Biologia et Biotechnologia

PUSHCHINO SCIENTIFIC CENTER for Biological Research of the RAS

UDK 504.064:576.8

Article

Development of an Adsorptive Bioremediation Method for Oil-Contaminated Mineral Soils of North-Western Siberia on the Example of Illuvial-Ferruginous Podzol

Vasilyeva G. K.[1,*], Mikhedova E. E.[1], Strijhakova E. R.[1], Akhmetov L. I.[2]

[1]Institute of Physical-Chemical and Biological Problems of Soil Science, the Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences"; soil@pbcras.ru

[2]G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, the Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences"; adm@ibpm.ru

*Responsible for correspondence: gkvasilyeva@rambler.ru

Citation: Vasilyeva G.K., Mikhedova E.E., Strizhakova E.R., Akhmetov L.I. Development of an adsorptive bioremediation method for oil-contaminated mineral soils of North-West Siberia by example of illuvial-ferruginous podzol. *Biologia et Biotechnologia* 2024, 1, 3. https://doi.org/10.61847/pbcras.bbt.2024.1.3

Received: 25.09.2024

Accepted: 18.10.2024

Published: 07.11.2024

Abstract: An important problem of modern time is the development of environmentally friendly methods for remediating soils contaminated with crude oil and petroleum products. Previously, we proved the effectiveness of an adsorptive bioremediation approach for remediation of petroleum-contaminated soils of the East-European Plain. The purpose of this work was to develop a similar method for the remediating podzolic soils common in North-Western Siberia, where the bulk of Russian oil is produced. It was concluded from the results of microfield and pot experiments with illuvial-ferruginous podzol, contaminated with 6 and 12% crude oil, that the method of the adsorptive bioremediation is highly effective for those soils. At the same time, in case of moderately contaminated soil, the best sorbent for that purpose is a mixed sorbent ACD based on granular activated carbon and diatomite, while a composite sorbent ACDP (a mixture of ACD and peat) demonstrated the best results for adsorptive bioremediation of the highly contaminated soils. Bioremediation of those soils on the background of optimal doses of the sorbents could reduce the concentration of petroleum products to the permissible level for recultivated soils of the Khanty-Mansiysk Autonomous Okrug intended for forest use by the end of the first or second warm seasons, and to the level for agricultural use – after two or three years of the treatment. Besides, the indicators of the soil integral toxicity will be reduced to a minimum to the end of the treatment. The mechanisms of the sorbents positive influence are explained by the reduction of soil toxicity due to the predominantly reversible sorption of petroleum hydrocarbons and their metabolites; due to maintaining optimal soil moisture and pH, as well as due to increasing resistance of microorganisms and plants to adverse factors. All these factors create optimal conditions for accelerated biodegradation of pollutants and minimizing the integral soil toxicity.

Keywords: sandy loam podzols, crude oil-pollution, sorbents, adsorptive bioremediation, biopreparations, phytotoxicity, biotoxicity.

Introduction

An important problem of our time is the development of environmentally friendly methods for protecting the natural environment from man-made pollution. One of the main sources of such pollution are the sites of extraction, processing and transportation of crude oil and petroleum products. According to the Statistical Bulletin of the Russian Federation, from 124 to 132 thousand hectares were contaminated annually due to accidental leakages during the transit of crude oil, gas and petroleum products in 2020-2022 [1].

The bulk of Russia's hydrocarbons are produced in Western Siberia, where the Khanty-Mansiysk Autonomous Okrug - Yugra (KhMAO) is the leader in oil production, with an area of ~534.8 thousand km^2. About 116 thousand km of oil and gas production pipelines pass through its territory. Therefore, the most common environmental problems are breaks in main oil and oil product pipelines. In 2021, for example, 942 accidents were registered in Yugra (*Fig. 1*), of which more than 68% of incidents occurred on oil pipelines [2].

Figure 1. A contaminated area after an accidental oil spill as a result of a rupture of a main oil pipeline in the KhMAO near the Samotlor oil field (author's photo by E.E. Mikhedova).

When crude oil enters the soil due to an accidental leakage, it has a strong negative impact on the soil properties and soil biota. The most common effects of oil include heating, hypoxia, oxidative and osmotic stress. All these factors cause changes in the composition of soil microbiota, inhibit plant growth, and have a detrimental effect on soil invertebrates. They directly or indirectly have a negative impact on human health, since oil pollution is highly toxic and has carcinogenic, mutagenic and teratogenic potential [3]. Federal Law No. 7 "On Environmental Protection" states that after the elimination of oil and oil product spills, reclamation and other restoration work must be carried out in accordance with the procedure established by the legislation of the Russian Federation [4].

Due to such rich hydrocarbon reserves in North-Western Siberia, the technogenic load on natural components is quite high. Oil pollution of soils leads to the withdrawal of vast territories from the national economy. These problems must be solved effectively and quickly, so the task of choosing the optimal way to eliminate the consequences of pollution often arises.

The most promising approach for remediating soils from crude oil is considered to be bioremediation, the method based on the ability of soil microorganisms to degrade and utilize petroleum hydrocarbons. Until now, most of the works on bioremediation of petroleum-contaminated soils were aimed at obtaining new biopreparations based on isolated strains of microorganisms able to degrade petroleum hydrocarbons [5–7]. However, this approach is generally recommended only for soils with hydrocarbons content no more than 5%, so bioremediation will be ineffective in solving the problems of emergency response in oil-producing regions where the concentration of petroleum hydrocarbons in the soil is significantly higher [8].

A number of analytical reviews examining the perspectives of bioremediation propose biopreparations based on degrading microorganisms immobilized on sorbents [9-11]. The more recent publications describe a positive effect of both the sorbents themselves [12-15] and plant residues [16] on the rate of bioremediation of soil ecosystems contaminated with crude oil and petroleum products.

In our and other recent studies, it has been shown that the most environmentally friendly approach for solving this class of problems is adsorptive bioremediation based on the use of sorbents in combination with classical bioremediation techniques [3, 17-21]. This technology can be used *in situ* and does not require the removal and disposal of contaminated soils. Soil is decontaminated to the required level within a relatively short period of time. At the same time, the landscape remains undisturbed; the native soil, valuable for the sensitive ecosystems of the northern territories, is preserved; the initial state of the soil cover is improved due to melioration; the development of native flora and fauna is stimulated. This strategy is the most economically attractive: additional costs associated with the purchase of sorbents and additional wages for workers are more than compensated by saving money necessary for excavating contaminated soil and delivering it to the disposal site, sometimes hundreds and thousands of kilometers away [22].

Previously, we demonstrated the high efficiency of the adsorptive bioremediation method for cleaning the main types of oil-contaminated soils common in the West-European Plain. In addition, it was proven that in the presence of natural sorbents, the migration of mobile and toxic hydrocarbon oxidation products into groundwater during soil treatment is sharply reduced. It was established that the positive effect of sorbents is associated mainly with the reversible sorption of toxic substances, as well as with the improvement of the physical and physico-chemical properties of petroleum-contaminated soils [17-23].

However, the territory of the main oil-producing region of Russia in the North of Western Siberia is localized within the tundra-taiga landscapes, where one of the main soils are mineral soils, namely podzols of light granulometric composition [24]. Sandy podzols are poorly provided with humus, poorly structured, and the vegetation is not diverse. Therefore, the development of effective methods for their purification from crude oil is very actual. To date, a number of technologies have been developed for the remediation of oil-contaminated areas with a cold climate [25-27], however, due to the low buffering of these soils, the use of the classical bioremediation is ineffective.

In our joint studies [12-13], the possibility of using the adsorptive bioremediation for cleaning folic albic podzol in a historically contaminated area located in the Subarctic region in the north of the Kola Peninsula was also proven. In addition, under conditions of pot experiments with gley-podzolic soil sampled near the city of Urengoy (Yamal-Nenets Autonomous Okrug), the positive effect of several natural sorbents on the rate of bioremediation of podzolic mineral soils contaminated with moderate doses of crude oil was also confirmed [28].

Currently, there are no generally accepted criteria for the permissible level of petroleum products in soils of the Russian Federation. However, according to the Decree of the Government of the Khanty-Mansiysk Autonomous Okrug of 2004 [29], local standards for the permissible residual TPH content in soils after reclamation and other restoration work have been established for the subzone of middle and northern taiga. For podzols, which belong to organic-mineral soils of the light granulometric composition (light loams, sandy loams), the maximal permissible levels have been established: for the upper accumulative horizons of sod-podzolic soils and podzols intended for forestry use, MPL_{forest}=15 g/kg; for similar soils intended for agricultural use, MPL_{agric}=5 g/kg.

In addition to these indicators, some authors point out to the need for an ecosystem approach to assessing the safe level of residual petroleum hydrocarbons in soil, taking into account not only their quantity but also – the soil toxicity [30, 31]. In accordance with the "Sanitary rules for determining the hazard class of toxic waste", for assessing the effectiveness of chemically contaminated soils recultivation, along with the requirement to reduce the pollutants residual concentrations in the soil to permissible levels, it is necessary to take into account the integral toxicity of the soil, based on the use of two standard methods. They include: 1) – estimation of the soil phytotoxicity on the length of the wheat or barley seedling roots or on the biomass of herbaceous plants; and 2) – estimation of biotoxicity of water soil extracts to aquatic organisms: small crustaceans *Daphnia magna* or protozoa *Paramecium caudatum* [32].

The purpose of this investigation was to study the influence of natural sorbents on the rate of bioremediation of illuvial-ferruginous podzol contaminated with moderate and high doses of crude oil, as well as to select optimal conditions for adsorptive bioremediation of podzolic soils of North-Western Siberia in a wide range of oil pollution and estimate the time needed for the soil remediation.

