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Assessment of the ecological state and health of oil-contaminated Luvic Phaeozems Albic, Gleyic Albeluvisols, and Greyic Phaeozems after remediation by biochar

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ABSTRACT

Oil pollution in soil endangers the health of the natural ecosystem on a larger scale. However, the application of biochar as an oil-contaminated soil rehabilitative amendment material is an ecologically friendly practice that supports soil ecological function. Thus, the research was carried out to evaluate ecological and environmental changes and the health condition of biochar-treated oil-polluted Luvic Phaeozems, Albic, Gleyic Albeluvisols, and Greyic Phaeozems Albic. A controlled laboratory experiment was set up for 30 days, during which the soils were artificially oil-contaminated at 5% of the soil weight and treated with biochar derived from birch at 10% of the soil weight. At the end of the experiment period, a range of physical, chemical, and biological properties indices were determined. The findings showed that the application of biochar raised the integral biological condition indicator by 77% in Gleyic Albeluvisols, 47% in Luvic Phaeozems Albic, and 18% in Greyic Phaeozems Albic relative to oil-contaminated untreated controls. Moreover, adding biochar resulted in petroleum hydrocarbon content reduction by 48%, 41%, and 33% in Gleyic Albeluvisols, Luvic Phaeozems Albic, and Greyic Phaeozems Albic soils, respectively. These results confirm the efficiency of biochar as an effective agent for the improvement of the oil-contaminated soil health.

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1. INTRODUCTION

Rapid and unselective application of petroleum hydrocarbons has made them an inevitable entrance into the environment, leading to massive ecological poisoning (Dike et al., 2022; 2021). Ground contamination by crude oil is one of the chronic environmental issues in countries engaged in bulk oil extraction (Barakhov et al., 2023). Crude oil includes organic and inorganic compounds, for example, polycyclic aromatic hydrocarbons that are carcinogenic and growth inhibitory (Wei et al., 2024). This type of contamination is highly harmful to the health of flora, fauna, and humans, apart from undermining environmental integrity (Cocârţă et al., 2017). High petroleum hydrocarbon concentrations in soil alter its physical, chemical, and biological characteristics (Daoud et al., 2019; Liang et al., 2020; Sani et al., 2023). These include the decline in microbial richness and overall microbial population density, alongside the increase in hydrocarbon-degrading microbial counts, ultimately disrupting biogenic element equilibrium, microbial ecology, and other ecosystem functions (Wei et al., 2024).

The extent of ecological disturbance is a function of the soil type; sandy and humus-deficient loamy soils are more vulnerable, whereas peat soils are comparatively less affected. The recoverability of soils from hydrocarbon contamination is a function of soil type, indigenous microbial populations, contaminant concentration, and exposure time (Karimullin et al., 2017). There is no universally accepted approach to remediate hydrocarbon pollution in different soil types currently, and there is significant heterogeneity concerning tolerable levels of contamination.

Consequently, heightened emphasis on creating cost-effective and sustainable soil treatment methods is required (Mo et al., 2022). Bioremediation represents a viable

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mechanism that enables degradation or immobilization of petroleum hydrocarbons using biosorption, bioaccumulation, and microbial metabolism, coupled with the involvement of organic substrates and inorganic nutrients (Wang et al., 2024). Of all these strategies, the application of biochar has been noteworthy in enhancing organic pollutants' degradation (Kong et al., 2018). Biochar is environmentally safe and effective in cleaning contaminated soils (Minnikova et al., 2023; Ruseva et al., 2024; Sani et al., 2023). It is applied in soil amendment and wastewater treatment processes to adsorb or immobilize contaminants, including heavy metals and organic substances (Wang & Wang, 2019; Wang et al., 2018; Ye et al., 2017; Yin et al., 2017). Due to its sorption capacity and active functional group interactions, together with its role of encouraging populations of bacteria that degrade hydrocarbons, it is making an emerging soil amendment material (Minnikova et al., 2022; Zahed et al., 2021).

Biochar's pH neutrality makes it compatible with both acidic and alkaline soils (Rubel et al., 2024; Susilowati et al., 2024). Biochar improves microbial activity, water and nutrient retention, and crop yields (Dike et al., 2022; Kumar et al., 2020; Wang et al., 2019; Zahed et al., 2021). Being a soil ameliorant, biochar is not only an oil residue adsorbent, however, it is also used as a biostimulant that enhances the activity and growth of native oil-degrading microbes (Kamali et al., 2022; Ruseva et al., 2023; Zhang et al., 2019). Research work at Southern Federal University has previously proven the efficacy of biochar from various feedstocks to detoxify heavy metal and organic-polluted soils (Barakhov et al., 2023; Burachevskaya et al., 2022; Minkina et al., 2022).

