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Bacterial Number in Petroleum-Contaminated Haplic Chernozem after Amelioration

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Abstract—Bacteria are a sensitive marker of the state of soils contaminated with oil and petroleum products. Introduction of ameliorants leads to the changes in the oil content in soil, which in turn affects the number of bacteria. The aim of our work is to estimate the number of bacteria in petroleum-contaminated Haplic chernozem after amelioration. Biochar, sodium humate, nitroammophoska (NPK fertilizer compound), and Baikal EM-1 microbiological fertilizer have been introduced into petroleum hydrocarbons-contaminated Haplic chernozem (5% petroleum hydrocarbons by weight of soil). After 90 days of the experiment, the number of bacteria in the soil was estimated on the basis of the total number of bacteria determined by the method of luminescent microscopy, the number of actinomycetes, and amylolytic and ammonifying bacteria determined by the method of inoculation on solid nutrient media. The integral indicator of the number of bacteria (INB) of the soil was calculated before and after the application of ameliorants. To assess the soil state of petroleum contaminated Haplic chernozem after remediation with biochar, it is possible to use the total number of bacteria; when it is remediated with nitroammophoska, the number of ammonifying bacteria; with sodium humate, the number of actinomycetes; and with Baikal EM-1, the number of amylolytic bacteria. According to the INB of soils, the most effective remediation dose for nitroammophoska, biochar, and Baikal EM-1 is $D_{0.5}$, while the most effective for remediation with sodium humate is D_2. It is advisable to use the results of the study in biodiagnostics and monitoring the state of petroleum contaminated Haplic chernozem after amelioration.

Keywords: soil, ecotoxicity, biochar, sodium humate, nitroammophoska (compound NPK fertilizer), Baikal EM-1, biodiagnostics

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INTRODUCTION

The South of Russia is an important strategic route for the transportation of oil and petroleum products in our country, as well as to neighboring countries. Thousands of kilometers of oil pipelines and highways have been laid through the territory of Azerbaijan and further along the South of Russia (Stavropol and Krasnodar kai; the Republics of Dagestan, Ingushetia, and Chechnya; and Rostov and Volgograd oblast). They are widely used for transportation of oil and petroleum products (Khoward et al., 2007; Ismailov, Nadzhafova, 2022). Because oil fields are mainly located on the sea shelf, the level of oil and petroleum pollution of the coastal waters of the Black Sea and Azov Sea exceeds the established standards by several times (Mironovk, Mironov, 2020). Sea water pollution also directly affects the pollution of coastal areas. Accidents resulting in soil pollution lead to disturbances and violations of the vegetation cover and the long-term alienation of lands. There are three sectors of oil refineries with a processing capacity of 27.9 million t of oil per year in the Southern Federal District: large refineries (Lukoil Volgograd Oil Refinery, 15.7 million t, and RN-Tuapse Refinery, 12 million t) and several small refineries, as well as miniplants (Slavyansk-ECO LLC, Afipsky, Ilsky, and Novoshakhtinsk oil refineries) (https://yfo.spr.ru/all/neftepererabativayuschie-zavodi; https://www.kommersant.ru/).

Remediants reduce the content of oil, petroleum products, and related compounds (such as benzo(a)pyrene) and are used to restore the ecological state of the soil (Minnikova et al., 2018, 2019; Nadzhafova, Bagirova, 2021; Smagin et al., 2021; Kovaleva and et al., 2022; Minnikova et al., 2021; Sozina, Danilov, 2023). Microbiological indicators that reflect the soil state at a microscopic level are sensitive markers of both soil disturbance and subsequent restoration (Vodopyanov et al., 2009; Kireeva et al., 2011; Kabirov et al., 2012). According to T. Dobrovolskaya (2015), an ordinary chernozem under optimal functioning conditions is dominated by fungi (87.8% of the total number) and fungal spores (8%), while the proportion of bacteria is only 2.8% (by biomass). Mostly fungi destruct plant material and do not fix nitrogen. However, anthropogenic activities disturb this balance and significantly change the cycles of carbon and nitrogen. A sufficient number of actinomycetes and amylolytic and ammonifying bacteria provide self-purification of the soil under various types of anthropogenic load (Artamonova et al., 2002; Akimenko et al., 2013, 2014).

The number of bacteria is the most important diagnostic indicator of the soil state under various types of anthropogenic load, including petroleum pollution (Zvyagintsev et al., 2002; Kolesnikov et al., 2010; Degtyareva et al., 2009). Changes in the abundance and composition of bacteria resulting from petroleum pollution are directly related to the physicochemical properties of the soil after pollution and affect the enzymatic activity of soils, as well as CO_2 emission (Vodyanitskii et al., 2016; Minnikova et al., 2019, 2021).