Materials and Methods

1. Materials

The experiments were carried out on ***illuvial-ferruginous podzol soil*** (AoA1-A2-Bf-Cf). A soil sample weighing about 500 kg was collected from the upper 30-cm layer in the background area of the Samotlor oil field near Nizhnevartovsk. Figure 2 shows the location of the illuvial-ferruginous podzol sample collection. The soil sample was delivered to the Institute of Physical-Chemical and Biological Problems of Soil Science of the RAS (t. Pushchino, Moscow region). After removing the forest litter, the soil was passed through a 1 cm sieve. Laboratory analyses showed that the soil was sandy loam (containing 87.5% sand, 12.5% clay, and 3.8% silt), slightly humified (C_{org} 0.86%), slightly acidic (pH_w 5.7, pH_{KCl} 4.3) and poorly supplied with biophilic elements: total nitrogen content – 0.7%, available phosphorus – 1.2 mg P_2O_5/100 g, exchangeable potassium – 9.5 mg K_2O/100 g.

Figure 2. The sampling location of illuvial-ferruginous podzol in the background area in the Samotlor oil field near the city Nizhnevartovsk (KhMAO) (author's photo by E.E. Mikhedova).

Table 1. Characteristics of the sorbents used in the experiments.

Sorbents		Codes	Fraction	Description, production
Mineral	Vermiculite	V	Granules 1–3 mm	Expanded vermiculite, GOST 12865-67, "Polymer" Limited, Irkutsk, RF
	Diatomite	D	Particles <0.5 mm	Diatomaceous earth, "Diatomaceous Plant" Limited, Ulyanovsk, RF
Carbonic	Granular activated carbon	GAC	Granules 2–3 mm	Granular activated carbon, Brand GAC VSK, Dzerzhinsk, RF
Organic	Spill-Sorb	SS	Particles <10 mm	Pyrolyzed peat sphagnum moss (water content 9%), "Terra-Ecology" Limited, Moscow region, RF
	Lowland peat	PeatL	Particles <10 mm	Neutralized lowland peat (pH 5.5–6.5) "Healthy Planet " Limited
	High moor peat	PeatH	Particles <15 mm	High moor peat (pH 3.1–4.0), (GOST P 51213–98), "FASKO" Limited, Moscow region, RF
Mixed	Sorbent ACD	ACD	various	Mixture of GAC and diatomite (4:1)
	Sorbent ACP	ACP	various	Mixture of GAC and high moor peat (1:1)
	Sorbent ACDP	ACDP	various	Mixture of ACD and high moor peat (1:1)

The experiments were performed using sample of ***crude oil*** obtained from the Moscow Oil Refinery "Kapotnya". The oil had medium density (0.87 g/cm^3), was sulfurous (sulfur content 0.99%), and had a high paraffin content (7.5%). Bioremediation of the crude oil-contaminated soils was performed using ***the biopreparation 'MicroBak'*** (BP) developed at G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms of the RAS. This BP contains a consortium of bacterial strains of the genera *Rhodococcu*s and *Pseudomonas* and can be used for bioremediation of soils with the oil content up to 15%, pH from 6 to 8, and temperature from 4 to 32°C. The *Pseudomonas* strains carry plasmids capable to degrade polycyclic aromatic hydrocarbons [33].

In course of the experiments, ***natural sorbents*** of three classes were used: mineral (diatomite and expanded vermiculite), organic (high-moor peat, lowland peat and Spill-Sorb), and carbonaceous - granular activated carbon (GAC). The composition and properties of all sorbents are given in Table 1. In addition, a mixed sorbent ACD (a mixture of GAC and diatomite, at a weight ratio of 4:1) was used, which showed the best results in previous experiments [23, 28]. Mixed sorbents based on high-moor peat and GAC were also used, including both with and without diatomite additives (ACDP and ACP, respectively). 'Azophoska' (AZP), containing 16% of the active substance in terms of $N_{total,}$ P_2O and K_2O, was used as a complex mineral fertilizer. Liming was carried out using dolomite powder (DP), which contains 40% $CaCO_3$ and 40% $MgCO_3$ (manufactured by "GERA" Limited, Moscow Region).

2. Experiment design

Two types of experiments were set up: in microfield and pot conditions.

2.1. Microfield experiment

The microfield experiment was carried out at the experimental site of the IPBP RAS, (Pushchino, Moscow Region). It was set up at the end of July 2020 and durated for 3 warm seasons until the end of October 2022, i.e. for 27 months. Thus, in the first year, the weather conditions of the KhMAO were simulated, which is characterized by a warm but short summer.

The experiment with illuvial-ferruginous podzol (hereinafter Podzol) was carried out in bottomless polyvinyl chloride vessels measuring 35×35×35 cm^3 (sample area 0.1 m^2), which were dug into the ground to a depth of 25 cm. The original soil was removed to a depth of 10 cm, the bottom was covered with a plastic mesh "sickle" and the vessel was filled with experimental soil - 10 kg each, as described in [19]. To simulate an oil spill, the soil was superficially contaminated with crude oil in an appropriate amount and left for 3 days at an air temperature of 5–22 °C, without precipitation. Then the soil was thoroughly mixed to the full depth (about 10 cm), analyzed for the initial content of petroleum products and immediately treated. The scheme of the microfield experiment is given in Table 2. Each variant was taken in 3 replications.

Two sets of samples (***Series P1*** and ***Series P2***) were prepared, which were contaminated with two different doses of crude oil, 6 and 12% on dry soil weight, respectively. In the ***Series P1***, two control samples were set up, including an untreated control (***UnK1***), which remained without any treatment, as well as a control (***K1***), which was treated through classical bioremediation. It included the introduction of the BP MicroBak, basic biophilic elements (NPK), liming with the help pf DP to maintain an optimal soil pH, and the creation of optimal aero-hydrothermal conditions through a periodical soil mixing. In addition, samples treated by adsorptive bioremediation were set up, where the soil was treated in the same way as in the control ***K1***, but on the background of additional application of sorbents. Vermiculite, GAC, ACD, high moor peat, lowland peat, Spill-Sorb were used as sorbents. Sorbents were applied in doses close to the optimal ones determined on the basis of our previous experiments [19, 28].

Due to the limited volume of the soil, only 2 samples were set up in the ***Series P2***: control ***K2*** without sorbents and the sample amended with 20% composite sorbent ACDP. It was assumed that in case of the highly contaminated low humified sandy loam soil, the effectiveness of bioremediation could be increased by introducing ACDP, consisting of a mixture of equal amount of high moor peat and the mixed sorbent ACD.

Additionally, a sample of uncontaminated background soil was set up, which was used as a background control (***BK***) to assess the phyto- and biotoxicity of contaminated soils. In each series of the experiments, all contaminated soil samples (controls and experimental samples with sorbents) were treated in the same way, with the exception of additional application of dolomite powder to some samples during the treatment.

At the first stage, sorbents were added to the vessels according to the scheme, the soil was thoroughly mixed, and after 24 hours, complex mineral fertilizers in the form of AZP were added. In the samples of ***Series P1*** and ***Series P2***, the total dose of AZP was 1000 and 1600 mg/kg, respectively, based on each active substance: N, P_2O_5, and K_2O. Fertilizers were applied in 4 stages: at the very beginning of treatment, as well as after 1.5, 9 and 11 months. Hereby, about 75% of Azophoska was applied during the 1st year, and the remaining amount - during the 2nd year. The total amount of mineral fertilizers was calculated based on the approximate ratio of the initial carbon content in petroleum products to the total content of biophilic elements: C:N:P:K=40:1:0.4:0.8.

Table 2. Scheme of the microfield experiment

<table>
<tr><th rowspan="2">Code</th><th rowspan="2">Variant/ Sorbent</th><th rowspan="2">Dose of crude oil, mass. %</th><th rowspan="2">Dose of sorbents, mass. %</th><th colspan="3">Total amount</th></tr>
<tr><th>BP, cells/g</th><th>NPK, mg a.i./kg</th><th>DP, g/kg</th></tr>
<tr><td>BK</td><td>Background control</td><td>-</td><td>-</td><td>-</td><td>150</td><td>0.5</td></tr>
<tr><td colspan="7">Series P1</td></tr>
<tr><td>UnK1</td><td>Untreated control</td><td rowspan="14">6.0</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>K1</td><td>Control 1</td><td>-</td><td rowspan="13">2x10^7</td><td rowspan="13">1000</td><td rowspan="7">3.0</td></tr>
<tr><td>V5</td><td rowspan="2">Vermiculite</td><td>5</td></tr>
<tr><td>V10</td><td>10</td></tr>
<tr><td>GAC5</td><td rowspan="2">GAC</td><td>5</td></tr>
<tr><td>GAC10</td><td>10</td></tr>
<tr><td>ACD5</td><td rowspan="2">ACD</td><td>5</td></tr>
<tr><td>ACD10</td><td>10</td></tr>
<tr><td>PeatL10</td><td rowspan="2">Lowland peat, neutralized</td><td>10</td><td>3.6</td></tr>
<tr><td>PeatL20</td><td>20</td><td>4.2</td></tr>
<tr><td>PeatH10</td><td rowspan="2">High moor peat</td><td>10</td><td>3.6</td></tr>
<tr><td>PeatH20</td><td>20</td><td>4.2</td></tr>
<tr><td>SS10</td><td rowspan="2">Spill-Sorb</td><td>10</td><td>6.1</td></tr>
<tr><td>SS20</td><td>20</td><td>7.5</td></tr>
<tr><td colspan="7">Series P2</td></tr>
<tr><td>K2</td><td>Control 2</td><td rowspan="2">12.0</td><td>-</td><td rowspan="2">2x10^7</td><td rowspan="2">1600</td><td>4.8</td></tr>
<tr><td>ACDP20</td><td>ACDP</td><td>20</td><td>6.2</td></tr>
</table>

Simultaneously with biophilic elements, dolomite powder (DP) was added to avoid strong acidification of the soil. The DP amount was calculated based on its three-fold weight to the weight of AZP in terms of active ingredients. After 2 and 12.5 months, an additional amount of DP was added to some samples in case of a strong acidification of the soil (to pH < 6.0), approximately one week after the addition of mineral fertilizers. Ultimately, the total dose of DP added to the samples with the maximum doses of organic sorbents was 1.5-2.5 times higher than those in the ***K1*** or in the samples amended with mineral and carbon sorbents. The doses of AZP and DP in the pure control ***BC*** were reduced to the levels recommended in agricultural practice.