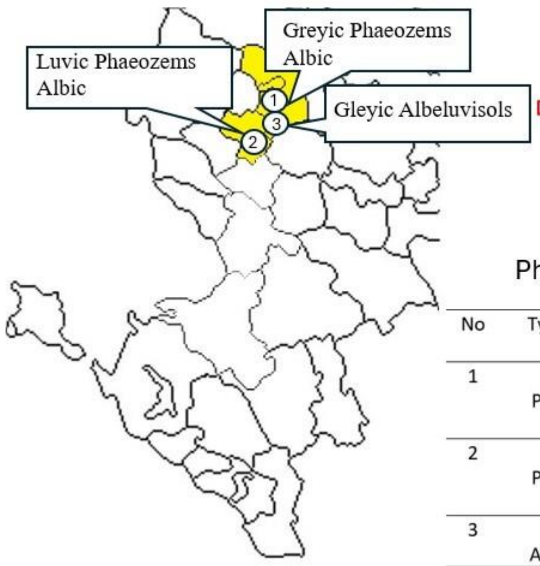
Over the last few years, there has been a sharp increase in petroleum accidents and oil spills in the world to a large extent, including Russia (Minnikova & Kolesnikov, 2025). Regions that are oil-rich in terms of infrastructure, i.e., pipelines and refineries, are particularly vulnerable to such environmental risk. Despite numerous investigations into oil-contaminated soil bioremediation (Cherdakova & Galchenko, 2020; Dike et al., 2022; dos Santos & Maranho, 2018; Sani et al., 2023), they have typically addressed single physicochemical or biological variables narrowly, with no comprehensive assessments of soil health. To generate a good assessment of remediation outcomes, both residual hydrocarbon levels and biological integrity and functionality of the soil must be considered, the focus of the present study.

This research aimed to evaluate ecological changes in oil-contaminated Luvic Phaeozems, Albic and Gleyic Albeluvisols, and Greyic Phaeozems soils following remediation using birch-derived biochar. A laboratory-controlled experiment was set up where the soils were artificially contaminated with crude oil and treated with biochar and incubated for 30 days. Biological markers (bacteria abundance, enzymatic activity in soil, germination of seeds, and growth of radishes), physical and chemical properties (residual oil concentration, pH, redox potential, salinity, hydrophobicity) were thoroughly evaluated.

2. MATERIAL AND METHODS

2.1. Soil Properties

Three soil types were selected for the current research: Luvic Phaeozems Albic, Gleyic Albeluvisols, and Greyic Phaeozems Albic, based on the World Reference Base classification (IUSS Working Group WRB, 2014). Sampling of Luvic Phaeozems Albic was conducted on arable land in the Tula Region (Venevsky District, settlement Ulyanovka), Gleyic Albeluvisols on a mixed forest in the Moscow Region (Domodedovo urban district, settlement Podmoskovie sanatorium), and Greyic Phaeozems Albic on arable land in the Moscow Region (Kashira urban district, village Zlobino). Sampling was carried out based on the diagonal envelope method (Kazeev et al., 2016), and five replicates were collected from the topsoil layer (0–10 cm) of each type of soil. Soil properties are shown in Figure 1.



Physical and chemical indicators of the studied soils:

No	Type of soils	Type of ecosystems	Coordinates of sampling points	pH	Soil organic content, %	Texture
1	Luvic Phaeozems Albic	Arable land	54°23'54.78" N; 38°25'08'60" E	6.5	4.7	heavy loamy
2	Greyic Phaeozems Albic	Arable land	54°44'1.19" N; 38°05'41.17" E	5.7	4.4	heavy loamy
3	Gleyic Albeluvisols	Mixed forest*	55°21'42.48" N; 37°46'32.05" E	4.9	7.1	heavy loamy

Note: *Mixed forest is a forest with deciduous and coniferous trees

Figure 1. Characteristics and physical and chemical indicators of the soil

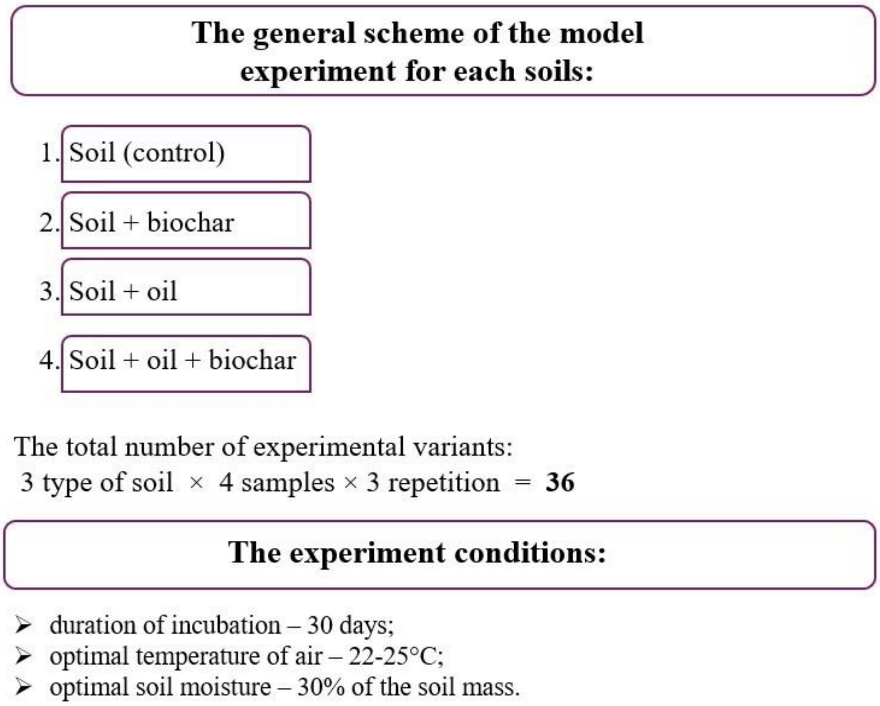


Figure 2. Scheme of a model experiment

2.2. Characteristics of Oil and Biochar

For the simulation of contamination, crude oil from the Novoshakhtinsk Oil Refinery was used. The light oil had the following characteristics: density 0.818 g/cm^3, mass fraction of sulfur 0.43%, mechanical impurity content 0.0028%, water content 0.03%, and content of chloride salts 40.1 mg/dm^3. Biochar as a remediation agent was introduced to increase the ecological status and functionality of oil-contaminated soils. The biochar employed in the present research was obtained by pyrolysis of white birch wood (Betula alba), grade A, according to GOST 7657–84 standards, with a carbon content of at least 85% (supplied by LLC "DianAGRO," Novosibirsk, Russia). Importantly, this biochar was characterized by a high degree of recalcitrance.