The aim of this study is to estimate the number of bacteria in petroleum-contaminated Haplic chernozem after remediation.

MATERIALS AND METHODS

The samples of the calcareous Haplic chernozem for the model experiment were taken from the arable area of Southern Federal University's botanical garden. Samples were taken from the plow horizon from a depth of 0–10 cm. The content of organic matter was determined by the method of wet burning of the soil with a dichromate mixture with a spectrophotometric analysis according to I. Tyurin (Tyurin, 1931), the reaction of the soil medium was analyzed in a soil-water extract (water : soil, 2.5 : 1) using a pH-150MI analyzer, and the granulometric composition of soil was determined by N. Kachinsky's method (Galeeva et al., 2012). The horizon is characterized by a humus content equal to 3.7%, pH = 7.8, and heavy loamy granulometric composition.

To evaluate bacterial number after remediation in laboratory conditions, biochar (5% by soil weight), nitroammophoska (NPK fertilizer compound) (0.375% by weight of soil), sodium humate (1% solution), and Baikal EM-1 (0.5% solution) were introduced to the modeled chernozem petroleum contamination (5% oil by soil weight) samples. Each remediant was introduced at the dose recommended by the manufacturer and based on the literature data (D), at a dose reduced by 50% ($D_{0.5}$) and at a doubled dose, 200% (D_2).

Biochar is pure birch (*Betula alba* Ehrh.) charcoal grade A (*GOST* (State Standard) 7657–84) with a carbon content of at least 85%. The product is produced by wood pyrolysis (800°C) in retort plants with no oxygen access (DianAGRO, Novosibirsk, Russia). The amount of carbon in 1 t of biochar is equivalent to 3 t of carbon (C) in carbon dioxide (CO_2). In relation to petroleum hydrocarbons, biochar serves as a sorbent and stimulator of native soil biota.

Nitroammophoska is a mineral nitrogen–phosphorus–potassium fertilizer. It is physiologically neutral and the most common fertilizer, with the N : P : K ratio of 15 : 15 : 15. In nitroammophoska, nitrogen and potassium are in the form of easily soluble compounds (NH_4NO_3, NH_4Cl, KNO_3, and KCl) and phosphorus is partly in the form of dicalcium phosphate, insoluble in water, but available to plants, and partly in the forms of water-soluble ammonium phosphate and calcium monophosphate. In the studies, we used nitroammophoska of AO Minudobreniya Co. (Rossosh, Voronezh oblast, Russia). Such nitrogen fertilizer is introduced because of the imbalance between carbon (C) and nitrogen (N) after the introduction of oil, an anthropogenic source of carbon.

Sodium humate was introduced into the soil in the form of a solution of GUMI-30 from NVP Bashinkom (Ufa, Bashkortostan, Russia). Sodium humate contains sodium salts of humic acids (up to 70%). It also contains trace elements (Mo, Cu, Co, Mg, and Zn) and heavy metals (Pb and Cd). Sodium humate is one of the best growth stimulants for vegetable and fruit crops.

Baikal EM-1 microbiological fertilizer contains 60 beneficial microorganisms, including bacterial strains (*Paenibacillus pabuli*, *Azotobacter vinelandii*, *Lactobacillus casei*, *Clostridium limosum*, *Cronobacter sakazakii*, *Rhodotorulla mucilaginosa*, and *Cryptococcus*), hybrid yeasts (*Saccharomyces*, *Candida lipolitica*, *Candida norvegensis*, and *Candida guilliermondii*), and fungi (*Aspergillus*, *Penicillium*, and *Actinomycetales*). The introduction of such a combination of microorganisms stimulates oil decomposition and contributes to the restoration of the ecological functions of the polluted soil.

Soil (300 g) that had been dried and sieved through a sieve with 3-mm openings was placed in numbered vegetative vessels of a certain variant of the experiment (three replicates) and moistened up to 25% by weight of soil. Oil with a concentration of 5% by weight of soil was added to each vessel, and the content was thoroughly mixed. This oil concentration in soil is the most common for oil spills. Sulfurous oil from the Novoshakhtinsk Oil Refinery (Novoshakhtinsk, Rostov oblast, Russia) was used for the model experiment. Oil characteristics are the following: density 0.861 kgm^3; sulfur content, 1.34%; mass fraction of water, 0.27%; concentration of chloride salts, 73 mg/dm^{-3}; mass fraction of mechanical impurities, 0.006%; mass frac-