One day after the 1st and 2nd additions of AZP and DP, the biopreparation 'MicroBak' (hereinafter BP) was added to all samples with contaminated soil (except ***UnK1***) in the amount of 10^7 cells/g as recommended in [33]. During the entire experiment, the soil in all vessels was watered with settled tap water to avoid drying. During the addition of additives or when collecting samples for analysis, the soil in the vessels was mixed to the full depth of the experimental sample. At the beginning of the 2nd and 3rd seasons, the soil in the vessels was sown with a mixture of seeds of red clover (*Trifolium pratense*) and tall ryegrass

(*Arrhenatherum elatius*) in a 1:1 ratio in the amount of 1 g/vessel; and after 2 or 3 months the plants were removed.

During the incubation, the soil was periodically mixed and samples were taken to determine various characteristics: the content of TPH and oxidized products of petroleum hydrocarbon biodegradation (OTPH) in the soil were periodically determined. Additionally, the number of hydrocarbon-oxidizing microorganisms (HOM) in the soil and its dehydrogenase activity (DHA), as well as water pH and field soil moisture were measured. After 11 months (30.04.2021) and 23 months (05.06.2023) from the experiment beginning, the integral toxicity of the soils was estimated by two standard methods. The phytotoxicity of soils was determined by the root length of wheat (*Triticum vulgaris*) seedlings, besides the biotoxicity in acute and chronic bioassays with *Daphnia magna* was determined also. The biotest on *Daphnia magna* was carried out additionally in the end of the experiment (02.10.2023) - in 27 months after the start of the treatment.

2.2. Pot experiment

The pot experiment of ***Series P2pot*** was conducted simultaneously with the experiment in microfield conditions. Its main objective was to evaluate the correctness of the taken composition and dose of the sorbent ACDP. For this purpose, samples of ***Series P2pot*** with soil contaminated with 12% crude oil were used, in which the results obtained with two doses (10 and 15%) of high moor peat or ACD were compared, with two doses (15 and 20%) of mixed peat-based sorbents: with the addition of diatomite (ACDP) and without it (ACP). Due to the complex composition of the sorbent ACDP, the term "composite" was applied to it.

The experiment was set up with 1 kg soil samples placed in 1.5 l plastic vegetation vessels with holes in the bottom and trays. The experimental setup is shown in Table 3. All soil treatments were carried out with practically the same BP doses as in the microfield experiment, but with slightly higher doses of AZP and DP, which were applied at approximately the same times as in the microfield experiment. The AZP doses were slightly increased, since a bit higher initial levels of TPH and their metabolites were found in these samples compared to those in the microfield experiment. All samples were set up in triplicate.

Table 3. Scheme of the pot experiment – ***Series P2pot***

Code	Variant/sorbent	Dose of crude oil, mass.%	Dose of sorbents, mass.%	Total amount		
				BP, cells/g	NPK, mg a.i./kg	DP, g/kg
BK	Background control	-	-	-	150	0.5
K2p	Control ***K2p***	12.0	-	$2x10^7$	1800	5.4
ACD10	ACD		10			
ACD15			15			
PeatH10	High moor peat		10			6.8
PeatH15			15			7.5
ACP15	ACP		15			6.0
ACP20			20			6.5
ACDP15	ACDP		15			6.0
ACDP20			20			6.5

During the vegetation period, the soil in the vessels was incubated on the experimental site under arches with covering material, and during the cold period - in greenhouse conditions at a temperature of 20-25 °C. The soil in the vessels was periodically moistened to avoid drying and mixed monthly, samples were taken periodically for analysis. In addition, after 4 and 14 months, the phytotoxicity of the soil was determined by standard methods: by the length of the roots of wheat seedlings and by the phytomass of 1-month-old ryegrass plants.

3. Analytical methods

The total content of petroleum hydrocarbons (TPH) in the soil was determined using the certified IR spectrometry method PND F 16.1:2.2.22-98 [34]. For this purpose, air-dried soil samples were extracted with carbon tetrachloride, the extract was purified by passing through a column with aluminum oxide, and the concentration of TPH in the purified extracts was measured by absorption at a wavelength of 3.42 µm using an IR spectrometer on a KN-2M concentration analyzer (Sibpribor, Russia). In addition, the total content of petroleum oxidation products (OTPH) was estimated using our own method, namely by the amount of polar C-H-containing compounds. This value was estimated from the difference between the total concentrations of C-H-containing compounds and their non-polar derivatives (in crude and purified extracts, respectively), as described in [18].

The number of hydrocarbon-oxidizing microorganisms (HOM) in the soil was determined by inoculating a soil suspension of the appropriate dilution onto a minimum agar medium, where diesel fuel vapor served as the source of carbon and energy [35]. The ***dehydrogenase activity*** of the soil was determined by the method described in [36]. The ***aqueous pH*** of the soil was measured in a suspension of soil in distilled water at a ratio of 1:2.5 using a pH-meter.

Soil phytotoxicity was determined by three methods. To select the sorbent dose and monitor the purification process, soil phytotoxicity was regularly determined by germination of white clover (*Trifolium repens* L.) seeds using our own express method [37]. In addition, at the end of the 2nd and 3rd seasons, soil phytotoxicity was determined by standard methods: on the root length of wheat (*Triticum vulgaris*) seedlings germinated under optimal conditions (at an air temperature of 20±2ºC, air humidity of 60±5% and soil moisture of 70±5% of the maximum permissible capacity) in accordance with ISO 11269-1:2012 [38], or by changes in plant phytomass. In the latter case, the soil in all vegetation vessels was sown with perennial ryegrass (*Lolium perenne*) seeds; after 1 month, the plants were removed, washed with water, dried at 40°C, and their dry weight was determined. Soil phytotoxicity was calculated based on the decrease in plant germination or growth rates compared to similar rates in the clean control.

Simultaneously with determining phytotoxicity based on plant growth parameters, ***soil biotoxicity*** was evaluated using biotests on aquatic organisms, namely *Daphnia magna* mortality in a water-soil extract, in accordance with standard method PND FT 14.1:2:4.12-06, T 16.1:2.3.3.9-06 [39].

4. Statistical analysis

All results are presented as mean values from 3 replicates with standard deviations. The content of TPH and OTPH in the soil, as well as various soil characteristics in each sample were compared with similar parameters in the corresponding control soil samples ***K*** using the Student's t-test and the STATISTICA10 program. Differences were considered significant at $p < 0.05$ (n=3).

Results

1. Results of the microfield experiment

Figures 3-5 show the results of the microfield experiment with irrigated podzol and two levels of oil pollution. Figure 3 shows the dynamics of changes in the concentrations of TPH and OTPH in the soil of ***Series P1*** contaminated with 6% oil, as well as the dynamics of various soil characteristics during bioremediation. The results of the experiments of ***Series P1*** showed that the initial TPH and OTPH concentrations in the soil, measured 3 days after the application of oil, were, respectively, 31.8±1.3 and 10.3±2.1 g/kg due to the rapid evaporation of volatile hydrocarbons and, possibly, due to partial microbial TPH degradation. During bioremediation of moderately contaminated soil, OTPH degradation in the ***control K1*** proceeded relatively quickly, and during the first 4 months the OTPH concentration in the soil decreased to 11.2±0.9 g/kg. Then the petroleum degradation slowed down, and by the end of the 2nd and 3rd seasons (after 14 and 27 months, respectively), the residual TPH concentrations in ***K1*** were 9.4±0.5 and 6.1±0.8 g/kg, and that one of OTPH were 9.0±0.9 and 4.4±0.4 g/kg, respectively. In the untreated control ***UnK1***, TPH concentration in the soil decreased only to 25.7±2.2 g/kg, and OTPH concentration even increased to 15.5±0.9 g/kg.