2.3. Model Experiment

Each soil sample was air dried and sieved through a mesh of 3.2 mm. Then 200 g of the wet soil was taken, separated from the plant pots to which oil of an equivalent concentration to 5% of soil weight was added. Biochar was incorporated into the oil-spiked soil to a concentration of 10% soil weight. A controlled laboratory experiment of 30 days was carried out with the prepared soil samples. Soils were dried after the experimental period, and analysis of the different physicochemical parameters was carried out together. Remediation studies carried out previously have proved that 30 days will be enough for the efficient remediation of oil-contaminated soils (Das et al., 2021; Shankar et al., 2014). The scheme and conditions of the experiment and indicators are presented in Figure 2.

In the experiment, the effect of biochar on oil-free soils was assessed by comparing samples with the control. At the same time, the effectiveness of the biochar for the remediation of contaminated soils was evaluated by comparing samples with oil-only soil.

2.4. Research Methods

On the 30th day from the initiation of the simulated laboratory experiment, soil samples were dried, and their physical, chemical, and biological parameters were analyzed simultaneously (Table 1).

The residual oil content in the soil was analyzed using infrared spectrometry with carbon tetrachloride as an extraction solvent. Soil extracts were analyzed by IKN-025 analyzer-concentrator (HDPE, 1998), which enables one to measure the content of petroleum hydrocarbons in soil following remediation (Zainulgabidinov et al., 2024). The change in concentration of oil was estimated through comparison of measurements made on day 1 and day 30 of the experiment. The pH of the soil was analyzed by potentiometric method, and the concentration of easily soluble salts was analyzed by conductometric method; in addition, redox potential (Eh) was analyzed by potentiometry, all three parameters being analyzed in extracts of soil water (Kazeev et al., 2016). Soil hydrophobicity properties were determined through the Water Drop Penetration Time (WDPT) and Ethanol Percentage (EP) tests; WDPT quantifies the duration for the penetration of a water droplet into the soil surface, and EP examines the surface tension characteristics through standard aqueous ethanol solutions (Caltabellotta et al., 2022; Tinebra et al., 2019). For the assessment of seed germination, shoot and root growth, the radish (*Raphanus sativus* 'Zhara' cultivar) was used as a bioindicator plant based on morphometric analysis.

Soil samples were placed in Petri dishes, watered, and seeded with 25 seeds per sample; germination rates and early growth (seedling and root length) were measured after seven days. Catalase activity (H_2O_2: H_2O_2 oxidoreductase) was assayed following the geometrical method of Galstyan (1978).

Table 1. Indicators of soil after adding biochar and oil

Physical and chemical indicators	
petroleum hydrocarbons residual content	determined by infrared spectrometry on analyzer-concentrator IKN-025
pH	determined in the soil extract (the ratio soil: water was 1:2.5) by the potentiometric method (HANNA HI-2211 analyzer, Germany)
hydrophobicity	determined by Ethanol Percentage and Water Drop Penetration Time tests
redox potential	determined in the soil extract by potentiometric method (ORP analyzer by HANNA HI-98120, Germany)
easily soluble salt content	determined in the soil extract by the conductometric method (HANNA inst. Total Dissolved HI-9034 conductometer, Germany)
Biological indicators	
radish roots and shoots length	determined by the intensity of initial growth radish (*Raphanus sativus* L.)
radish seeds germination	determined by development intensity of seed radish (*Raphanus sativus* L.)
catalase activity	determined by the gasometrical method according to Galstyan (1978)
dehydrogenases activity	determined according to the method by Galstyan (1978)
bacteria total number	counting the number of soil bacteria using a Carl-Zeiss Axio Lab fluorescent microscope
Calculations	
integrated indicator of biological state	determined by the average value of biological indicators
sensitivity	determined by the degree of change in biological parameters relative to control when applying oil and biochar («1, 2» – the most sensitive, «5, 6» – the least sensitive)
Statistical processing	
one-factor analysis of variance	performed with the Statistica 13.3 software package and MS Excel (2016) (mean value (M ± m), standard deviation (s), and standard error of the mean (SE)

Dehydrogenase activity assessment, with NADF-oxidoreductase as the substrate and measurement by triphenyl tetrazolium chloride reduction according to Galstyan (1978) technique. These enzyme activities are sensitive indicators of anthropogenic stress and are involved in organic carbon compound transformation. The number of bacteria was counted by luminescent microscopy in incident light according to the method described by M. Zvyagintsev (Dadenko et al., 2021; Khaziev, 2018). A known volume of soil suspension was applied to 2 cm² duplicate slides, air-dried, and stained with acridine orange. In addition, the sensitivity of the biological parameters measured was examined. Based on the above-mentioned biological characteristics of soils, the integrated indicator of biological state (IIBS) of soils, developed at the Department of Ecology and Nature Management of the Southern Federal University, was calculated (Kazeev et al., 2016). To calculate the IIBS, the data of the control variants of Luvic Phaeozems Albic, Greyic Phaeozems Albic, and Gleyic Albeluvisols were taken as the maximum value of each characteristic (100%). The relative values of this indicator for other variants were calculated using Equation 1.

$$S_1 = \frac{S_x}{S_{max}} \times 100\% \quad [1]$$

where S_1 is the relative score of the biological indicator; S_x is the actual value of the biological indicator; S_{max} is the maximum value of the biological indicator (control).