Table 1. Methods for evaluation of number of bacteria

No.	Title	Method	Units
1	Total number of bacteria	Fluorescence microscopy according to D. Zvyagintsev	1 billion/1 g soil
2	Number of actinomycetes	Deep plating on dense nutrient acidified Czapek-Dox agar	thousand CFU/ 1 g soil
3	Number of ammonifying bacteria	Deep plating on dense beef extract agar	thousand CFU/ 1 g soil
4	Number of amylolytic bacteria	Deep plating on dense KAA agar	thousand CFU/1 g soil

tion of paraffin, 4.46%. Oil introduction was followed by the introduction of remediants. Vessels were incubated in a Binder KBW 240 climate chamber at controlled temperature (25°C) and soil moisture (25% of soil weight) for 90 days. At the end of the exposure period the number of bacteria in the soil was assessed via fluorescence microscopy and direct plating onto soil nutrient media (Table 1) (Kazeev et al., 2016).

The total number of bacteria was estimated by fluorescent microscopy according to (Zvyagintsev et al., 2002). The total number of bacteria is expressed as 10^9 bacteria per gram of dry soil mass:

$$M = \frac{b \times A \times H \times T}{P}, \quad (1)$$

where M is the number of cells in 1 g of fresh soil; A is the average number of cells within one field of view; b is the magnification factor (b = 4); H is the dilution index; T is the conversion factor in billions of bacteria per 1 g of soil (T = 1010); and P is the area of the field of view, μm^2.

To assess the state of soils after remediation with biochar, sodium humate, nitroammophoska, and Baikal EM-1, an integral indicator of the number of bacteria (INB) in Haplic Chernozem was calculated for all microbiological indicators (Kolesnikov et al., 2019). In the sample, the control value of each of the indicators is taken as 100%, with the value of the microbiological indicator in the remaining samples being expressed as a percentage in relation to it. Relative values were calculated for all indicators: the total number of bacteria, the number of actinomycetes, number of ammonifying bacteria, and number of amylolytic bacteria. The stage of calculating the relative value (B_1) is presented using indicator No. 1 as an example:

$$S_1 = \frac{S_{x1}}{S_{\max 1}} \times 100, \quad (2)$$

where S_1 is the relative score of indicator No. 1, S_{x1} is the actual value of indicator No. 1, and $S_{\max 1}$ is the maximum value of indicator No. 1.

After that, we calculated the average estimated score of all the studied microbiological indicators for each variant, which absolute values cannot be summed up, since they have different units of measurement (1 billion/g; thousand CFU/1 g of soil).

$$S_{av} = \frac{S_1 + S_2 + S_3 + S_4}{4}. \quad (3)$$

The INB in the soil was calculated according to Eq. (4):

$$INB = \frac{S_{av}}{S_{av.\max}} \times 100, \quad (4)$$

where S_{av} is the average score of all indicators and $S_{av.max}$ is the maximum score of all indicators.

Statistical processing of the obtained data was carried out using the STATISTICA 12.0 software package. The statistics (means and variance) was determined and the reliability of the various samples was established using analysis of variance (Student *t*-test).

RESULTS

Changes in Total Number of Bacteria

The independent effect of remediants on the total number of bacteria in uncontaminated chernozem was studied (Fig. 1). After contamination, the number of bacteria decreased by 76% relative to the control. It was found that the total number of bacteria decreases with the introduction of all remediants by 32–81% (relative to the control). An increase in the bacterial number was observed after introduction of D_2 sodium humate; it was 30% higher than in the control. However, the introduction of remediants into the petroleum-contaminated Haplic Chernozem has a multidirectional effect on the total number of bacteria. The number of bacteria was the highest after the introduction of $D_{0.5}$ biochar and nitroammophoska (52 and 11% higher than with petroleum pollution, respectively).

The introduction of Baikal EM-1 at the recommended increases the number of bacteria in petroleum-contaminated Haplic Chernozem by 26% relative to petroleum pollution. According to the total number of bacteria, we compiled a series of effectiveness of the remediants in chernozem:

biochar > Baikal EM-1 > sodium humate
> nitroammophoska.

