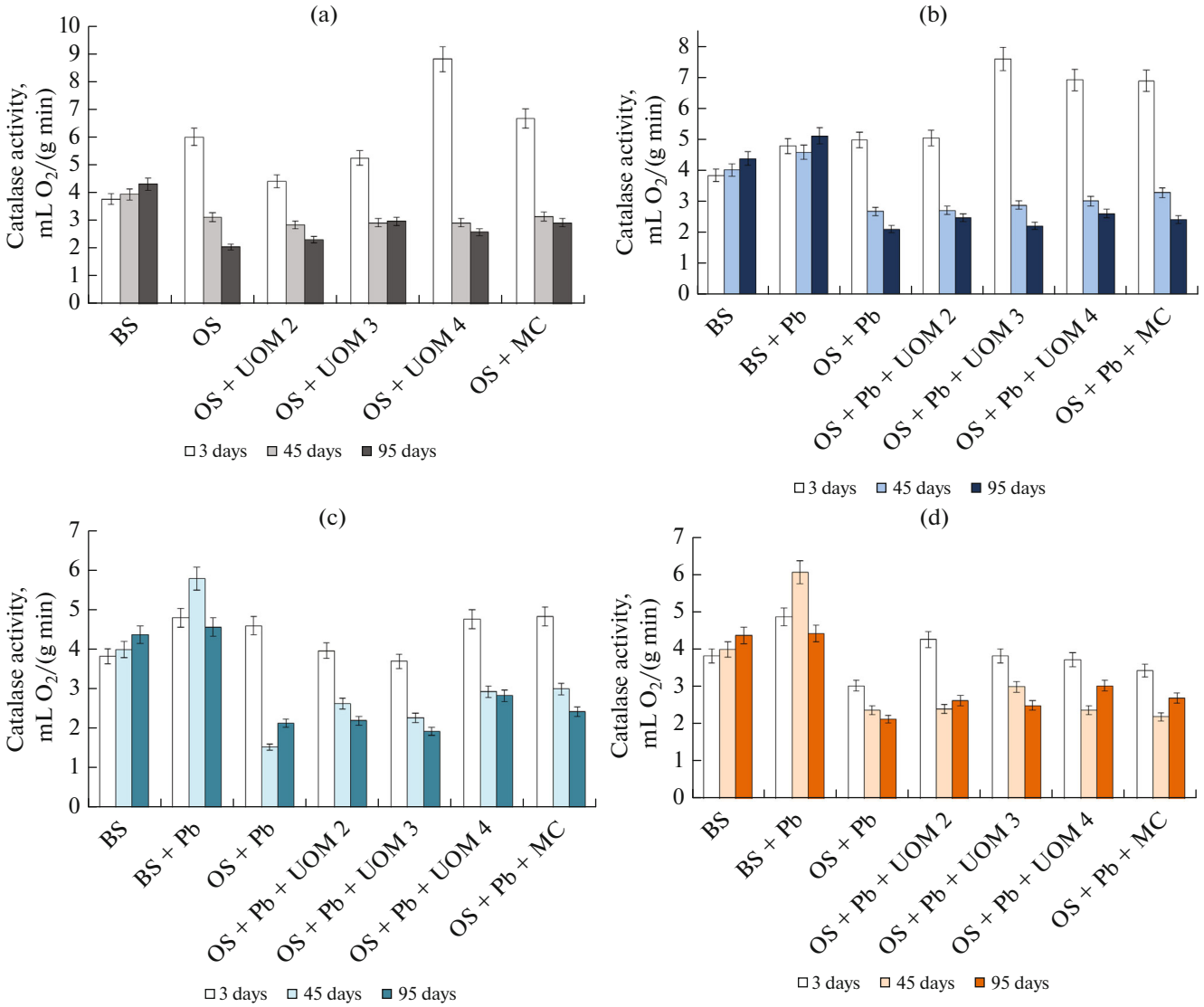

Fig. 1. Catalase activity of the soil contaminated with (a) oil and lead at the concentrations of (b) lead 450 mg/kg soil, (c) 900 mg/kg soil, and (d) 1800 mg/kg soil (d) and with both pollutants.Here and in the following figures: BS is background soil, OS is oil-polluted soil, and Pb is lead acetate.

Thus, the soil contaminated with oil and with oil and lead was characterized by a general tendency to a decrease in the CA. The individual application of lead, on the contrary, stimulated the CA. The use of microorganisms was favorable for the CA: it was higher than in the variant without bacterization.

Invertase activity. Invertase is one of the enzymes resistant to the excessive content of HMs and oil in soil [8, 17]. Despite this, its activity is considered informative for the integral assessment of the soil status [5, 78].

In our experiment, the invertase activity (IA) decreased in the oil-contaminated soil, and this was also observed after the application of bacteria (Fig. 2a).

This could be related to the destruction of bacterial cells by toxic substances in the pollutant [14]. Since the invertase origin is extracellular, the death of some bacteria could cause a decrease in its synthesis and in the IA of the soil [65]. The only exception was observed in the variant with strain UOM 2: the IA exceeded that in the control by 10% on the 95th day. A decrease in the IA with time was also seen for the soil with combined contamination (Figs. 2b–2d). In most cases, the IA was higher in the variants with lead than in the control (mainly during the first half of the experiment). The application of the bacterial strain UOM 4 to the oil-contaminated soil with lead (450 mg/kg) caused an increase in the IA to 5 mg of glucose/g of soil by the end of the experiment (Figs. 2c and 2d). The restoration of the IA level after initial inhibition could be related to the growth of the introduced microbial population as a result of adaptation [41] and to the ability of this strain to utilize a wider range of oil hydrocarbons.