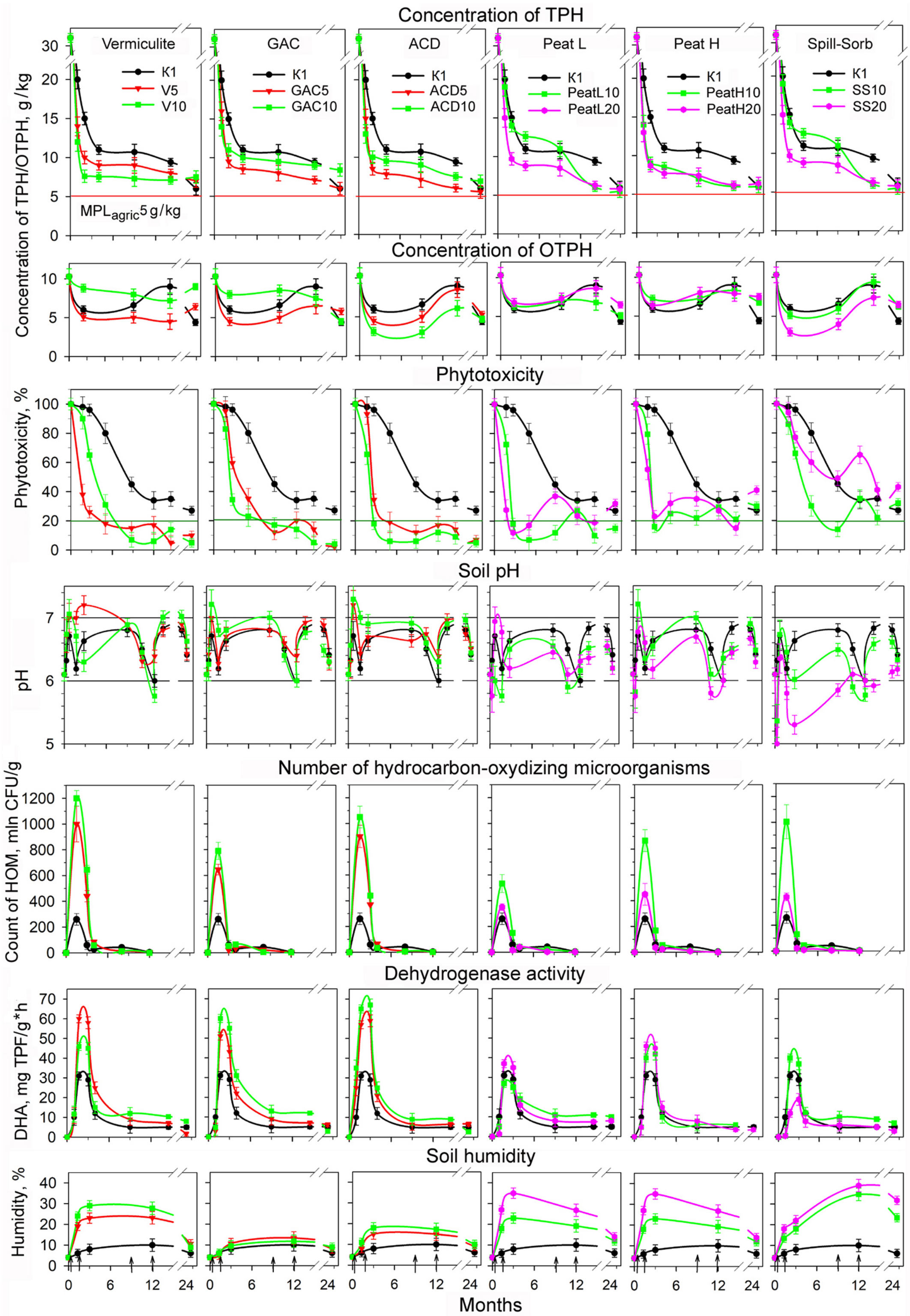

Figure 3. Influence of two doses (5 and 10%) of mineral, carbon and mixed sorbents (V, GAC, ACD) and two doses (15 and 20%) of organic sorbents (PeatL, PeatH, SS) on the dynamics of the TPH and OTPH contents in soil, as well as on various soil characteristics during bioremediation of Podzol contaminated with 6% oil, under the conditions of the microfield experiment ***Series P1.*** Here and below, the arrows indicate the time of application of AZP and DP; the red lines indicate the levels of MPL, and the green lines indicate the levels of minimal phyto- and biotoxicity of soils.

The introduction of the sorbents of all three classes significantly accelerated the petroleum hydrocarbon degradation, but this phenomenon was observed mainly in the first 4 months, then the process slowed down in all soil samples. By the end of the 3rd season, the TPH concentrations in some samples with sorbents (ACP5, PeatL10 and SS10) varied in the range of 5.0-5.9 g/kg, and in the remaining samples - in the range of 6.0-8.4 g/kg. At the same time, the difference between the TPH concentrations in the experimental samples and control ***K1*** was statistically insignificant in many cases. The difference between the OTPH content in almost all experimental samples differed insignificantly from the control level, and only in the samples ACP10 and SS20, amount of OTPH accumulated was half that in the control ***K1.***

More significant differences in some soil properties, especially its ***phytotoxicity***, was observed between the experimental and control samples. In the first 2 months in the control ***K1***, white clover seeds (*Trifolium repens*) almost completely perished; then the phytotoxicity gradually decreased, but even by the end of the 2nd and 3rd seasons it remained quite high: up to 35±5 and 27±3%, respectively. At the same time, the introduction of all the studied sorbents (except for 20% SS) sharply reduced the soil phytotoxicity in the first 3-4 months to a low-toxic level (9-25%). However, in the presence of all organic sorbents, especially Spill-Sorb, the phytotoxicity of the soil again temporarily increased to 29-64% depending on the sorbent.

Changes in soil phytotoxicity are consistent not only with a decrease in pollutant concentrations, but also with the dynamics of ***soil pH*** changes. In the control ***K1***, as well as in the presence of both doses of vermiculite, GAC and ACP, the soil pH level fluctuated mainly within the range from 6.0 to 7.3. At the same time, additions of organic sorbents at a dose of 10% and, even more, 20% led to a sharp acidification of the soil to pH 5.0-5.8 depending on the sorbent. The pH decreased most significantly in the SS20 variant. In most cases, soil acidification was accompanied by an increase in phytotoxicity, and only timely introduction of an additional amount of DP ensured neutralization of excess soil acidity, which was accompanied by a decrease in the phytotoxicity level. In total, to maintain soil pH close to neutral, in samples with organic sorbents, it was necessary to apply approximately 1.5–2.5 times higher doses of DP (Table 2).

During the period of the most rapid decrease in the TPH concentration, a virtually symmetrical increase in the number of ***hydrocarbon-oxidizing microorganisms*** was observed, which in all samples reached a maximum in the first 1.5-3 months of treatment. In the control ***K1***, the count of HOM increased to 260±33 million CFU/g, while in most variants with sorbents, the maximum count of HOM was several times higher. At the same time, the highest increase in the average maximum count of HOM (up to 900-1 200 million CFU/g) was observed in variants with both doses of vermiculite and ACP. In samples with 10% organic sorbents, the number of HOM also increased significantly, but in the presence of high doses (20%) of organic sorbents, the HOM density decreased to 350-450 million CFU/g and approached the average level in control ***K1***.

The value of soil ***dehydrogenase activity*** changed similarly. After 2 or 3 months, DHA increased to a maximum: 31.2±3.0 mg TPF/g*h in the ***K1*** control, 51-65 mg TPF/g*h - in the samples with mineral/carbon sorbents, and 28-46 mg TPF/g*h with organic sorbents. By the end of the warm season, the DHA value decreased, and subsequently only a slight excess of DHA was recorded compared to background soil: within 2.7-5.0 and 0.6-0.7 mg TPF/g*h, respectively.

A different picture was observed when measuring the ***field soil moisture.*** According to their ability to retain moisture in oil-contaminated soil, the sorbents were arranged in the following order: GAC < ACD < vermiculite < PeatL = PeatH < SS, and the field soil moisture became higher with an increase in the dose of sorbents. In the ***K1*** control, the soil moisture in the middle of the 1st and 2nd seasons remained low (from 8 to 10%); in the presence of GAC and ACD, it increased to 11-12 and 13-18%, respectively, and up to 22-28% in the variants with vermiculite. But the highest soil moisture (up to 22-38%) was maintained in all variants with organic sorbents.

Figure 4 shows the dynamics of changes in the same soil characteristics of ***Series P2*** with a high level of pollution (***K2*** and ACDP20), where they are compared with the results of 3 samples of ***Series P1*** (***K1,*** ACD5 and ACD10). Comparison of the obtained results indicated that the TPH degradation in the control ***K2*** proceeded much slower compared to the moderately contaminated soil. In the first 4 months, TPH content decreased from 67.0±2.9 to 38.8±4.4 g/kg, after which the degradation process slowed down sharply. By the end of the 3rd warm season, TPH concentration remained relatively high (22.3±2.8 g/kg), whereas in the ACDP20 sample they decreased to 7.2±2.5 g/kg.

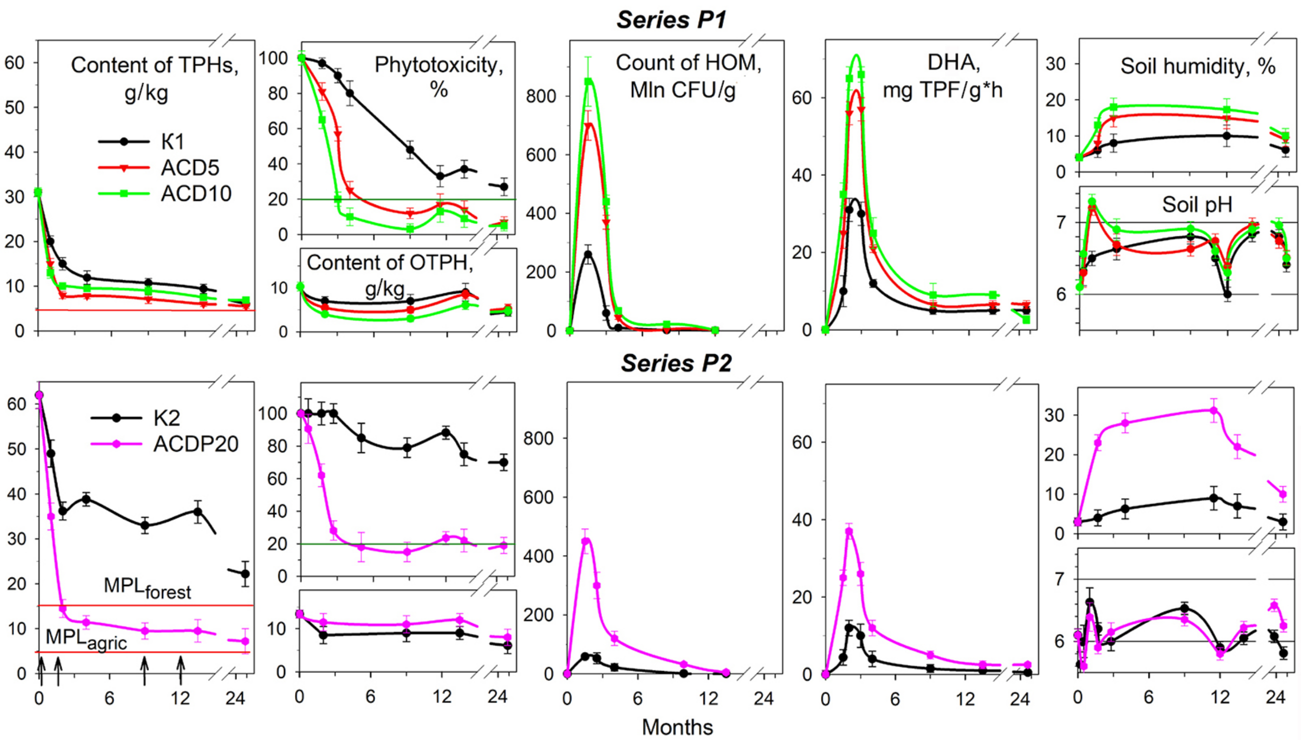

Figure 4. Influence of two doses (5 and 10%) of the mixed sorbent ACP and the composite sorbent ACDP at a dose of 20% on the dynamics of TPH and OTPH concentrations in Podzol samples contaminated with 6% and 12% oil, respectively, as well as the sorbents influence on various characteristics of the contaminated soils during a three-year microfield experiment in the best variants of ***Series P1*** and both two variants of ***Series P2***, respectively.