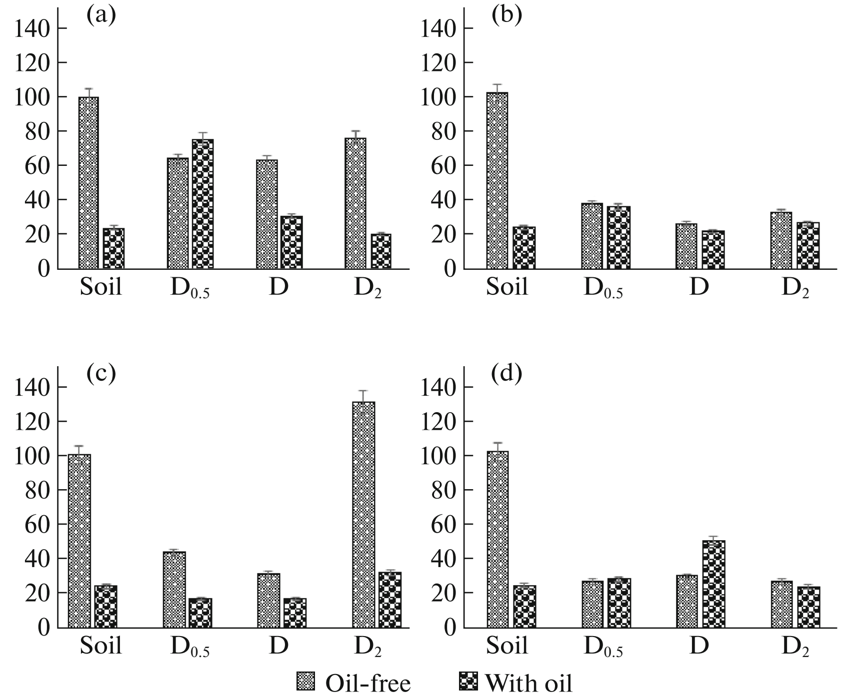

Fig. 1. Changes in total number of bacteria in oil-contaminated chernozem after introduction of remediants, % of control: (a) biochar, (b) nitroammophoska, (c) sodium humate, and (d) Baikal EM-1.

Changes in Number of Actinomycetes

Oil pollution led to an 81% decrease in the number of actinomycetes (relative to the control). The number of actinomycetes in the soil after the introduction of $D_{0.5}$ biochar and D_2 sodium humate increased by 19% and reached the control level, respectively (Fig. 2). On the contrary, the number of actinomycetes in the petroleum-contaminated chernozem decreased by 8% with the introduction of biochar (relative to the remediant-free oil-contaminated chernozem). Introduction of D and D_2 of sodium humate increased this indicator by 20–22% compared with the remediant-free contaminated soil. The introduction of Baikal EM-1 already at a dose of $D_{0.5}$ had a positive effect on the number of actinomycetes (by 12% of oil pollution).

According to the number of actinomycetes, we compiled a series of effectiveness of the remediants in the chernozem:

sodium humate > Baikal EM-1 > biochar > nitroammophoska.

Changes in Number of Amylolytic Bacteria

Oil-contaminated soil was characterized by a decreased number of amylolytic bacteria (75% less than in the control). Biochar at concentrations of $D_{0.5}$ and D_2 increased the number of amylolytic bacteria by 28 and 17% above the control, respectively (Fig. 3). Nitroammophoska at a concentration of $D_{0.5}$ increased the number of amylolytic bacteria by 18% relative to the control.

According to the number of amylolytic bacteria in the oil-contaminated chernozem after remediation, the series of effectiveness of the remediants is the following:

Baikal EM-1 > nitroammophoska > biochar > sodium humate.

Changes in Number of Ammonifying Bacteria

The number of ammonifying bacteria in oil-free soil after introduction of $D_{0.5}$ biochar was increased by 11% compared to the control (Fig. 4). Introduction of each studied remediant at other concentrations (D and D_2) led to a decrease in the number of ammonifying bacteria by 60–98% relative to the control. Petroleum contamination of the soil resulted in 98% decrease in the number of ammonifying bacteria in the soil (relative to the control).

Introduction of biochar into oil-contaminated chernozem led to an increase in the number of ammonifying bacteria (35–80% relative to oil pollution). However, introduction of the increased concentrations of biochar tended to decrease the number of bacteria. An increase in the number of ammonifying bacteria in the oil-contaminated chernozem was also found with an introduction of nitroammophoska (22–64%) and sodium humate (26–51%) (relative to the