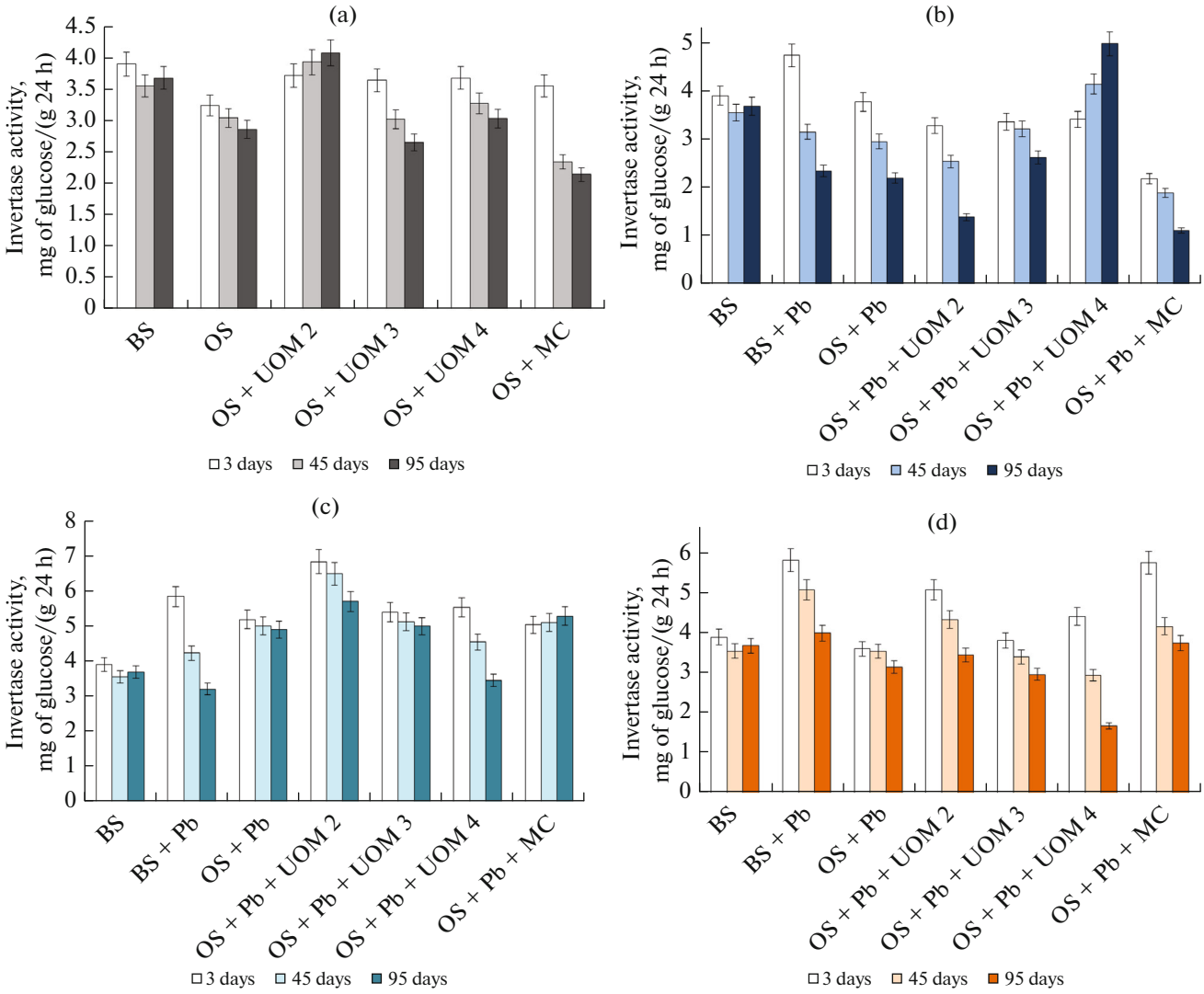

Fig. 2. Invertase activity of the soil contaminated with (a) oil and lead at the concentrations of (b) lead 450 mg/kg soil, (c) 900 mg/kg soil, and (d) 1800 mg/kg soil (d) and with both pollutants, during bioremediation in particular.

It was revealed that the IA directly depends on the amount of soil microbes [45], because it slightly correlates with the number of heterotrophic microorganisms ($r = 0.35$, $p > 95\%$) by the end of this experiment.

Thus, oil suppressed the IA. Soil contamination with lead, on the contrary, increased the activity of this enzyme at the initial stage of contamination. The combined pollution caused various reactions of soil invertase, however, bioaugmentation sometimes exerted a positive effect on the IA in the soil with both oil and combined contamination.

Urease activity. The urease activity (UA) in soil characterizes the potential of urea mineralization. The enzyme forms stable complexes (urease–humus) and contributes much to soil fertility [55]. Its activity may be an adequate indicator of combined contamination (HMs, polycyclic aromatic hydrocarbons, etc.), at the early stages, in particular [72, 81].

Oil exerted a negative impact on the UA of the soil (Fig. 3a).

The addition of microorganisms stimulated the UA at the initial stage, but it decreased with time though remained higher than that in the soil without bioremediation and generally close to that in the control. The strain UOM 2 caused an increase in the UA by 64% on the third day and by 25% by the end of the experiment relative to the control.