In the highly contaminated soil, the elevated initial level of OTPH of (13.4±0.9 g/kg) was detected, the concentration of which slowly decreased during the entire period. By the end of the treatments, their content in the sample ACDP20 and control ***K2*** decreased to 6.1±0.6 and 8.2±0.8 g/kg, respectively, and the difference in OTPH content between these variants was statistically insignificant at most sampling points. Thus, in the ***K2*** and ACDP20 soils, by the end of the observations, TPH were degraded by 67% and 89%, respectively, while OTPH concentration in both samples decreased by 40-55%.

Other soil characteristics of ***Series P2*** changed similarly to those of ***Series P1,*** but in the highly contaminated soil the difference between the experimental and control samples was more significant for a number of parameters. Until the end of the 3rdwarm season, the ***phytotoxicity*** of the soil ***K2*** remained very high (>70%), whereas in the sample ACDP20 it decreased to a minimum (<20%) already in the first months of treatment and further remained at a level of 19-25%. In the highly contaminated control ***K2,*** the density of HOM was lower than in ***K1;*** however, in the ACDP20 variant it was much higher than in ***K2***: 450.3±42.1 and 58.4±4.6 million CFU/g, respectively.

A similar picture was observed for the maximum values of dehydrogenase activity (DHA). In the experiment and control ***K2***, it reached 37.2±3.1 and 10.2±1.4 mg TPF/g per hour, respectively. In the first months of treatment in ***Series P2***, the soil was acidified significantly up to pH 5.5. Later, due to the additional introduction of DP in the ACDP20 sample, it was possible to maintain the soil pH level close to neutral, although peat was also present in this composite sorbent. A particularly noticeable difference was observed between the ***field soil moisture*** values. In the ACDP20 samples, it varied within the range of 23-31%, while in the control ***K2*** it did not exceed 10% due to the high hydrophobicity of the heavily contaminated soil.

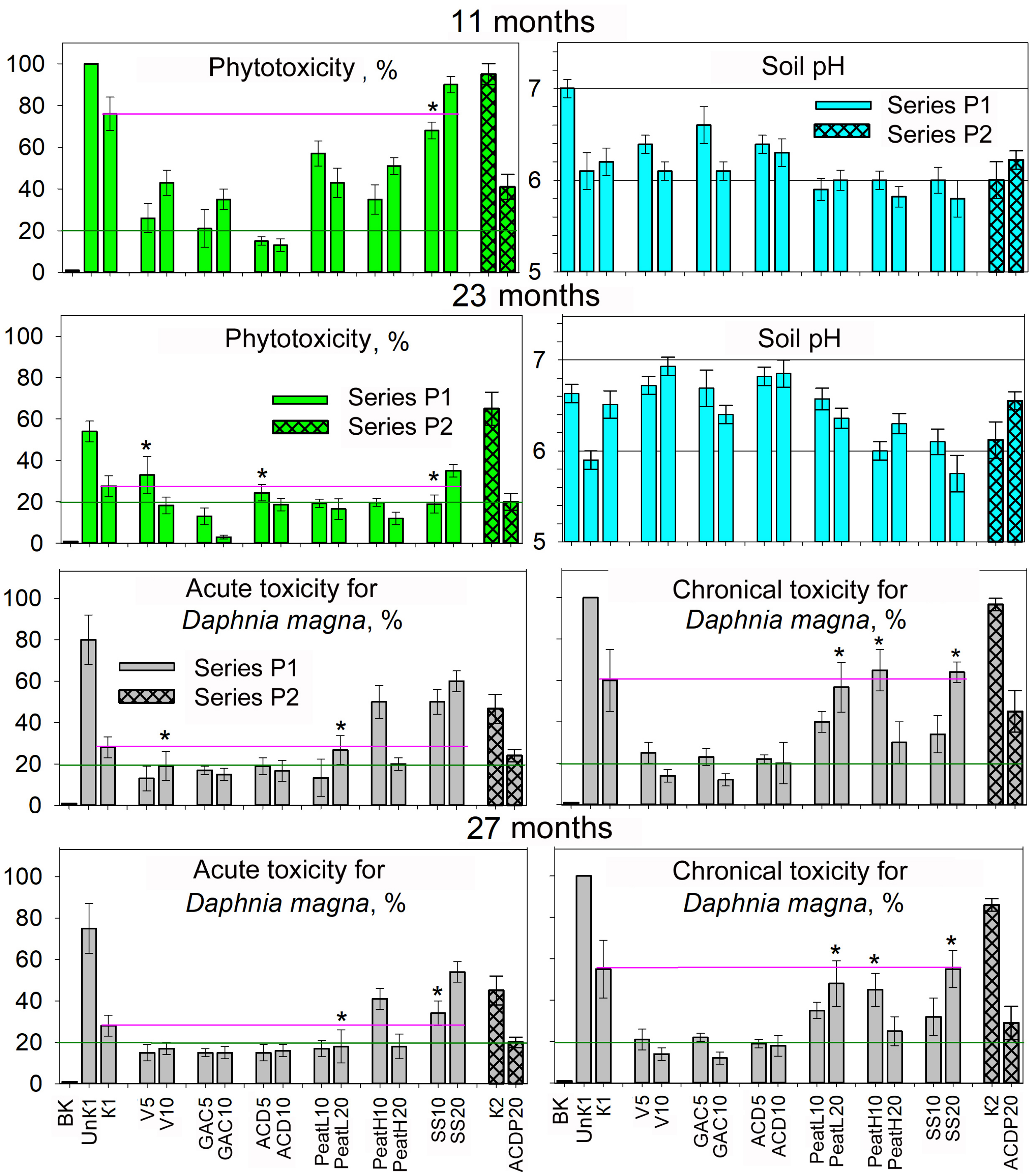

Figure 5. Effect of one or two doses of sorbents (vermiculite V, GAC, ACP, PeatL and PeatH, SS, ACDP) on the integral toxicity and pH of the Podzol soil contaminated with 6 and 12% oil, assessed 11, 23, and 27 ***months after the start of treatment under the conditions of the microfield experiment Series P1 and Series P2.*** The integral toxicity of the soil was assessed by the length of the roots of wheat seedlings (*Triticum vulgaris*), as well as by biotoxicity in acute and chronic biotests with *Daphnia magna.* Asterisks mark the variants in which the difference between the experimental and control variants was insignificant ($p < 0.05$). Codes correspond to the variants given in Table 2. Crimson lines mark the level of phyto- and biotoxicity of the soil in the control ***K1.***

Figure 5 shows the data on the ***integral soil toxicity*** at the end of the 2nd and 3rd seasons and their comparison with the soil pH values. The data on soil phytotoxicity determined by the certified method based on the length of wheat seedling roots are presented, as well as on the biotoxicity determined basing on the

Daphnia magna mortality in acute and chronic experiments. In the 2nd season (after 11 months), the sown seeds completely died in the soil from the untreated control ***UnK1;*** in the 3rd year, root growth was inhibited by 54±4%. In the control ***K1,*** in the 2nd and 3rd warm seasons, the soil ***phytotoxicity,*** assessed by the certified method, decreased to 74±6 and 25±4%, respectively, while in the control ***K2*** this index remained very high (66±7%) until the end of the 3rd year.

On the other hand, in ***Series P1***, already in the 2nd season, the phytotoxicity of soils with ACP additives decreased to a minimum (12-15%) and to 21-43% in the presence of vermiculite and GAC, while in the variants with all organic sorbents it remained high: from 35 to 95%. The increased phytotoxicity of soil samples with organic sorbents coincided with a decrease in soil pH to 6.0 and less. However, in the 3rd season, in most variants with sorbents, soil phytotoxicity approached a minimum (<20%), with the exception of the soil sample SS20 variant with pH <6.0, where the length of seedling roots was still significantly lower by 32-34% than in the pure control. In ***Series P2,*** the phytotoxicity of the ACDP20 soil decreased to a minimum in the middle of the 3rd warm season, while in the ***K2*** control it remained very high (66±5%).

The data on phytotoxicity are confirmed by the results of ***biotests on aquatic organisms Daphnia magna.*** The results obtained with daphnia show an even higher level of soil biotoxicity than with phytotesting. The most prominent differences are observed when determining the chronic toxicity of water-soil suspensions assessed by the reproduction of daphnia. In the variants of ***Series P1*** in the last season, the biotoxicity of the sample ***UnK1***, both acute and chronic, was very high and varied within 75-80 and 98-99%, respectively. In the control ***K1***, these indicators decreased only to 27-28 and 55-60%, respectively. The addition of mineral and carbon sorbents provided a reliable reduction in the mortality of daphnia compared to the control ***K1*** after 23 months. Finally, after 27 months, in both the acute and chronic biotests, this indicator decreased to a minimum of <20%. However, the same indicators for most samples with organic sorbents remained elevated until the end of the observations.

2. Results of the pot experiment

The results of the pot experiment are shown in Figures 6 and 7. Figure 6 shows the dynamics of changes of TPH and OTPH contents in soil, as well as of various soil properties during the entire observation period. The presented data show that all the studied sorbents had a positive effect on the rate of oil degradation. By the end of the 3rd warm season, the TPH concentration in the control ***K2p*** decreased from 71.2±3.4 to 16.3±1.1 g/kg. At the same time, significant OTPH amounts initially accumulated in the soil – 20.9±3.8 g/kg, then their concentration in the control first increased slightly, and by the end of the experiment decreased to 13.3±0.7 g/kg.