Afterwards, the relative values of the studied biological indicators were summed up, and for each option, the average score was calculated using Equation 2.

$$S_{av} = \frac{S_1+S_2+\ldots+S_n}{N} \quad [2]$$

where S_{av} is the average assessment score of the indicators; $S_1 \ldots S_n$ is the relative score of the indicator; N is the number of indicators.

The final value of the IIBS was calculated using Equation 3.

$$\text{IIB} = \frac{S_{av}}{S_{ref}} \times 100\% \quad [3]$$

where S_{av} is the average assessment score of the biological indicator, S_{ref} is the control value averaged over all biological indicators.

The reliability of the results was assessed using a single-factor analysis of variance.

3. RESULTS

3.1. Physical and Chemical Characteristics of Soils

Residual oil content in soil. A study of the residual oil content in soils without biochar showed that due to natural processes in the soil, the amount of oil decreased in Gleyic Albeluvisols Luvic to 31%, in Greyic Phaeozems Albic to 33%, and in Greyic Phaeozems Albic to 32%, relative to the initial oil content in the soils (Fig. 3).

Determining the residual oil content in the soil after introducing biochar revealed a decrease in oil content by 33–48% relative to its concentrations in the studied soils on the first day (Fig. 4).

Input of biochar in Gleyic Albeluvisols decreased oil content to 48%, in Luvic Phaeozems Albic this index was noted to 41%, and in Greyic Phaeozems Albic it was 33% relative to the initial oil content in the soils on the first day of the experiment.

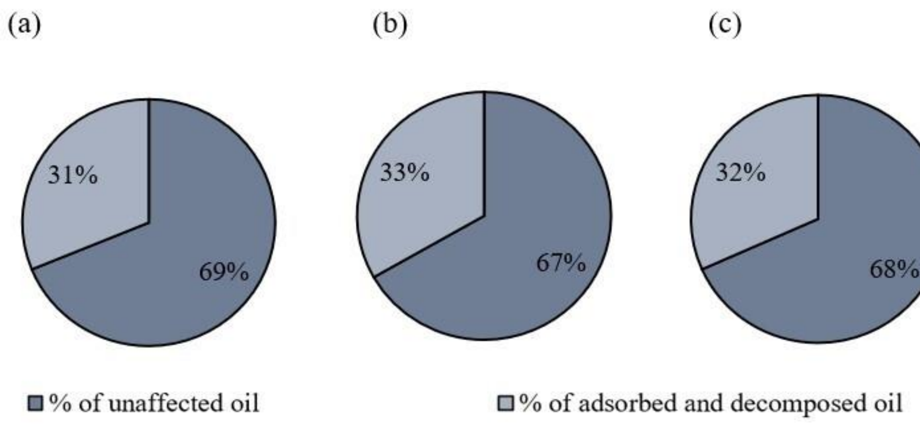


Figure 3. Change in the residual oil content in soils without the application of biochar, % of the initial content on the 1st day of the experiment: (a) Gleyic Albeluvisols; (b) Luvic Phaeozems Albic; (c) Greyic Phaeozems Albic

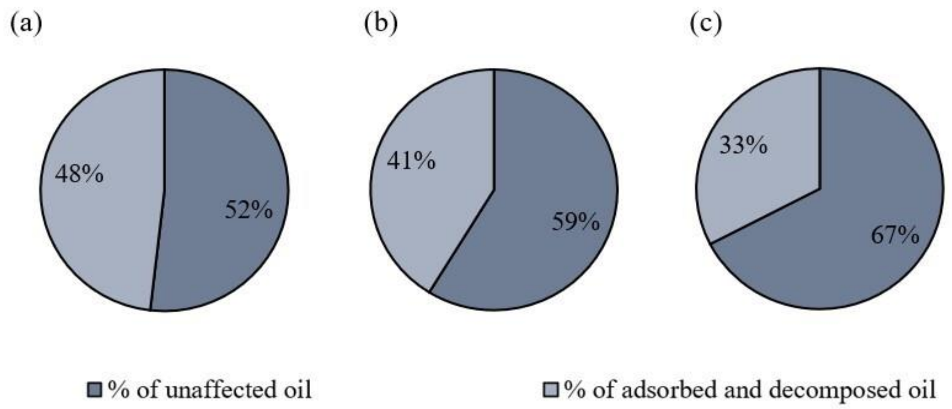


Figure 4. Change in the residual oil content in soils after the application of biochar, % of the initial content on the 1st day of the experiment: (a) Gleyic Albeluvisols; (b) Luvic Phaeozems Albic; (c) Greyic Phaeozems Albic

Table 2. Changes in the physical and chemical parameters of soil due to oil pollution and the input of biochar (M±m)