Soil contamination with lead acetate inhibited soil urease. In the variant with combined contamination, the UA was lower that in the control and in the variants with only one pollutant. It is known that the UA decreases due to changes in the enzyme molecular structure caused by inhibitors. It is assumed that HMs bind sulfhydryl groups of the active center forming equivalents of metal sulfides, while organic ecotoxicants (including oil) can denature the entire protein

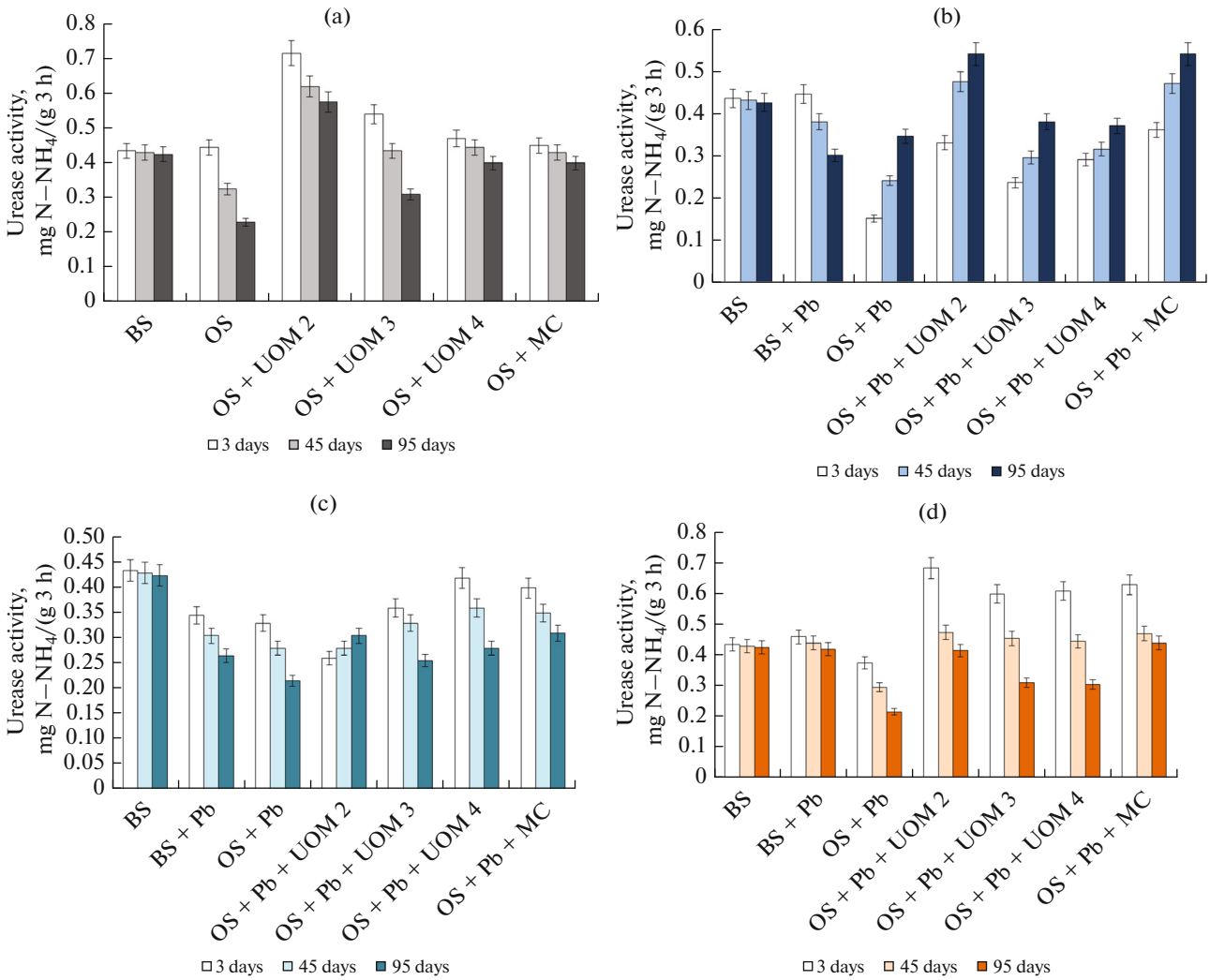

Fig. 3. Urease activity of soil contaminated with (a) oil and lead at the concentrations of (b) lead 450 mg/kg soil, (c) 900 mg/kg soil, and (d) 1800 mg/kg soil (d) and with both pollutants.

structure [71]. The combined effect of xenobiotics probably resulted in a synergistic effect, which caused faster inhibition of the UA.

Bioaugmentation exerted a favorable effect on the UA, which became maximum after the application of the strain UOM 2 and MC: 0.54 N-NH_4/g by the end of the experiment in both variants contrary to 0.43 N-NH_4/g in the control (Fig. 3b). In the soil with oil and lead in the amount of 900 and 1800 mg/kg, the UA on the 95th day was two times lower as compared to the control (Figs. 3c and 3d). The use of microorganisms in these variants initially stimulated urease, but its activity decreased with time, which was probably explained by the depletion of the substrate for bacteria. The UA in samples with bacteria application was higher than in the untreated variants and sometimes reached control values.

Thus, the combined soil contamination with oil (5%) and lead at concentrations of 450–1800 mg/kg inhibited the UA stronger than in the soil contaminated with lead only. In the variant with combined contamination with oil and lead in the amount of 450 mg/kg, the UA initially sharply dropped and then gradually increased. A rise in the lead content caused a slow decrease in the UA. The use of strains of microorganisms exerted a favorable effect on the restoration of the UA both in the oil-contaminated soil and in the soils with combined contamination.