In the presence of all sorbents, the TPH degradation accelerated, especially in the first months, and after 26 months, the residual TPH concentrations in these soils were 1.5-2 times lower compared to the control ***K2p***. As expected, the most rapid TPH degradation occurred in the ACDP15 and ACDP20 samples, in which the residual TPH concentrations at the end of the 1st season varied within 13.9-17.3 g/kg, and by the end of the 3rd year - within 4.9-6.1 g/kg. Hereby, the difference between the samples with various ACDP doses in both cases was statistically insignificant. In the remaining samples with sorbents, the TPH degradation proceeded slightly more slowly, and at the end of the 3rd year, the residual TPH concentrations varied in the range of 8.4-10.5 g/kg. That is, in the variants with sorbents, final TPH concentrations decreased by 85-92% compared to 77% in ***K2p,*** and in the best variants (with ACDP) it reached the level of MPL_{agric}.

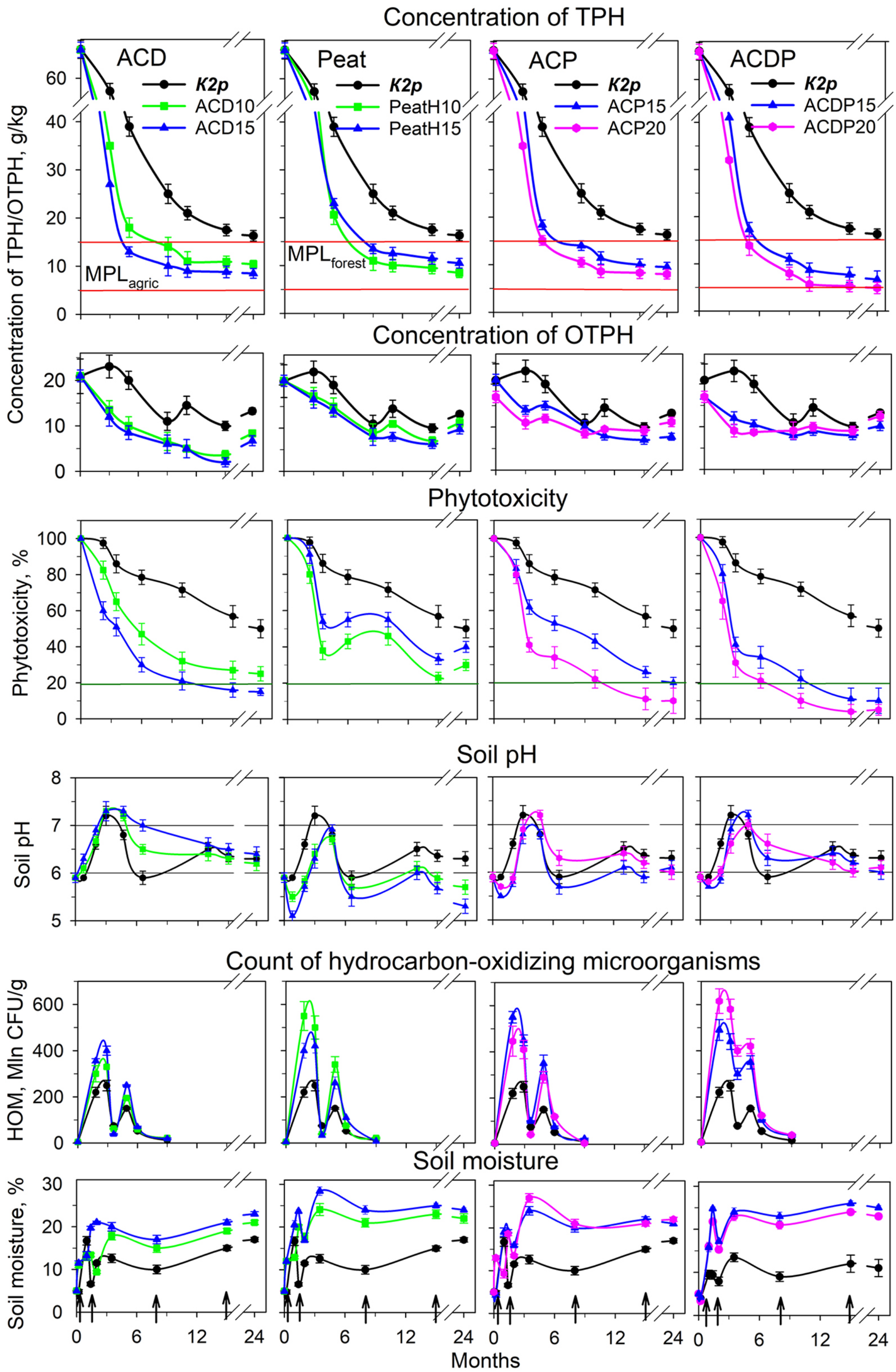

Figure 6. Influence of two doses (10 and 15%) of ACD and PeatH, and two doses (15 and 20%) of ACP and ACDP on the dynamics of the TPH and OTPH concentrations in the Podzol contaminated with 12% oil, as well as on changes in soil characteristics under the conditions of the pot experiment of ***Series P2pot***: soil phytotoxicity assessed by the express method, count of HOM, aqueous pH and soil moisture.

The phytotoxicity of soils with sorbents in ***Series P2p*** decreased significantly faster than in the control ***K2p***; the best results were observed also in variants with two doses of the composite sorbent ACDP, where the minimal level of phytotoxicity (<20%) was achieved after 7-12 months. In soil with other mixed sorbents it decreased only after 13-26 months. However, the samples amended with peat alone, as well as the control samples ***K2p***, retained the increased phytotoxicity (up to 35-50%) until the end of observations. In the pot experiment, as well as in the microfield experiment, the increased phytotoxicity of samples with both doses of peat correlated with a significant decrease in ***soil pH,*** namely <6.0.

The pH values of the samples with the mixed sorbent ACP were also lower in some periods of time, but to a lesser extent than with peat alone. On the other hand, in the samples amended with of ACD and ACDP, the soil pH varied mainly in the range from 6.0 to 7.4.

In all samples of the pot experiment, the count of HOM increased in the first months after the start of treatment. In this case, 2 maxima of their number were observed: after 2 and 5 months, i.e. approximately 1.5-2 months after the first two applications of AZP and DM. The maximum of HOM count was observed in the ACDP20 samples (615±54 million CFU/g), while in the other samples with sorbents, the maximum of HOM count varied within 298-554 million CFU/g.

In addition, in samples with peat-based sorbents, the highest ***soil moisture*** content was maintained (from 20 to 28%); in samples with ACP additives it varied within 17-23%, however, in most cases this indicator reliably exceeded the moisture content of the control soil ***K2p,*** which varied within 6-16%.

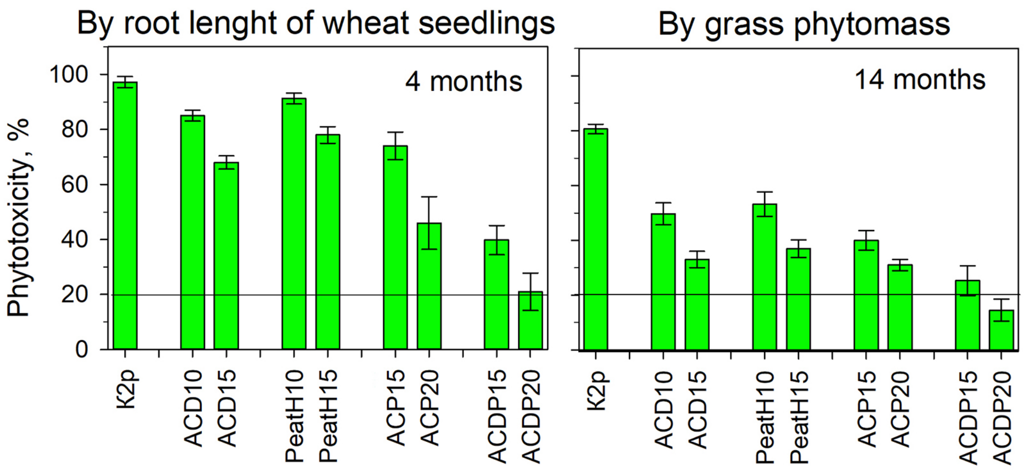

Figure 7. Influence of two doses (10 and 15%) of the sorbents ACD and PeatH and two doses (15 and 20%) of the sorbents of ACP and ACDP on the phytotoxicity of Podzol contaminated with 12% oil in the pot experiment of ***Series P2pot*** after 4 months (assessed by the root length of wheat (*Triticum vulgaris*) seedling) and after 14 months (assessed by the dry phytomass of 1-month-old plants of perennial ryegrass (*Lolium perenne)*).

Figure 7 shows the ***soil phytotoxicity*** for ***Series P2pot*** determined by standard methods. After 4 and 14 months, the phytotoxicity of the control soil ***K2p*** remained very high: 97.2±2.0 and 80.7±1.8%, respectively. At the same time, the phytotoxicity of the sorbent amended soils decreased in the next row: Peat H > ACD > ACP > ACDP. Hereby, the phytotoxicity of the sorbent amended soils was significantly lower compared to the control ***K2p***. For soils with a higher sorbent dose, this indicator was lower compared to its lower dose. Moreover, after 14 months, the phytotoxicity of the soil in the ACDP20 sample decreased to a minimum (<20%) and in the ACDP15 sample - to 26.3±6.4%; in the other samples with sorbents, the phytotoxicity of the soils was higher and varied within 31-49%.