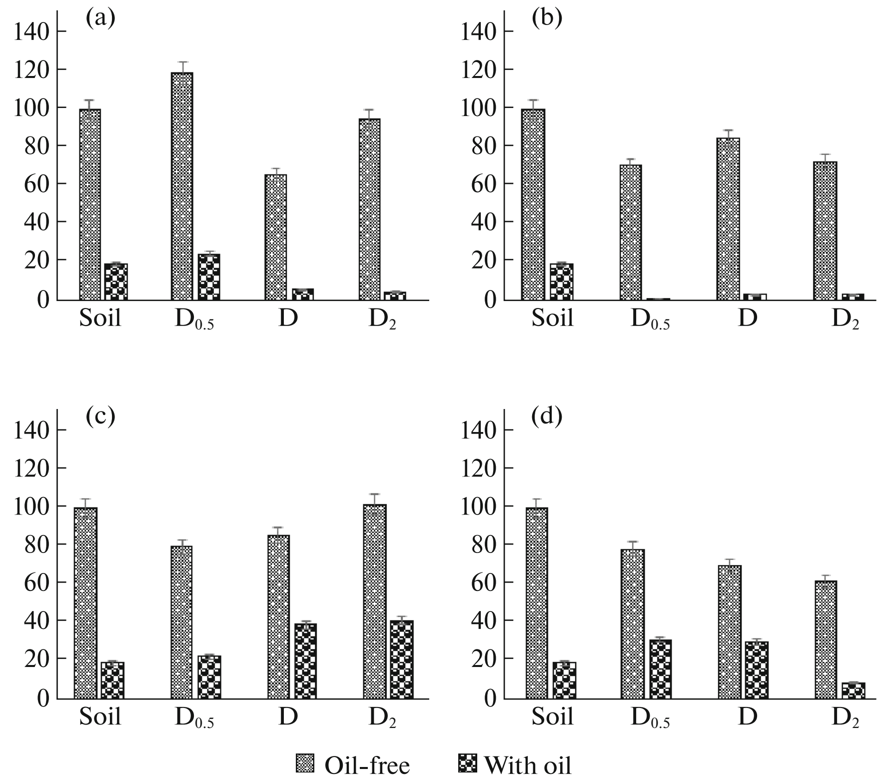

Fig. 2. Changes in number of actinomycetes in oil-contaminated chernozem after introduction of remediants, % of control: (a) biochar, (b) nitroammophoska, (c) sodium humate, and (d) Baikal EM-1.

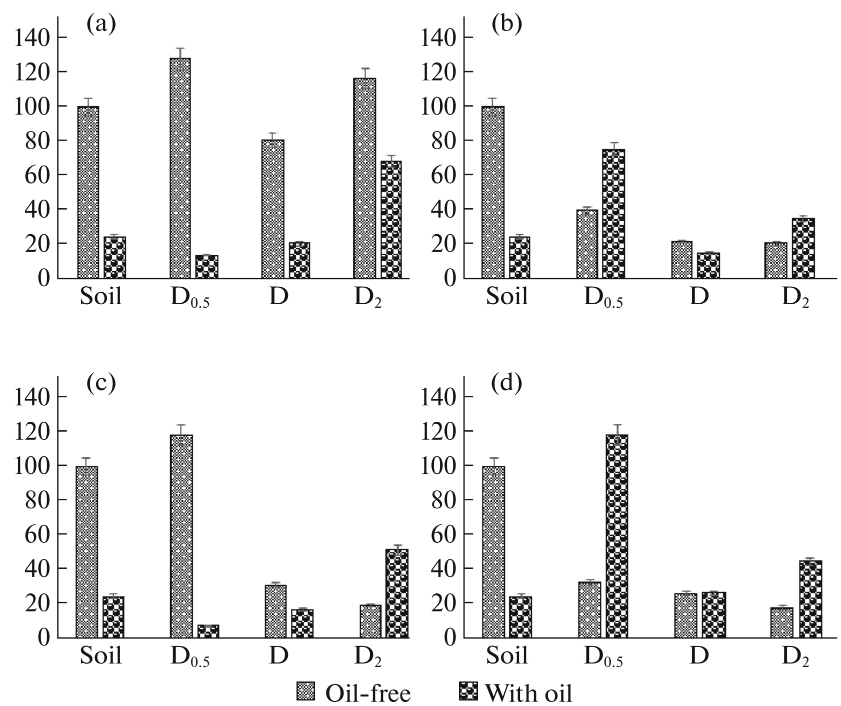

Fig. 3. Changes in number of amylolytic bacteria in oil-contaminated chernozem after introduction of remediants, % of control: (a) biochar, (b) nitroammophoska, (c) sodium humate, and (d) Baikal EM-1.