Type of soil	Variants	pH	Total content of readily soluble salts, ppm	Redox potential, mV
Gleyic Albeluvisols	Control	4.9±0.01	69±3.5	329±1.2
	B	4.8±0.02	136±2.3	331±2.6
	5%O	4.8±0.01	60±0.6	331±0.9
	5%O + B	4.9±0.00	61.5±0.3	327±1.4
Greyic Phaeozems Albic	Control	5.7±0.02	174±4.6	337±2.9
	B	6.2±0.00	193±2.3	333±1.2
	5%O	6.5±0.02	38±4.0	346±0.6
	5%O + B	6.7±0.01	62.5±0.9	292±1.4
Luvic Phaeozems Albic	Control	6.5±0.02	78.5±4.3	249±0.6
	B	7.1±0.01	113.5±0.3	251±1.4
	5%O	6.5±0.09	29±2.9	292±0.3
	5%O + B	6.8±0.01	68±2.3	277±2.0

Notes: B – biochar; 5%O – soil contaminated with oil at a concentration of 5% by weight of the soil. Significant differences are significant at $p < 0.05$

Soil environment reaction. The results of determining pH showed that Gleyic Albeluvisols and Greyic Phaeozems Albic had a strongly acidic reaction, while Luvic Phaeozems Albic was specified by a slightly acidic reaction (Table 2).

Biochar provided an increase in the indicator values of Greyic Phaeozems, Albic and Luvic Phaeozems, however, the pH value in Gleyic Albeluvisols changed slightly under the ameliorant effect. Oil contamination increased the indicator level only in Greyic Phaeozems Albic. Concurrently, in contaminated Greyic Phaeozems Albic and Luvic Phaeozems Albic, biochar provided a change in the reaction of the environment to a neutral value (Table 2).

Concentration of easily soluble salts. The content of salt was assessed through its concentration in the soil solution. It is known that soils with total salt concentration in the soil (in terms of the amount of dense residue) exceeding 0.3% are considered saline (Kallas & Maron, 2018). This study showed that the introduction of oil and biochar impacted the examined indicator; however, the salt concentration did not reach the level at which the soils could be regarded as saline (Table 2).

Oxidation-reduction potential. In addition to all the above-mentioned examinations, the oxidation-reduction potential (Eh) of the soils was assessed. According to the research data, under normal conditions, the value of Eh in Luvic Phaeozems Albic was 400–600 mV. In Gleyic Albeluvisols, the indicator was 550–750 mV, whereas in Greyic Phaeozems Albic, it was 350–450 mV. Moreover, this value was below the established standards in all the examined soils. A decrease in Eh to 350 mV and below may indicate the development of denitrification processes in the soils (Kazeev et al., 2016). Application of biochar and oil did not lead to significant changes in the indicator values.

Hydrophobicity. Based on the Dekker (1998) scale, 5 classes of soil hydrophobicity are usually distinguished: moistened, non-hydrophobic (class 0, WDPT ≤ 5 seconds), slightly hydrophobic (class 1, WDPT = 5–60 seconds), strongly hydrophobic (class 2, WDPT = 60–600 s), very strongly hydrophobic (class 3, WDPT = 600–3600 seconds), and extremely strongly hydrophobic (class 4, WDPT > 3600 seconds).

The examination of soil hydrophobicity showed that Luvic Phaeozems, Albic and Greyic Phaeozems were non-hydrophobic. In the latter, biochar somewhat increased the hydrophobicity level, however, it did not change its class. Concurrently, Gleyic Albeluvisols, according to the WDPT test, were slightly hydrophobic, and biochar slightly reduced their level. The oil contamination changed the hydrophobicity class in sod-podzolic and dark gray forest soils to a very strong hydrophobic level, while Luvic Phaeozems Albic soil became strongly hydrophobic (Table 3).

Biochar led to a decrease in hydrophobicity in soils contaminated with oil; more specifically, in Gleyic Albeluvisols and Greyic Phaeozems Albic, the hydrophobicity level changed its class to strongly hydrophobic. It was noted that upon the introduction of oil and biochar in soils, the intensity of hydrophobicity according to the EP test decreased in all variants with an increase in the concentration of ethanol in the solution (Table 3). After biochar input, hydrophobicity decreased as follows: in Gleyic Albeluvisols, its level dropped by 3.3 times, in Greyic Phaeozems, Albic fell by 1.6 times, and in Luvic Phaeozems, it reduced by 2.2 times.

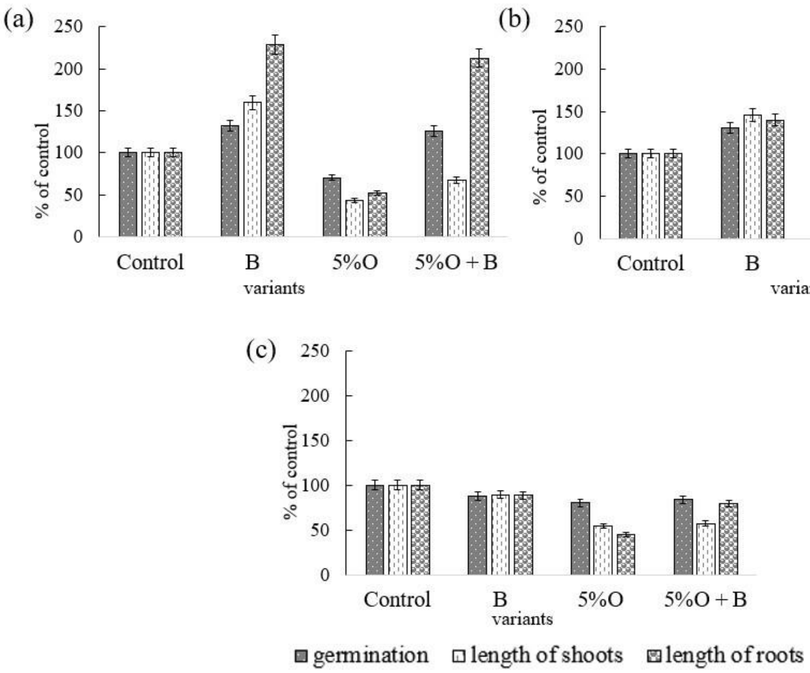
3.2. Biological Characteristics of Soils