Discussion

Thus, the results of the microfield experiment showed that when carrying out bioremediation of moderately contaminated soils of ***Series P1*** (6% oil), both with the classical method (control ***K1)*** and by adsorptive bioremediation on the background of various natural sorbents, the TPH concentration decreased from 31.8±1.3 g/kg to levels below the permissible ones for forestry soils (MPL_{forest}) by the end of the 1st warm season. However, by the end of the 3rd year, the TPH concentration decreased to 5.5±0.5 g/kg (i.e. practically to the level of MPL_{agric}) only in the samples ACP5, PeatL10, and SS15/20. The residual TPH concentrations in samples with organic sorbents and in control ***K1*** varied within the range of 6.0-8.4 g/kg, and thus also approached the level of MPL_{agric}. Moreover, the difference between some variants and the control ***K1*** was statistically insignificant.

However, in the highly contaminated soil of ***Series P2*** (12% oil), a similar decrease in TPH concentration to the specified permissible levels was observed only when adsorptive bioremediation was carried out with the addition of 20% ACDP, whereas in the final TPH concentration in ***K2*** (22.2±1.8 g/kg) this value remained significantly higher than both permissible standards.

The results of the pot experiment of ***Series P2p*** confirmed the high efficiency of the adsorptive bioremediation of highly contaminated soil with the use of the composite sorbent ACDP. Although all the studied components of ACDP and their mixtures (peat, ACP and ACD), introduced in doses of 10, 15 or 20%, influenced positively on the rate of bioremediation of the highly contaminated Podzol, while the best results were obtained with the addition of the composite sorbent ACDP. Adsorptive bioremediation of Podzol with 15% and 20% ACDP ensured a decrease in TPH concentration from 71.2±3.4 g/kg to the level of MPL_{forest} in 4 months, while after 14 months the residual TPH concentrations in these soils approached the level of MPL_{agric}. Moreover, TPH degradation was accompanied by the accumulation of the lowest (in comparison with other samples) concentrations of oxidized microbial metabolites of hydrocarbons – OTPH.

At the same time, comparison of soil phyto- and biotoxicity values showed different results. In ***Series P1*** soils, despite the decrease in TPH residual concentrations to acceptable levels, the phyto- and biotoxicity dropped to a minimum only in soils with both doses (5 and 10%) of mineral or carbon sorbents (ACD, GAC, and vermiculite). The remaining soils (control ***K1*** and samples with organic sorbents) still demonstrated significant phytotoxicity up to the end of the treatment. In variants with organic sorbents, increased phytotoxicity correlated with a decrease in soil pH to 5.6-6.1. The fact of high toxicity of these soils can be explained by the presence of increased concentrations of the petroleum metabolites, which demonstrated high toxicity than that of the hydrocarbons themselves [18]. Moreover, due to the decrease in the sorption of these polar oxygen-containing compounds in soils with low pH, their toxic effect on plants and, especially, on aquatic organisms can increase even more.

These conclusions are confirmed by the results obtained with heavily contaminated soil of ***Series P2.*** After 3 years of treatment by the classical bioremediation method, residual concentrations of TPH significantly exceeded both MPL levels in control ***K2.*** This indicates that the toxic effect on plant growth, as well as on the survival and reproduction of daphnia, may be exerted by the hydrocarbons themselves, the residual concentrations of which in this soil exceed the critical level. At the same time, the higher level of biotoxicity assessed by the mortality and reproduction of daphnia in aqueous extracts, compared to phytotoxicity assessed by plant growth in soil, may also indicate an additional toxic effect of OTPH metabolites, which, due to their relatively high water solubility, have higher mobility and toxicity than the original hydrocarbons. The results obtained confirm the conclusions of a number of authors about importance to take into account the integral indicators of toxicity of recultivated soils along with achieving an acceptable level of petroleum products [30, 31].

Based on the data obtained, it is also possible to draw a conclusion about a high efficiency of the adsorptive bioremediation method for cleaning sandy loam podzol from crude oil. In the case of moderately contaminated soil, the best sorbent for adsorptive bioremediation is the mixed sorbent ACD, the additional introduction of which in a dose close to the initial level of contamination can ensure a rapid reclamation of illuvial-ferruginous podzol. During the first warm season, the TPH concentration can decrease to the level of MPL_{forest}, and by the end of the 2nd or 3rd seasons – to the level of MPL_{agric}. By the end of the treatment, the integral toxicity indicators of these soils will decrease to the required minimum, i.e. $<20\%$.

On the other hand, for remediating the highly contaminated Podzol, it is recommended to apply the composite sorbent ACDP in a dose that is twice as high as the initial level of petroleum products. This is explained by the increased hydrophobicity of highly contaminated sandy loam podzolic soil due to its low buffering capacity. At the same time, in highly contaminated soil, amended with composite sorbent ACDP, there are more favorable conditions for the development of petroleum-degrading microorganisms. This is confirmed by the maximum increase in the number of HOM as well as dehydrogenase activity of these soils, which was observed during the period of maximum rate of hydrocarbon degradation.

The acceleration of oil hydrocarbon degradation in all the studied samples in the presence of ACD or ACDP can be explained by the creation of the most favorable conditions for the activation of oil-degrading microorganisms due to several factors. This may be a result of the decreased soil toxicity due to the predominantly reversible sorption of hydrocarbons and less amounts of toxic products of TPH oxidation. The main role in this process is apparently plays the ***activated carbon***, which has a high absorption capacity to many organic compounds.

The positive role of ***diatomite*** additives in ACD and ACDP sorbents can be explained by the ability of this mineral to release mono- and polysilicon acids, which increase plant resistance to stress and reduce the strong binding of highly aromatic PAHs that are part of oil. This effect was demonstrated in our previous studies on bioremediation of petroleum-contaminated soils of the Russian Plain [18, 19, 23], and gley-podzolic soil sampled in the Yamal-Nenets Autonomous Okrug [28].

Another mechanism of the positive influence of sorbents ACD and, even more of ACDP, on the rate of the contaminated Podzol bioremediation is due to the maintenance of optimal soil moisture during its treatment, which is especially important at a high level of pollution. In this case, the most effective are peat additives, which significantly increase the moisture capacity of oil-contaminated soils [19]. At the same time, peat together with high doses of mineral fertilizers can promote the soil acidification caused by the degradation of plant residues. However, the added activated carbon can reduce acidification of the soil due to the presence of ash elements Ca and Mg. This is also evidenced by a lower dose of dolomite powder introduced to neutralize acidification in the soil with ACDP, compared to the peat itself.

Conclusion

The results of the studies conducted in the conditions of microfield and pot experiments with illuvial-ferruginous podzol contaminated with 6 and 12% oil demonstrated a higher efficiency of the adsorptive bioremediation compared to the classical bioremediation method with the use of only biopreparations based on isolated strains of oil-degrading microorganisms. At the same time, important factors influencing the rate of microbial degradation of hydrocarbons are the choice of optimal dose and form of the sorbent, as well as the doses of complex mineral fertilizers and dolomite powder, which should maintain the optimal level of hydrocarbons available to microorganisms in the soil, the required ratio of carbon and biophilic elements, and also ensure a soil acidity level close to neutral.

Thus, the experiments with illuvial-ferruginous podzol contaminated with crude oil demonstrated the potential of using the adsorptive bioremediation method to eliminate emergency situations caused by oil leaks on the surface of mineral soils common in the oil-producing region of North-Western Siberia. It was found that ACD (a mixture of granulated activated carbon and diatomite, 4:1) was the best sorbent for adsorptive bioremediation of moderately contaminated soils. With heavily contaminated soil, the best results were obtained with composite sorbent based on ACP and peat (1:1).

Adsorptive bioremediation of oil-contaminated podzolic soils with the use of optimal doses of these sorbents can, within one or two vegetation seasons, reduce the concentration of oil products to a level acceptable for reclaimed soils of the Khanty-Mansiysk Autonomous Okrug intended for forest use, and after 2-3 seasons of treatment - for agricultural soils. At the same time, the integral toxicity of soils will be reduced to a minimum.

The mechanism of the positive action of these sorbents is explained by the reduction of soil toxicity due to the predominantly reversible sorption of oil hydrocarbons and their metabolites, maintenance of optimal soil moisture and pH, and increasing the resistance of microorganisms and plants to unfavorable factors. All this creates the conditions for the accelerated decomposition of pollutants and minimization of integral soil toxicity.