CONCLUSIONS

In a model experiment, soil pollution with oil, lead, and their combination, as well as bioaugmentation by cultures of hydrocarbon-oxidizing microorganisms (HCOMs) *Thalassospira xiamenensis* UOM 2, *Enterobacter* sp. UOM 3, and *Pseudomonas songnenensis* UOM 4 and by their microbial composition (MC) affected the biological activity of leached chernozem.

A pronounced phytotoxic effect of the pollutants was manifested in all the studied variants of pollution as judged from a poorer radish germination and inhibition of the growth of its roots. The application of HCOMs reduced phytotoxicity. The most efficient microorganisms were represented by the bacterial strain *T. xiamenensis* UOM 2 for the oil-contaminated soil; by the strains *Enterobacter* sp. UOM 3 and *P. songnenensis* UOM 4 for the soil with combined contamination at the lead concentration of 450 mg/kg; and by *T. xiamenensis* UOM 2 and the MC at higher lead concentrations. The use of bacteria enabled the transformation of the contaminated leached chernozem into the category of moderately phytotoxic soil.

The introduced HCOM survived well in the soil contaminated with both pollutants. In the oil-contaminated soil, the degradation of hydrocarbons was more intensive than in the soil with combined contamination without the introduction of bacteria. Bioremediation of leached chernozem enhanced the destruction of these xenobiotics. Their biodegradation in the oil-contaminated soil was maximal, when *T. xiamenensis* UOM 2 and *P. songnenensis* UOM 4 were applied. Under combined pollution, the decomposition of hydrocarbons was the greatest under the effect of *Enterobacter* sp. UOM 3 and *P. songnenensis* UOM 4 in the soil with the lead content of 450 mg/kg and of *T. xiamenensis* UOM 2 and MC in the soil with the lead content of 900 and 1800 mg/kg.

Oil and combined soil pollution caused an increase in the number of heterotrophic microorganisms, obviously due to the intensive development of hydrocarbon-oxidizing microbiota. Actinomycetes were most sensitive microorganisms to oil and lead pollution.

Soil with oil and combined contamination was characterized by a tendency to a decrease in the catalase activity. The individual introduction of lead acetate in the amount of 450, 900, and 1800 mg/kg into the soil stimulated the catalase activity. The use of microbial strains for bioremediation exerted a positive effect on this parameter.

At the initial stage of pollution, oil caused suppression of the invertase activity, while lead, on the contrary, increased it. The combination of pollutants resulted in different reactions of soil invertase. Bioaugmentation of the soil contaminated with oil and with oil and lead exerted a positive effect on the invertase activity in some cases.

The combined effect of pollutants inhibited the urease activity stronger than in the case of lead contamination. Application of microbial strains was generally favorable for the urease activity restoration both in the oil-contaminated soil and in the soil with combined pollution.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. V. N. Grishko and O. V. Syshchikova, "Streptomyces communities in soils polluted with heavy metals," Eurasian Soil Sci. **42**, 217–224 (2009).
2. G. M. Zenova, Yu. V. Zakalyukina, V. V. Selyanin, and D. G. Zvyagintsev, "Isolation and growth of acidophilic soil actinomycetes from the *Micromonospora* genus," Eurasian Soil Sci. **37**, 737–742 (2004).
3. I. B. Ivshina, A. V. Krivoruchko, M. S. Kuyukina, L. V. Kostina, T. A. Peshkur, and K. D. Kanningkhem, "Bioremediation of soils disturbed by hydrocarbons and heavy metals using biosurfactants and immobilized *Rhodococcus*," Agrar. Vestn. Urala, No. 8, 65–68 (2012).
4. R. R. Kabirov, N. A. Kireeva, T. R. Kabirov, I. Ye. Dubovik, A. B. Yakupova, and L. M. Safiullina, "Evaluating the biological activity of oil-polluted soils using a complex index," Eurasian Soil Sci. **45**, 157–161 (2012).
5. K. Sh. Kazeev, A. V. Trushkov, M. Yu. Odabashyan, and S. I. Kolesnikov, "Postagrogenic changes in the enzyme activity and organic carbon content in chernozem during the first three years of fallow regime," Eurasian Soil Sci. **53**, 995–1003 (2020).
6. N. A. Kireeva, A. S. Grigoriadi, and E. F. Khaibullina, "Associations of hydrocarbon-oxidizing microorganisms for bioremediation of petroleum-contaminated soils," Vestn. Bashkir. Gos. Univ., No. 2, 391–394 (2009).
7. E. N. Kozlova, A. L. Stepanov, and L. V. Lysak, "The influence of bacterial-humus preparations on the biological activity of soils polluted with oil products and heavy metals," Eurasian Soil Sci. **48**, 400–409 (2015). https://doi.org/10.1134/S1064229315020052
8. S. I. Kolesnikov, D. K. Aznaurian, K. Sh. Kazeev, and V. F. Val'kov, "Biological properties of south Russian soils: tolerance to oil pollution," Russ. J. Ecol. **41**, 398–404 (2010).
9. S. I. Kolesnikov, M. G. Zharkova, K. Sh. Kazeev, I. V. Kutuzova, L. S. Samokhvalova, E. V. Naleta, and D. A. Zubkov, "Ecotoxicity assessment of heavy metals and crude oil based on biological characteristics of chernozem," Russ. J. Ecol. **45**, 157–166 (2014). https://doi.org/10.1134/S1067413614030059
10. S. I. Kolesnikov, K. Sh. Kazeev, M. L. Tatosyan, and V. F. Val'kov, "The effect of pollution with oil and oil products on the biological status of ordinary chernozems," Eurasian Soil Sci. **39**, 552–556 (2006).
11. T. V. Koronelli, "Principles and methods for raising efficiency of biological degradation of hydrocarbons in