The characteristics of the initial growth intensity and germination of radish seeds. The results of the assessment of germination and intensity of initial growth of seeds revealed that introducing biochar promoted an increase in the length of radish roots in Gleyic Albeluvisols and Luvic Phaeozems Albic by 239 and 39%, respectively. In Greyic Phaeozems Albic, on the contrary, an increase in phytotoxicity was observed, which was attested by a decrease in the indicator value of 12% relative to the control. In the first two mentioned soils, upon introducing biochar, the length of shoots also increased by 60% and 46%, respectively, while in the Greyic Phaeozems Albic, the indicator level decreased by 11%. Oil contamination led to a significant decrease in the length of roots by 40–55% and shoots by 46–57% in all studied soils compared to the control samples (Fig. 5).

Table 3. Change in hydrophobicity (EP and WDPT tests) of soils when polluted with oil and added biochar, sec (M±m)

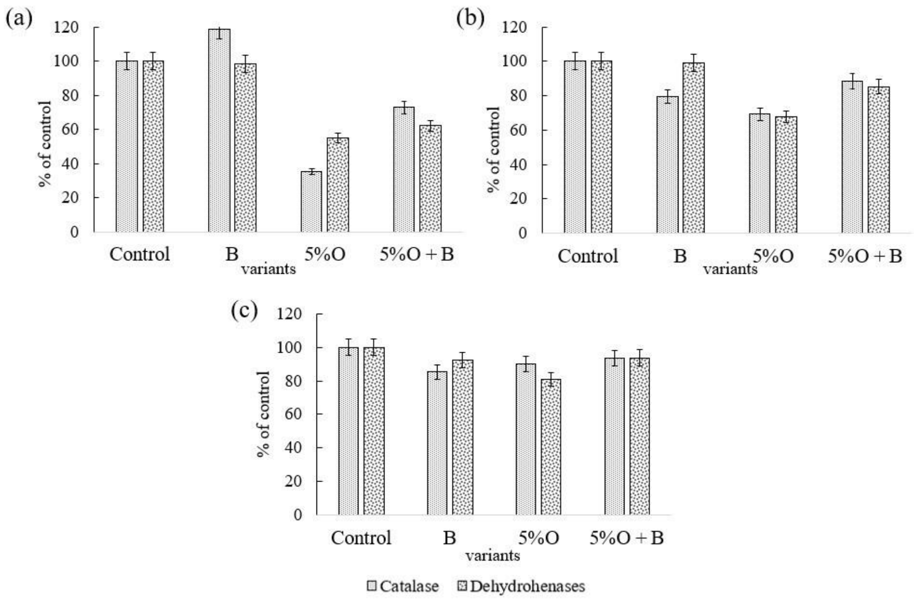
Type of soil	Variants	Ethyl alcohol content in solution, %						
		0	5	10	15	25	30	35
Gleyic Albeluvisols	Control	13±1.1	9±2.2	8±1.2	6±1.3	5±1.0	3±0.8	2±1.0
	B	12±1.3	5±1.1	5±1.1	5±1.0	5±1.5	3±2.1	3±0.9
	5%O	1020±1.2	1001±1.5	900±1.0	890±0.7	603±1.1	350±1.2	216±1.2
	5%O + B	400±1.3	325±1.3	243±1.4	180±1.2	118±1.6	120±1.2	120±2.2
Greyic Phaeozems Albic	Control	2±0.6	2±0.1	2±0.6	2±0.8	3±0.1	2±0.3	2±0.1
	B	4±0.8	2±0.6	2±0.1	2±0.6	2±0.5	2±0.5	1±0.1
	5%O	700±1.1	703±1.5	351±1.1	141±1.2	16±1.4	9±2.0	7±1.7
	5%O + B	455±1.4	480±1.5	106±2.1	70±0.6	63±1.5	10±1.2	2±2.3
Luvic Phaeozems Albic	Control	2±0.2	2±0.4	2±0.3	2±0.1	1±0.6	1±0.4	1±0.5
	B	2±0.3	2±0.3	2±0.9	1±0.1	1±0.4	1±3.0	1±0.1
	5%O	349±1.7	207±1.1	249±0.9	270±0.5	103±0.4	20±0.3	9±0.4
	5%O + B	255±1.9	156±1.6	60±1.8	48±1.7	17±1.1	18±2.5	3±0.8

Notes: B – biochar; 5%O – soil contaminated with oil at a concentration of 5% by weight of the soil. Significant differences are significant at p <0.05



Notes: see Table 2 & 3. Significant differences are significant at $p < 0.05$

Figure 5. Change in the length of roots and shoots in soils with oil pollution and the addition of biochar, % of control: (a) Gleyic Albeluvisols; (b) Luvic Phaeozems Albic; (c) Greyic Phaeozems Albic