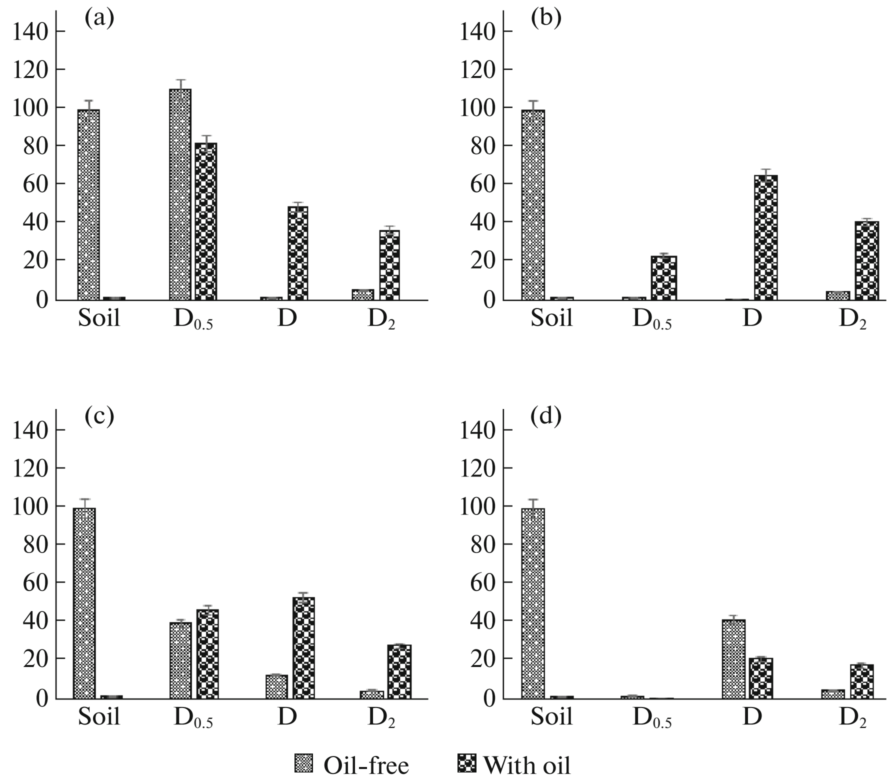

Fig. 4. Changes in number of ammonifying bacteria in oil-contaminated chernozem after introduction of remediants, % of control: (a) biochar, (b) nitroammophoska, (c) sodium humate, and (d) Baikal EM-1.

oil-contaminated remediant-free soil). Baikal EM-1 caused an increase in the number of ammonifying bacteria in oil-contaminated chernozem only at the recommended (D) and doubled (D_2) concentrations by 19 and 16% relative to oil pollution, respectively. According to the number of ammonifying bacteria in the oil-contaminated chernozem after remediation, the series of effectiveness of the remediants is

biochar > sodium humate
> nitroammophoska > Baikal EM-1.

Evaluation of Sensitivity of Bacteria

The sensitivity of each analyzed type of microorganism was assessed by the correlation ratio with the oil content in the soil after remediation. For biochar introduction into oil-contaminated chernozem, the series of the most biologically sensitive indicators is

total number of bacteria = ammonifying bacteria
> actinomycetes = amylolytic bacteria.

The highest sensitivity, i.e., information content, estimated by the degree of restoration of the number of microorganisms after biochar introduction into the oil-contaminated chernozem was established for the total number of bacteria, while the lowest was established for the number of actinomycetes and amylolytic bacteria.

For nitroammophoska introduction into oil-contaminated chernozem, the most biologically sensitive indicators arranged as

ammonifying bacteria = amylolytic bacteria
> actinomycetes = total number of bacteria.

The highest sensitivity, i.e., information content, estimated by the degree of restoration of the number of bacteria in the oil-contaminated chernozem after introduction of nitroammophoska was established for the number of ammonifying and amylolytic bacteria, while the lowest was established for the total number of bacteria in Haplic chernozem after remediation.

When sodium humate is introduced into oil-contaminated chernozem, the range of sensitivity of microbiological indicators is the following:

actinomycetes > ammonifying bacteria
> total number of bacteria > amylolytic bacteria.

The highest sensitivity estimated by the degree of restoration of the number of bacteria after introduction of sodium humate was established for actinomycetes, while the lowest was established for amylolytic bacteria.