the environment: a review," Appl. Biochem. Microbiol. **32**, 519–525 (1996).
12. T. Yu. Korshunova, S. P. Chetverikov, M. D. Bakaeva, E. V. Kuzina, G. F. Rafikova, D. V. Chetverikova, and O. N. Loginov, "Microorganisms in the elimination of oil pollution consequences (review)," Appl. Biochem. Microbiol. **55**, 344–354 (2019).
13. E. I. Novoselova and O. O. Volkova, "Impact of heavy metals on catalase activity in different soil types," Izv. Orenb. Gos. Agrar. Univ., No. 2, 190–193 (2017).
14. E. I. Novoselova, O. O. Volkova, and R. R. Tur'yanova, "Enzymatic transformation of organic residues in soils contaminated by heavy metals," Ekol. Urban. Territ., No. 1, 75–81 (2019). https://doi.org/10.24411/1816-1863-2019-11075
15. E. I. Novoselova, N. A. Kireeva, and M. I. Garipova, "The role of the enzymatic activity of soils in the trophic function affected by petroleum pollution," Vestn. Bashkir. Gos. Univ., No. 2, 474–479 (2014).
16. E. V. Pleshakova, E. G. Kabantseva, and V. S. Chernovol, "Dehydrogenase activity in petroleum-contaminated soils as a tool for monitoring of bioremediation technologies," Izv. Sarat. Gos. Univ., Ser. Khim., Biol., Ekol., No. 1, 40–46 (2010).
17. E. V. Pleshakova, M. V. Reshetnikov, K. T. Ngun, and E. P. Shuvalova, "Microbiological and biochemical indication of soils in Mednogorsk city," Agrokhimiya, No. 1, 65–72 (2016).
18. Yu. M. Polyak and V. I. Sukharevich, "Soil enzymes and soil pollution: biodegradation, bioremediation, and bioindication," Agrokhimiya, No. 3, 83–93 (2020). https://doi.org/10.31857/S0002188120010123
19. *Practical Manual on Microbiology*, Ed. by A. I. Netrusov (Akademiya, Moscow, 2005) [in Russian].
20. G. F. Rafikova, E. V. Kuzina, E. A. Stolyarova, S. R. Mukhamatd'yarova, and O. N. Loginov, "Complexes of micromycetes of leached chernozem under oil pollution and introduction of oil-destructive microorganisms," Mikol. Fitopatol., No. 2, 107–115 (2020). https://doi.org/10.31857/S0026364820020099
21. J. Szegi, *Talajmikrobiológiai Vizsgálati Módszerek* (Mezőgazdasági Könyvkiadó, Budapest, 1979; Kolos, Moscow, 1983).
22. Kh. F. Khaziev, *Methods of Soil Enzymology* (Nauka, Moscow, 2005) [in Russian].
23. S. P. Chetverikov, M. D. Bakaeva, T. Yu. Korshunova, E. V. Kuzina, G. F. Rafikova, D. V. Chetverikova, L. B. Vysotskaya, and O. N. Loginov, "New strain *Enterobacter* sp. UOM 3 as oil destructor and producer of indoleacetic acid," Estestv. Tekh. Nauki, No. 7, **37–40** (2019). https://doi.org/10.25633/ETN.2019.07.13
24. I. E. Sharapova, E. M. Lapteva, S. P. Maslova, G. I. Tabalenkova, and A. V. Garabadzhiu, "Use of the integral coefficient of biological activity and phytotoxicity index for assessment of phytoremediation of oil-contaminated soils," Teor. Prikl. Ekol., No. 2, 67–73 (2015).
25. A. T. Adetunji, F. B. Lewu, R. Mulidzi, and B. Ncube, "The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review," J. Soil Sci. Plant Nutr. **17** (3), 794–807 (2017). https://doi.org/10.4067/S0718-95162017000300018