Authors' contributions

Vasilieva G.K. planned the research and wrote the main text of the article. Mikhedova E.E. conducted soil analyses and wrote the results of the experiments. Strizhakova E.R. performed soil analyses and wrote the section Analysis Methods. Akhmetov L.I. counted the number of destructor microorganisms and participated in the discussion of the results. All the authors have read and agreed with the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. Key indicators of environmental protection. Statistical bulletin. // [Electronic resource]. Federal State Statistics Service, M., 2023. -105 p. Available at: https://rosstat.gov.ru/storage/mediabank/oxr_bul_2023.pdf (visit date 01.10.2023).
2. Report "On the environmental situation in the Khanty-Mansiysk Autonomous Okrug - Yugra in 2021" [Electronic resource] - Khanty-Mansiysk, 2022. Available at: https://prirodnadzor.admhmao.ru/upload/iblock/fa0/MAKET-DOKLADA-2021.docx (visit date 15.09.2024).
3. Vasilyeva G. K., Strijakova E. R., Ortega-Calvo J-J. Remediation of soils polluted by oil industries. *In* Soil Remediation, Science and Technology, Ed. by J-J. Ortega-Calvo, F. Coulon (Germany, Springer-Nature, 2024, pp. 191-239. DOI: 10.1007/698_2024_1080.
4. Federal Law of 10.01.2002 No. 7-FZ "On Environmental Protection" (Electronic resource). Article 46, paragraph 14, subparagraphs 4-5. Available at: https://www.consultant.ru/document/cons_doc_LAW_34661/66adaab0989b794d736d9eb8e3a3ac44bf40a039 (visit date 31.01.2023).
5. Naeem U., Qazi M. A. Leading edges in bioremediation technologies for removal of petroleum hydrocarbons. *Environ Sci Pollut Res Int*., **27** (22), 27370–27382 (2020). DOI: 10.1007/s11356-019-06124-8.
6. Ławniczak Ł., Wozniak-Karczewska M., Loibner A. P., Heipieper H. J., Chrzanowski Ł. Microbial degradation of hydrocarbons – basic principles for bioremediation: a review. *Molecules,* **25** (4), 856 (2020). DOI: 10.3390/molecules25040856.
7. Yuniati M. D. Bioremediation of petroleum-contaminated soil: a review. *Earth Environ Sci.*, **118** (012063) (2018). DOI: 10.1088/1755-1315/118/1/012063.
8. De la Huz R., Lastra M., López J. Other environmental health issues: oil spill. *In* Encyclopedia of Environmental Health, Ed. by J. Nriagu (Burlington, NJ, USA, Elsevier, 2018), pp. 251–255. DOI: 10.1016/B978-0-12-409548-9.11156-X.
9. Bashkin V. N., Luzhkov V. A., Trubitsina O. P. Development of a biosorbent to eliminate the consequences of hydrocarbon pollution at oil and gas facilities. Problems of Risk Analysis, 18 (1), 40-51 (2021). DOI: 10.32686/1812-5220-2021-18-1-40-51.
10. Fedorova O. S., Bondar P. N., Ryazanova T. V. Effect of the biosorbent "unisorb-bio" with immobilized microflora of the genera Bacillus and Trichoderma on soil restoration under conditions of oil pollution. Journal of SFU. Chemistry. 15 (2), 289-297 (2022). DOI: 10.17516/1998-2836-0293.
11. Cui J.-Q., He Q.-S., Liu M.-H., Chen H., Sun M.-B., Wen J.-P. Comparative study on different remediation strategies applied in petroleum-contaminated soils. *Int J Environ Res Public Health,* **17** (5), 1606 (2020). DOI: 10.3390/ijerph17051606.
12. Myazin V. A., Isakova E. A., Vasilyeva G. K. Effect of granular activated carbon on the rate of bioremediation of soils of the Murmansk region historically contaminated with oil products. *Problems Regional Ecol*., **2**, 20-26 (2020). DOI: 10.24411/1728-323X-2020-12020.
13. Myazin V. A., Korneikova M. V., Chaporgina A. A., Fokina N. V., Vasilieva G. K. The effectiveness of biostimulation, bioaugmentation and sorption-biological treatment of soil contaminated with petroleum

products in the Russian Subarctic. *Microorganisms*, **9**, 1722 (2021). DOI: 10.3390/microorganisms9081722.
14. Minnikova T., Kolesnikov S., Ruseva A., Kazeev K., Minkina T., Mandzhieva S., Sushkova S. Influence of the biochar on petroleum hydrocarbon degradation intensity and ecological condition of haplic chernozem. *Eur J Soil Sci*, **11** (2), 157–166 (2022). DOI: 10.18393/ejss.1037798.
15. Zavgorodnyaya Y. A., Stepanov A. A., Trofimov S. Y., Farkhodov Y., Pervakova V. N., Sokolova T. A., Aptikaev R. S. Effect of introduction of clays, mineral fertilizers, and soil ameliorants on decomposition of organic pollutants in oil-polluted sand under conditions of model experiment. *Moscow University Soil Sci Bull.,* **72** (1), 35–41 (2017). DOI: 10.3103/S0147687417010070.
16. Ukpaka C. P., Eno O. N. Modeling of *Azadirachta indica* leaves powder efficiency for the remediation of soil contaminated with crude oil. *Chem Internat,,* **7** (1), 62–70 (2021). DOI: 10.5281/zenodo.4032367.
17. Vasilyeva, G.K., Strizhakova E.R., Bocharnikova E.A., Semenyuk N.N., Yatsenko V.S., Slyusarevsky A.V., Baryshnikova E.A. Oil and oil products as soil pollutants. Technology of combined physical and biological treatment of contaminated soils. Russian Chemical Journal, **57** (1), 79-104 (2013).).
18. Vasilyeva G., Kondrashina V, Strijakova E., Ortega-Calvo J.-J. Adsorptive bioremediation of soil highly contaminated with crude oil. *Sci Tot Environ.,* **706**, 135739 (2020). DOI: 10.1016/j.stotenv.2019.135739.
19. Vasilyeva G., Mikhedova E., Zinnatshina L., Strijakova E., Akhmetov L., Sushkova S., Ortega-Calvo J.-J. Use of natural sorbents for accelerated bioremediation of grey forest soil contaminated with crude oil. *Sci Tot Environ.,* **850**, 157952 (2022). DOI: 10.1016/j.stotenv.2022.157952.
20. Al-Muqbel D., Otifi M., Aly R., Darra R., Al-Othman A., Tawalbeh M., Halalsheh N. Bioremediation of petrochemical sludge from soils. *In* Metagenomics to Bioremediation. Ed. by V. Kumar, M. Bilal, S. K. Shahi, V. K. Carg (Elsevier Science, 2022), pp. 379–402. DOI: 10.1016/B978-0-323-96113-4.00009-3.
21. Sozina I. D., Danilov A. S. Microbiological remediation of oil-contaminated soils. *J Mining Inst*., **260**, 297–312 (2023). DOI: 10.31897/PMI.2023.8.
22. Slyusarevsky A. V., Zinnatshina L. V., Vasilyeva G. K. Comparative ecological and economic analysis by the method of reclamation of oil-contaminated soils by means of bioreclamation in situ and mechanical replacement of soil. *Ecol. Ind. Russia*, **22** (11), 40-45 (2018). DOI: 10.18412/1816-0395-2018-11-40-45.
23. Kondrashina V., Strijakova E., Zinnatshina L., Bocharnikova E., Vasilyeva G. Influence of activated carbon and other additives on bioremediation rate and characteristics of petroleum-contaminated soils. *Soil Sci. (Special Issue)*, **138** (4), 150–159 (2018). DOI: 10.1097/SS.0000000000000234.
24. Seredina V. P., Sadykov M. E., Blokhina S. L. Physical state of background soils of oil fields of the middle taiga of Western Siberia. Bulletin of Tomsk State University. Biology. 4 (16), 17-29 (2011).
25. Bashkin V.N., Galiulin R.V. Reclamation of disturbed soils on the Tazovsky Peninsula. Earth Life, 2, 153–159 (2020). DOI 10.29003/m1385.0514-7468.2020_42_2/153-159.
26. Chaudhary D. K., Kim J. New insights into bioremediation strategies for oil-contaminated soil in cold environments. *Biodegradation,* **142**, 58–72 (2019). DOI: 10.1016/J.IBIOD.2019.05.001.
27. Korneykova, M. V., Myazin V. A., Fokina N. V., Chaporgina A. A. Bioremediation of soil of the Kola Peninsula (Murmansk region) contaminated with diesel fuel. *Geography Environment Sustainability*, **14** (1), 171–176 (2021). DOI: 10.24057/2071-9388-2019-170.
28. Mikhedova E. E., Vasilyeva G. K., Strizhakova E. R., Lankin A. V., Akhmetov L. I., Uzorina M. I. Using biotesting to assess the sorption bioremediation of oil-contaminated podzolic soil in Western Siberia. Natural Resources of the Arctic and Subarctic, **28** (4), 595-605 (2023). DOI: 10.31242/2618-9712-2023-28-4-595-605.
29. Resolution of the Government of the Khanty-Mansiysk Autonomous Okrug - Yugra of December 10, 2004 N 466-p "On approval of the regional standard "permissible residual content of oil and oil products in soils after reclamation and other restoration work on the territory of the Khanty-Mansiysk Autonomous Okrug - Yugra".

30. Kapelkina L. P., Bardina T. V., Chugunova M. V., Bakina L. G., Gerasimov A. O., Bardina V. I., Malyshkina L. A. Ecotoxicological assessment of drill cuttings from oil fields by biological methods. Instruments, 3 (165), 50-55 (2014).
31. Terekhova V. A. Soil biotesting: approaches and problems. Soil Science, 2, 190-198 (2011). SP 2.1.7.1386-03. Sanitary rules for determining the hazard class of toxic production and consumption waste, 2003. 20 p.
32. SP 2.1.7.1386-03. Sanitary rules for determining the hazard class of toxic production and consumption waste, 2003. 20 p.
33. Filonov A., Akhmetov L., Puntus I., Solyanikova I. Removal of oil spills in temperate and cold climates of Russia: experience in the creation and use of biopreparations based on effective microbial consortia. *In:* Biodegradation, pollutants and bioremediation principles. Ed. by E. D. Bidoia, R. N. Motagnolli (Boca Raton, CA, USA, Taylor's & Francis CRC Press), pp. 137–159 (2021). DOI:10.1201/9780429293931-7.
34. PND F 16.1:2.2.22-98. Quantitative chemical analysis of soils. Methodology for measuring the mass fraction of petroleum products in mineral, organogenic, organomineral soils and bottom sediments using IR spectrometry. Moscow, 2006, 21 p.
35. Tepper E. Z., Shilnikova V. K., Pereverzeva G. I. Practical training in microbiology: 5th edition, V. K. Shilnikova. (Moscow: Drofa, 2004). 256 p.
36. Khaziev F. Kh. Methods of soil enzymology. (Moscow: Nauka, 2005). 252 p.
37. Vasilyeva G. K., Kondrashina V. S., Strijakova E. R., Pinsky D. L. Express-phytotest for choosing conditions and following process of soil remediation. *Environ Geochem Health*., **44** (2) 433-445 (2020), DOI: 10.1007/s10653-020-00727-8.
38. ISO 11269-1:2012 - Soil quality. Determination of the effects of pollutants on soil flora, 2012.
39. PND FT 14.1:2:4.12-06, T 16.1:2.3.3.9-06. Toxicological methods of analysis. Methodology for determination of acute toxicity of drinking, fresh natural and waste water, aqueous extracts from soils, sewage sludge and waste by mortality of daphnia (*Daphnia magna Straus*), approved by FGU "FCAO" on 19.04.2011. 33 p.