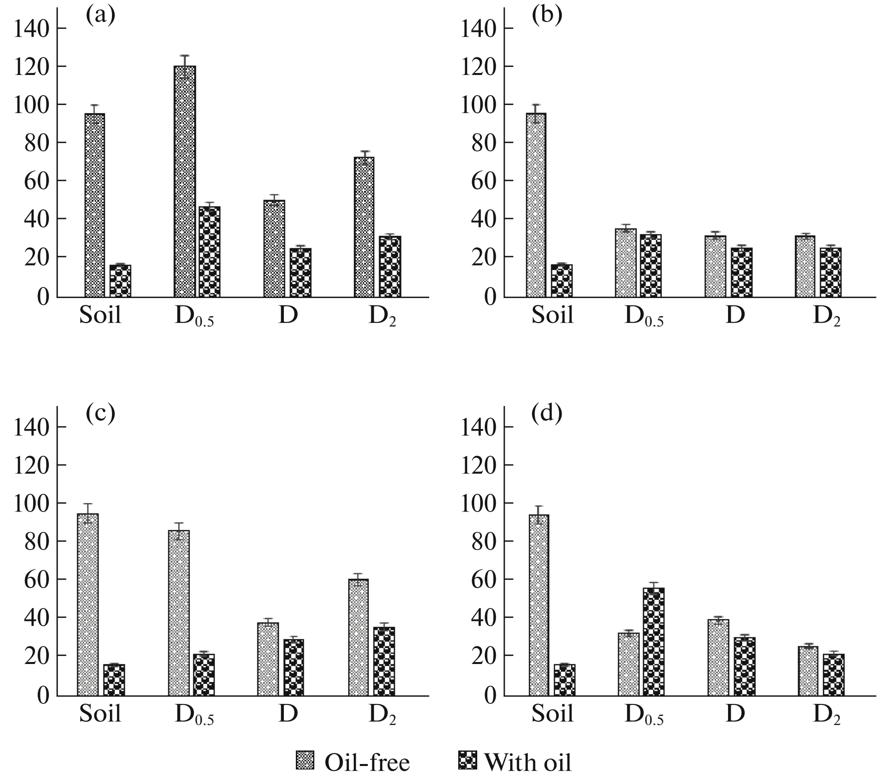

Fig. 5. Integral indicator of number of bacteria (INB) in oil-contaminated chernozem after introduction of remediants, % of control: (a) biochar, (b) nitroammophoska, (c) sodium humate, and (d) Baikal EM-1.

When Baikal EM-1 was introduced into oil-contaminated chernozem, the sensitivity of microbiological indicators can be represented as

amylolytic bacteria > actinomycetes
= total number of bacteria
> ammonifying bacteria.

The highest information content estimated by microbiological indicators when introducing Baikal EM-1 was established for the number of amylolytic bacteria, while the smallest was established for the number of ammonifying bacteria.

Changes in the Integral Indicator of the Number of Bacteria in Chernozem

The result of INB calculation according to microbiological indicators is shown in Fig. 5. The introduction of remediants into oil-free soil contributed to a decrease in the number of bacteria. However, when remediants were introduced into petroleum-contaminated soil, there was an increase in the number of bacteria and actinomycetes by 20–57% relative to the control.

The introduction of biochar $D_{0.5}$ increases the INB by 32%; an increase in the dose (D and D_2) leads to some decrease in bacterial number (an increase by 10 and 16% relative to the oil-contaminated background). A minimum dose ($D_{0.5}$) of nitroammophoska and Baikal EM-1 led to an increase in the INB by 17 and 42%, respectively. At the recommended dose (D), an increase in INB was established after the introduction of nitroammophoska, sodium humate, and Baikal EM-1—by 10, 14, and 15% relative to the petroleum-contaminated background, respectively.

Thus, according to the INB, for remediation of the petroleum-contaminated chernozem, the most effective dose for nitroammophoska, biochar, and Baikal EM-1 is $D_{0.5}$, while for sodium humate it is D_2.

DISCUSSION

According to our study, petroleum contamination of the soil resulted in a decrease in the number of all bacterial species analyzed in the following order: ammonifying bacteria > actinomycetes > amylolytic bacteria = total number of bacteria. Petroleum pollution reduces the number of most microorganisms in the soil, in particular, leads to a decrease in the number of dominant species: *Thiothrix*, *Sphingomonas*, *Gemmatimonas*, *Pseudomonas*, *Acinetobacter*, and *Pedobacter* (Liang et al., 2016; Xu et al., 2021; Shi et al., 2022). This results from the changes in the content of total organic carbon, sulfur, as well as changes of pH, which are limiting factors for the growth of the representatives of the dominant genera *Sphingomonas*, *Thiothrix*, and *Pseudomonas*. (Kabirov et al., 2012) previously proposed a list of indicators of biological activity and recovery of petroleum-contaminated chernozem (1, 4, and 8% of oil by weight of soil) after the application of microbiological preparations, which, in addition to catalase activity, also includes the number of carbon-oxidizing microorganisms, heterotrophic microorganisms, and microscopic fungi. The number of heterotrophs and microscopic fungi increased with the introduction of *Belvitamil* to the soil contaminated with oil at a concentration of 8% of the soil mass. Introduction of the biological product *Metabolite* into the oil-contaminated soil under sugar beet stimulated the total number of heterotrophs by ten and four times in the beet rhizosphere and edaphosphere, respectively (Kireeva et al., 2011). Cellulose-degrading bacteria in the edaphosphere were stimulated by a factor of 3 when the biopreparation was introduced (compared to contamination). This effect can be explained by intensification of the processes of fiber decomposition and improvement of carbohydrate nutrition of plants resulting from the activity of metabolites of the endomycorrhizal fungus, a component of the biological product.