26. A. C. Agnello, M. Bagardc, E. D. van Hullebuscha, G. Esposito, and D. Huguenota, "Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation," Sci. Total Environ. **563–564**, 693–703 (2016). https://doi.org/10.1016/j.scitotenv.2015.10.061
27. M. H. Ali, M. T. Sattar, M. I. Khan, M. Naveed, M. Rafique, S. Alamri, and M. H. Siddiqui, "Enhanced growth of mungbean and remediation of petroleum hydrocarbons by *Enterobacter* sp. MN17 and biochar addition in diesel contaminated soil," Appl. Sci. **10**, 8548 (2020). https://doi.org/10.3390/app10238548
28. N. Al-Mutairi, A. Bufarsan, and F. Al-Rukaibi, "Ecorisk evaluation and treatability potential of soils contaminated with petroleum hydrocarbon-based fuels," Chemosphere **74** (1), 142–148 (2008). https://doi.org/10.1016/j.chemosphere.2008.08.020
29. H. Aponte, J. Medina, B. Butler, S. Meier, P. Cornejo, and Y. Kuzyakov, "Soil quality indices for metal (loid) contamination: an enzymatic perspective," Land Degrad. Dev. **31** (17), 2700–2719 (2020). https://doi.org/10.1002/ldr.3630
30. H. Athar, S. Ambreen, M. Javed, M. Hina, S. Rasul, Z. U. Zafar, H. Manzoor, C. C. Ogbaga, M. Afzal, F. Al-Qurainy, and M. Ashraf, "Influence of sub-lethal crude oil concentration on growth, water relations and photosynthetic capacity of maize (*Zea mays* L.) plants," Environ. Sci. Pollut. Res. Int. **23**, 18320–18331 (2018). https://doi.org/10.1007/s11356-016-6976-7
31. M. Bakaeva, E. Kuzina, L. Vysotskaya, G. Kudoyarova, T. Y. Arkhipova, G. Rafikova, S. Chetverikov, T. Korshunova, D. Chetverikova, and O. Loginov, "Capacity of *Pseudomonas* strains to degrade hydrocarbons, produce auxins and maintain plant growth under normal conditions and in the presence of petroleum contaminants," Plants **9**, 379 (2020). https://doi.org/10.3390/plants9030379
32. V. P. Beškoski, G. Gojgić-Cvijović, J. Milić, M. Ilić, S. Miletić, T. Šolević, and M. M. Vrvić, "Ex situ bioremediation of a soil contaminated by mazut (heavy residual fuel oil)—a field experiment," Chemosphere **83** (1), 34–40 (2011). https://doi.org/10.1016/j.chemosphere.2011.01.020
33. X. Chen and V. Achal, "Biostimulation of carbonate precipitation process in soil for copper immobilization," J. Hazard. Mater. **368**, 705–713 (2019). https://doi.org/10.1016/j.jhazmat.2019.01.108
34. J. Cheng, Z. Sun, Y. Yu, X. Li, and T. Li, "Effects of modified carbon black nanoparticles on plant-microbe remediation of petroleum and heavy metal co-contaminated soils," Int. J. Phytoremed. **21** (7), 634–642 (2019). https://doi.org/10.1080/15226514.2018.1556581
35. S. Chetverikov, L.Vysotskaya, E. Kuzina, T. Arkhipova, M. Bakaeva, G. Rafikova, T. Korshunova, D. Chetverikova, G. Hkudaygulov, and G. Kudoyarova, "Effects of association of barley plants with hydrocarbon-degrading bacteria on the content of soluble organic compounds in clean and oil-contaminated sand," Plants **10**, 975 (2021). https://doi.org/10.3390/plants10050975
36. I. Declercq, V. Cappuyns, and Y. Duclos, "Monitored natural attenuation (MNA) of contaminated soils: state of the art in Europe—a critical evaluation," Sci. Total