Notes: see Table 2 & 3. Significant differences are significant at $p < 0.05$

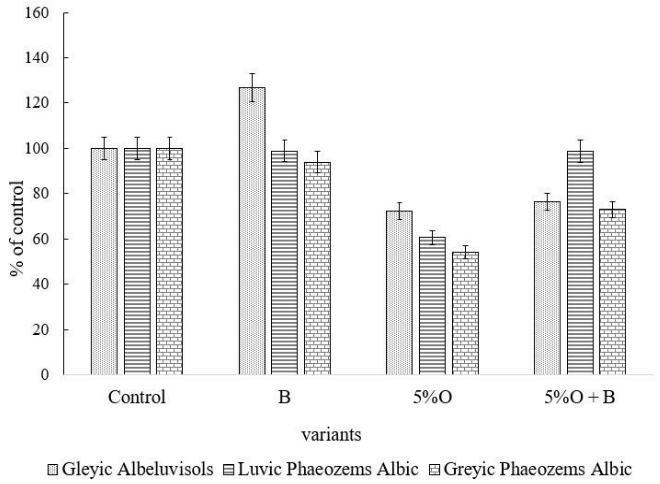
Figure 6. Changes in the activity of catalase and dehydrogenases in soils under oil pollution and the addition of biochar, % of control: (a) Gleyic Albeluvisols; (b) Luvic Phaeozems Albic; (c) Greyic Phaeozems Albic

Application of biochar into oil-contaminated soils caused stimulation of the length of roots and shoots in Gleyic Albeluvisols by 310 and 56%, in Luvic Phaeozems Albic by 74 and 67%, and in Greyic Phaeozems Albic by 77 and 5%, respectively, as compared to samples containing the contaminant under the ameliorant-free conditions (Fig. 5).

Biochar also stimulated the germination of radish seeds in Gleyic Albeluvisols and Greyic Phaeozems Albic by 32 and 30%, respectively, but decreased this indicator level in Luvic Phaeozems Albic by 12% relative to the control. Meanwhile, oil contamination reduced the indicator level in soils by 12–29% compared to the control.

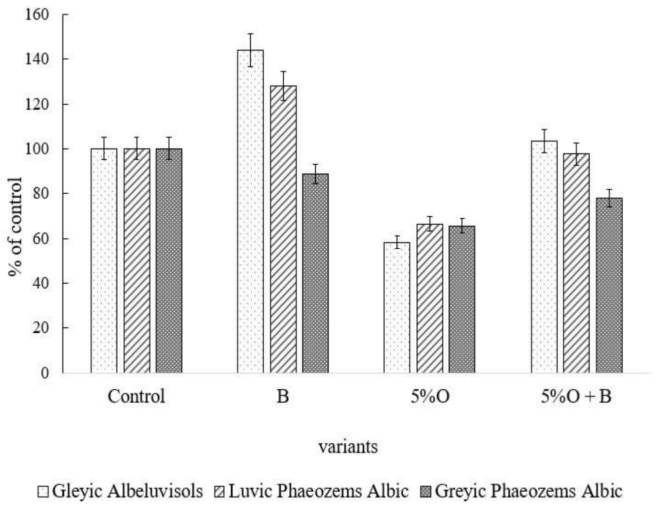
Biochar stimulated germination in all oil-contaminated soils; the greatest significant increase was revealed in Gleyic Albeluvisols and Luvic Phaeozems Albic, where indicator values changed by 79 and 38%, respectively, compared to the control.

Soil enzyme activity. The examination of enzyme activity showed that biochar led to significant stimulation of catalase in Gleyic Albeluvisols, namely by 19% compared to the control. In all other experimental variants and soil types, the inhibition of catalase and dehydrogenases activities was revealed. Oil contamination suppressed catalase activity by 10–65% and dehydrogenases by 19–45% (Fig. 6).



Notes: see Table 2 & 3. Significant differences are significant at p <0.05

Figure 7. Change in the total number of bacteria in soils with oil pollution and the addition of biochar, % of control: (a) Gleyic Albeluvisols; (b) Luvic Phaeozems Albic; (c) Greyic Phaeozems Albic



Notes: see Table 2 & 3. Significant differences are significant at p <0.05

Figure 8. Integral indicator of the biological state of soils with oil pollution and the introduction of biochar, % of control: (a) Gleyic Albeluvisols; (b) Luvic Phaeozems Albic; (c) Greyic Phaeozems Albic

Table 4. Ranking of biological indicators of oil-contaminated soils after the application of biochar according to sensitivity: "1, 2" – the most sensitive, "5, 6" – the least sensitive

Type of soils	G	LSh	LR	A_{cat}	A_{deh}	Bc
Gleyic Albeluvisols	5	**2**	**1**	3	4	6
Greyic Phaeozems Albic	4	**1**	3	5	5	**2**
Luvic Phaeozems Albic	4	**1**	5	3	**2**	6

Notes: G – germination; LSh – length of shoots; LR – root length; A_{cat} – catalase activity; A_{deh} – dehydrogenases activity; Bc – total number of bacteria.

Biochar stimulated enzyme functioning; the most significant increase was revealed in catalase activity, which was boosted by 106 and 28% in Gleyic Albeluvisols and Luvic Phaeozems Albic, respectively. Concurrently, the activation of dehydrogenases was also significant, and their levels depended on soil type: the increase was 26% in Luvic Phaeozems Albic, 16% in Greyic Phaeozems Albic, and 13% in Gleyic Albeluvisols compared with contaminated samples.