The number of actinomycetes is closely related to the carbon content in the soil; at low levels of oil pollution, it can even increase due to an additional source of nutrition, i.e. individual oil hydrocarbons (Lednev, Skvortsova, 2017). High levels of soil pollution with oil (more than 50 g/kg) decrease the number of actinomycetes. In the present study, the number of actinomycetes tended to increase after the application of sodium humate in all doses, Baikal EM-1 in $D_{0.5}$ and D, as well as biochar in $D_{0.5}$. This is associated with the close relationship between the content of anthropogenically introduced carbon in the soil and the number of actinomycetes (Vinogradova, Kozhevin, 2011; Polyak, Sukharevich, 2021). However, the number of actinomycetes is a less sensitive indicator than the number of ammonifying and amylolytic bacteria. Our study found a decrease in the number of ammonifying and amylolytic bacteria in oil-contaminated chernozem by 75–98% relative to the control. The most significant decrease in the number of microorganisms affected by oil pollution was found for ammonifying bacteria (98%), which resulted from the C : N imbalance due to an introduction of oil carbon (Ismailov, 1983; Kolesnikov et al., 2007; Korshunova, 2019). A significant decrease in the number of soil ammonifying bacteria related to oil pollution is associated with the introduction not only of an additional amount of organic matter available to soil microorganisms, but also toluene, benzene, xylene, naphthalene, heavy metals, and a number of other compounds toxic to bacteria, as was previously established (Ismailov, 1983; Pikovskii, 1993; Zvyagintsev et al., 2002; Sharkova et al., 2011).

Both individual groups of microorganisms and the total number of bacteria directly depend on the residual oil content in the soil after remediation. As can be seen from the series of sensitivity of microbiological indicators obtained in our study, the total number of bacteria is the most sensitive indicator for the soil state after the introduction of biochar into oil-contaminated chernozem. Previously, it was found that introduction of biochar in two concentrations (10 and 20% of the soil mass) into oil-contaminated Haplic chernozem led to an increase in the number of bacteria with a decrease in oil content and an increase in the intensity of soil respiration (r = –0.60–1.00) (Minnikova et al., 2022). An increase or decrease in the number of microorganisms depends on the concentration and type of a remediant. So, for remediation of oil pollution with biochar, Baikal EM-1, and nitroammophoska, the recommended dose is $D_{0.5}$, while the dose for remediation with sodium humate is D_2. The highest increase in the number of bacteria, including amylolytic and ammonifying bacteria, was observed after the introduction of biochar and Baikal EM-1. Other remediants were found to be less effective. The introduction of nitroammophoska provides restoration of the number of amylolytic and ammonifying bacteria, and the use of sodium humate is beneficial for actinomycetes and ammonifying bacteria. The total number of bacteria increased only after biochar introduction into the oil-contaminated chernozem. The high efficiency of nitroammophoska, as a source of mineral nitrogen, in restoring the number of amylolytic and ammonifying bacteria in oil-contaminated chernozem is associated with the nitrogen requirements of microorganisms, as nitrogen content sharply decreased after introduction of a large amount of oil carbon. An increase in the number of actinomycetes after the introduction of sodium humate into oil-contaminated chernozem is associated with an increase in the consumption of organic and mineral substrates of humate (Kuznetsova et al., 1989; Bezuglova et al., 2016).

The results obtained show that the number of microorganisms can be used as a diagnostic criterion for the state of oil-contaminated soil after the introducing various remediants. More than that, these remediants may use different mechanisms for the soil remediation: a direct increase in the number of bacteria due to the introduction of nutrients (sodium humate and biochar), optimization of the C : N ratio (nitroammophoska) or introduction of other microorganisms that are potential petroleum destructors (Baikal EM-1).

CONCLUSIONS

The use of the studied remediants in remediation of oil pollution affects the abundance of soil bacteria. To assess the ecological state of soils by the abundance of bacteria, the total number of bacteria can be used after soil treatment with biochar, the number of ammonifying bacteria after treatment with nitroammophoska, the number of actinomycetes after sodium humate, and the number of amylolytic bacteria after application of Baikal EM-1. According to the INB of soils, the most effective dose for remediation with nitroammophoska, biochar, and Baikal EM-1 is $D_{0.5}$, while it is D_2 for remediation with sodium humate. The results of the study can be used in biodiagnostics and monitoring of the ecological state of petroleum-contaminated chernozem after remediation.

AUTHOR CONTRIBUTIONS

T.V. Minnikova and S.I. Kolesnikov, design of the experiments, general management; A.S. Ruseva and D.A. Trufanov, conducting experiments, data processing, manuscript preparation; T.V. Minnikova, text editing, preparation of figures and tables. All authors participated in the discussions.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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