Environ. **426**, 393–405 (2012). https://doi.org/10.1016/j.scitotenv.2012.03.040

37. M. del Carmen Cuevas-Díaz, Á. Martínez-Toledo, O. Guzmán-López, C. P. Torres-López, A. D. C. Ortega-Martínez, and L. J. Hermida-Mendoza, "Catalase and phosphatase activities during hydrocarbon removal from oil-contaminated soil amended with agro-industrial by-products and macronutrients," Water, Air Soil Pollut. **228** (4), 159 (2017). https://doi.org/10.1007/s11270-017-3336-2
38. A. Dwivedi, S. Chitranshi, A. Gupta, A. Kumar, and J. L. Bhat, "Assessment of the petroleum oil degradation capacity of indigenous bacterial species isolated from petroleum oil-contaminated soil," Int. J. Environ. Res. **13**, 735–746 (2019). https://doi.org/10.1007/s41742-019-00210-y
39. L. El Fels, M. Zamama, A. El Asli, and M. Hafidi, "Assessment of biotransformation of organic matter during co-composting of sewage sludge-lignocelullosic waste by chemical, FTIR analyses, and phytotoxicity tests," Int. Biodeterior. Biodegrad. **87**, 128–137 (2014). https://doi.org/10.1016/j.ibiod.2013.09.024
40. H. Etesami, "Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: mechanisms and future prospects," Ecotoxicol. Environ. Saf. **147**, 175–191 (2018). https://doi.org/10.1016/j.ecoenv.2017.08.032
41. C. Feng, Y. Ma, X. Jin, Z. Wang, Y. Ma, S. Fu, and H. Y. Chen, "Soil enzyme activities increase following restoration of degraded subtropical forests," Geoderma **351**, 180–187 (2019). https://doi.org/10.1016/j.geoderma.2019.05.006
42. O. E. Giwa and F. O. Ibitoye, "Bioremediation of heavy metal in crude oil contaminated soil using isolated Indigenous microorganism cultured with *E. coli* DE3 BL21," Int. J. Eng. Appl. Sci. **4** (6), 67–70 (2017).
43. M. T. Gómez-Sagasti, I. Alkorta, J. M. Becerril, L. Epelde, M. Anza, and C. Garbisu, "Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation," Water, Air Soil Pollut. **23** (6), 3249–3262 (2012). https://doi.org/10.1007/s11270-012-1106-8
44. W. Hou, J. Wang, Z. Nan, M. J. Christensen, C. Xia, T. Chen, Z. Zhang, and X. Niu, "*Epichloe gansuensis* endophyte-infection alters soil enzymes activity and soil nutrients at different growth stages of *Achnatherum inebrians*," Plant Soil **455** (1), 227–240 (2020). https://doi.org/10.1007/s11104-020-04682-2
45. J. Hu, X. Lin, J. Wang, J. Dai, R. Chen, J. Zhang, and M. H. Wong, "Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer," J. Soil. Sediments **11** (2), 271–280 (2011). https://doi.org/10.1007/s11368-010-0308-1
46. A. Iyer, K. Mody, and B. Jha, "Biosorption of heavy metals by a marine bacterium," Mar. Pollut. Bull. **50** (3), 340–343 (2005). https://doi.org/10.1016/j.marpolbul.2004.11.012
47. I. Jerin, M. S. Rahi, T. Sultan, M. S. Islam, S. A. Sajib, K. M. F. Hoque, and M. A. Reza, "Diesel degradation efficiency of *Enterobacter* sp., *Acinetobacter* sp., and *Cedecea* sp. isolated from petroleum waste dumping site: a bioremediation view point," Arch. Microbiol. **203**, 5075–5084 (2021). https://doi.org/10.1007/s00203-021-02469-2
48. S. I. Kolesnikov, M. A. Myasnikova, T. V. Minnikova, T. A. Ter-Misakyants, R. S. Kazeev, and Y. V. Akimenko, "Assessment of meadow soil resistance of the Azov Sea region to pollution with heavy metals and oil," Ecol. Environ. Conserv. **23** (4), 2346–2351 (2017).
49. M. V. Korneykova, V. A. Myazin, and N. V. Fokina, "Restoration of oil-contaminated soils in mountain tundra (Murmansk region, Russia)," in *Proceedings of the Smart and Sustainable Cities Conf. "Green Technologies and Infrastructure to Enhance Urban Ecosystem Services"* (Springer-Verlag, Cham, 2020), pp. 187–198. https://doi.org/10.1007/978-3-030-16091-3_21
50. M. Kuyukina, A. Krivoruchko, and I. Ivshina, "Hydrocarbon- and metal-polluted soil bioremediation: progress and challenges," Microbiol. Austr. **39**, 133–136 (2018). https://doi.org/10.1071/MA18041
51. M. Li, X. Cheng, and H. Guo, "Heavy metal removal by biomineralization of urease producing bacteria isolated from soil," Int. Biodeterior. Biodegrad. **76**, 81–85 (2013). https://doi.org/10.1016/j.ibiod.2012.06.016
52. X. Li, X. Lan, W. Liu, X. Cui, and Z. Cui, "Toxicity, migration and transformation characteristics of lead in soil-plant system: effect of lead species," J. Hazard. Mater. **395**, 122676 (2020). https://doi.org/10.1016/j.jhazmat.2020.122676
53. Q. Lin, Z. Wang, S. Ma, and Y. Chen, "Evaluation of dissipation mechanisms by *Lolium perenne* L. and *Raphanus sativus* for pentachlorophenol (PCP) in copper co-contaminated soil," Sci. Total Environ. **368** (2–3), 814–822 (2006). https://doi.org/10.1016/j.scitotenv.2006.03.024
54. Y. L. Ma, W. Lu, L. L. Wan, and N. Luo, "Elucidation of fluoranthene degradative characteristics in a newly isolated *Achromobacter xylosoxidans* DN002," Appl. Biochem. Biotechnol. **175**, 1294–1305 (2019). https://doi.org/10.1007/s12010-014-1347-7
55. J. H. Makoi and P. A. Ndakidemi, "Selected soil enzymes: examples of their potential roles in the ecosystem," Afr. J. Biotechnol. **7** (3), 181–191 (2008).
56. V. Masindi and K. L. Muedi, "Environmental contamination by heavy metals," in *Heavy Metals* (IntechOpen, London, 2018), pp. 115–132.
57. M. Megharaj and R. Naidu, "Soil and brownfield bioremediation," Microb. Biotechnol. **10** (5), 1244–1249 (2017). https://doi.org/10.1111/1751-7915.12840
58. S. Mishra, J. Jyot, R. C. Kuhad, and B. Lal, "In situ bioremediation potential of an oily sludge-degrading bacterial consortium," Curr. Microbiol. **43** (5), 328–335 (2001). https://doi.org/10.1007/s002840010311
59. S. Mustafa, A. Al-Douseri, K. Majki, and E. Al-Saleh, "Potential of crude oil-degrading bacteria to co-resist heavy metals in soil," WIT Trans. Ecol. Environ. **173**, 697–705 (2013). https://doi.org/10.2495/SDP130581
60. D. Neina, "The role of soil pH in plant nutrition and soil remediation," Appl. Environ. Soil Sci. **2019**, 5794869 (2019). https://doi.org/10.1155/2019/5794869

61. M. Nie, Y. Wang, J. Yu, M. Xiao, L. Jiang, J. Yang, C. Fang, J. Chen, and B. Li, "Understanding plant–microbe interactions for phytoremediation of petroleum polluted soil," PLoS One **6**, e17961 (2011). https://doi.org/10.1371/journal.pone.0017961
62. A. Nosheen, H. Yasmin, R. Naz, A. Bano, R. Keyani, and I. Hussain, "*Pseudomonas putida* improved soil enzyme activity and growth of kasumbha under low input of mineral fertilizers," Soil Sci. Plant Nutr. **64** (4), 520–525 (2018). https://doi.org/10.1080/00380768.2018.1461002
63. A. Oliveira and M. E. Pampulha, "Effects of long-term heavy metal contamination on soil microbial characteristics," J. Biosci. Bioeng. **102** (3), 157–161 (2006). https://doi.org/10.1263/jbb.102.157
64. Y. M. Polyak, L. G. Bakina, M. V. Chugunova, N. V. Mayachkina, A. O. Gerasimov, and V. M. Bure, "Effect of remediation strategies on biological activity of oil-contaminated soil—A field study," Int. Biodeterior. Biodegrad. **126**, 57–68 (2018). https://doi.org/10.1016/j.ibiod.2017.10.004
65. F. Raiesi and S. Salek-Gilani, "The potential activity of soil extracellular enzymes as an indicator for ecological restoration of rangeland soils after agricultural abandonment," Appl. Soil Ecol. **126**, 140–147 (2018). https://doi.org/10.1016/j.apsoil.2018.02.022
66. B. Ravindran and P. N. S. Mnkeni, "Bio-optimization of the carbon-to-nitrogen ratio for efficient vermicomposting of chicken manure and waste paper using *Eisenia fetida*," Environ. Sci. Pollut. Res. **23** (17), 16965–16976 (2016). https://doi.org/10.1007/s11356-016-6873-0
67. R. L. Raymond, "Microbial oxidation of *n*-paraffinic hydrocarbons," Dev. Ind. Microbiol. **2** (1), 23–32 (1961).
68. R. Sánchez-Cruz, I. Tpia Vázquez, R. A. Batista-García, E. W. Méndez-Santiago, M. D. R. Sánchez-Carbente, A. Leija, V. Lira-Ruan, G. Hernández, A. Wong-Villarreal, and J. L. Folch-Mallol, "Isolation and characterization of endophytes from nodules of *Mimosa pudica* with biotechnological potential," Microbiol. Res. **218**, 76–86 (2019). https://doi.org/10.1016/j.micres.2018.09.008
69. P. Satapute, M. K. Paidi, M. Kurjogi, and S. Jogaiah, "Physiological adaptation and spectral annotation of arsenic and cadmium heavy metal-resistant and susceptible strain *Pseudomonas taiwanensis*," Environ. Pollut. **251**, 555–563 (2019). https://doi.org/10.1016/j.envpol.2019.05.054
70. G. Saxena, R. Kishor, and R. N. Bharagava, "Application of microbial enzymes in degradation and detoxification of organic and inorganic pollutants," in *Bioremediation of Industrial Waste for Environmental Safety*, Vol. 1: *Industrial Waste and Its Management* (Springer-Verlag, Singapore, 2020), pp. 41–51.
71. G. Shen, Y. Lu, and J. Hong, "Combined effect of heavy metals and polycyclic aromatic hydrocarbons on urease activity in soil," Ecotoxicol. Environ. Saf. **63** (3), 474–480 (2006). https://doi.org/10.1016/j.ecoenv.2005.01.009
72. G. Shen, Y. Lu, Q. Zhou, and J. Hang, "Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme," Chemosphere **61**, 1175–1182 (2005). https://doi.org/10.1016/j.chemosphere.2005.02.074
73. S. Sun, Q. Liu, S. Chen, W. Yu, C. Zhao, and H. Chen, "Optimization for microbial degradation of petroleum hydrocarbon (TPH) by *Enterobacter* sp. S-1 using response surface methodology," Petrol. Sci. Technol. **37**, 821–828 (2019). https://doi.org/10.1080/10916466.2019.1566256
74. H. I. Tak, F. Ahmad, and O. O. Babalola, "Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals," Rev. Environ. Contam. Toxicol. **223**, 33–52 (2013). https://doi.org/10.1007/978-1-4614-5577-6_2
75. N. F. Y. Tam and S. Tiquia, "Assessing toxicity of spent pig litter using a seed germination technique," Resour. Conserv. Recycl. **11** (1–4), 261–274. 1994.
76. S. J. Varjani, "Microbial degradation of petroleum hydrocarbons," Bioresour. Technol. **223** (1), 277–286 (2017). https://doi.org/10.1016/j.biortech.2016.10.037
77. X. Wang, J. Zheng, Z. Han, and H. Chen, "Bioremediation of crude oil-contaminated soil by hydrocarbon-degrading microorganisms immobilized on humic acid-modified biofuel ash," J. Chem. Technol. Biotechnol. **94** (6), 1904–1912 (2019). https://doi.org/10.1002/jctb.5969
78. Y. Xian, M. Wang, and W. Chen, "Quantitative assessment on soil enzyme activities of heavy metal contaminated soils with various soil properties," Chemosphere **139**, 604–608 (2015). https://doi.org/10.1016/j.chemosphere.2014.12.060
79. R. Xiao, S. Wang, R. Li, J. J. Wang, and Z. Zhang, "Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China," Ecotoxicol. Environ. Saf. **141**, 17–24 (2017). https://doi.org/10.1016/j.ecoenv.2017.03.002
80. X. Yang, J. Liu, K. McGrouther, H. Huang, K. Lu, X. Guo, L. He, X. Lin, L. Che, Z. Ye, and H. Wang, "Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil," Environ. Sci. Pollut. Res. Int. **23** (2), 974–984 (2016). https://doi.org/10.1007/s11356-015-4233-0
81. Z. X. Yang, S. Q. Liu, D. W. Zheng, and S. D. Feng, "Effects of cadmium, zinc and lead on soil enzyme activities," J. Environ. Sci. **18** (6), 1135–1141 (2006). https://doi.org/10.1016/s1001-0742(06)60051-x
82. J. Zhang, Q. Shi, S. Fan, Y. Zhang, M. Zhang, and J. Zhang, "Distinction between Cr and other heavy-metal-resistant bacteria involved in C/N cycling in contaminated soils of copper producing sites," J. Hazard. Mater. **402**, 123454 (2021). https://doi.org/10.1016/j.jhazmat.2020.123454
83. C. Zhou, N.Ge, J.Guo, L. Zhu, Z. Ma, S. Cheng, and J. Wang, "*Enterobacter asburiae* reduces cadmium toxicity in maize plants by repressing iron uptake-associated pathways," J. Agric. Food Chem. **67**, 10126–10136 (2019). https://doi.org/10.1021/acs.jafc.9b03293

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