The total count of bacteria. The examination of the total count of bacteria showed that biochar increased this indicator in samples where there was no oil, only in Gleyic Albeluvisols; the augmentation amounted to 27% relative to the control. Concurrently, soil contamination with oil reduced the number of bacteria by 28–46% compared with the control (Fig. 7).

The introduction of biochar into oil-contaminated soils increased the indicator values by 6–63%; the most significant stimulation of oil-contaminated samples was revealed in Luvic Phaeozems, Albic and Greyic Phaeozems.

Integrated indicator of the biological state of soils. Application of biochar in Gleyic Albeluvisols and Luvic Phaeozems Albic resulted in an increase in IIBS by 44% and 28%, respectively; in Greyic Phaeozems Albic, on the contrary, a decrease in the IIBS by 11% relative to the control was noted under this condition (Fig. 8). The contamination with oil reduced the IIBS in Gleyic Albeluvisols by 42% and by 34% in both Luvic Phaeozems Albic and Greyic Phaeozems Albic. Whereas Gleyic Albeluvisols and Luvic Phaeozems Albic worked towards reaching the IIBS control level (100%); however, in Greyic Phaeozems Albic, on the contrary, the IIBS value was lower than the control by 22%.

The IIBS level relative to oil-contaminated samples in Gleyic Albeluvisols by 77%, in Luvic Phaeozems Albic by 47%, and in Greyic Phaeozems by 18% was noted in biochar applied treatments. It is important to emphasize that after biochar input, a decrease in oil concentration was revealed in Gleyic Albeluvisols that reached 48%; in Greyic Phaeozems Albic, the reduction was by 33%; and in Luvic Phaeozems Albic, it was by 41% relative to the initial levels. Based on the IIBS changes due to biochar application in oil-contaminated soils, their sensitivity levels were assessed and shown in Table 4.

In Gleyic Albeluvisols, the most sensitive indicator was the length of radish roots; however, in Luvic Phaeozems Albic and Greyic Phaeozems Albic, radish shoot length was the most sensitive indicator. The least sensitive indicator was the total number of bacteria; in Greyic Phaeozems Albic, the activity of catalase and dehydrogenases under biochar implementation. Based on the degree of a decrease in residual oil content in the soil, as well as on the stimulation of biological indicators, the efficacy of biochar for the remediation of oil-contaminated soils was assessed. According to the ameliorant efficacy, the soils were arranged in a series from the highest to the lowest efficacy as follows: Gleyic Albeluvisols> Luvic Phaeozems Albic > Greyic Phaeozems Albic.

4. DISCUSSION

The application of biochar was the most effective and environmentally suitable in Gleyic Albeluvisols, with the efficacy declining progressively in Luvic Phaeozems Albic and Greyic Phaeozems Albic, respectively (Table 4). Residual oil hydrocarbon analysis revealed that biochar significantly reduced oil content in all soil types compared to untreated controls. Gleyic Albeluvisols registered the greatest decrease, and this may have been due to heightened biological activity initiated by biochar supplementing the poor initial biological potential of the soil. Slight decrease, however, was registered for Greyic Phaeozems Albic, possibly because the fairly brief duration of 30 days was insufficient for effective remediation.

Lower contents of organic matter and buffer capacity in Greyic and Luvic Phaeozems may also be a reason for the reduced effectiveness of biochar compared to Gleyic Albeluvisols. These differences are perhaps due to soil structure, as agricultural soils can permit more penetration by oil than forest soils. Biochar had a highly significant impact on biological indicators, as reflected in a 77% increase in the Integral Indicator of Biological State (IIBS) of Gleyic Albeluvisols compared with 47% and 18% increases in Luvic and Greyic Phaeozems Albic, respectively (Fig. 7). It was noted that the pine biochar, at 5–10% soil weight, lowered hydrocarbon content by more than 50% within 60 days (Mukome et al., 2020).

Biochar had a lesser effect on soil pH, Eh, and salinity than it did on hydrocarbon content, hydrophobicity, and biological markers. Biochar reduced hydrophobicity considerably in Gleyic Albeluvisols, whereas the latter type of soil initially recorded the highest hydrophobicity under clean conditions. Redox potential, reflecting current oxidation-reduction relations, shifts in the direction of a reducing state under oil pollution, influencing humic substance transformation and mineralization processes (Kazeev et al., 2016). Additionally, oil pollution can enhance soil salinity (Nosova et al., 2023).

It is shown that the biochar also increased phytotoxicity indices. In non-contaminated soils, it promoted radish seed germination and shoot/root growth, especially in Gleyic Albeluvisols and Luvic Phaeozems Albic. In oil pollution, root development increased markedly—by 310% in Gleyic Albeluvisols, 74% in Luvic Phaeozems Albic, and 77% in Greyic Phaeozems Albic—most likely due to the direct contact between roots and biochar and pollutants. The findings agree with earlier research using phytotests in contaminated soil remediation (Cherdakova & Galchenko, 2020; Ruseva et al., 2023).

Soil enzyme activity, a significant parameter of biochemical processes, and the extent of contamination a functions of texture, structure, and organic matter (Karimullin et al., 2017). Activity of enzymes, particularly catalase and dehydrogenases, is often used to follow the restoration of hydrocarbon-contaminated soil. Catalase activity in unpolluted Gleyic Albeluvisols increased in this study, while catalase and dehydrogenase activity increased in all contaminated soils upon addition of biochar. This corresponds with previous studies into enzyme sensitivity to anthropogenic factors (Dadenko et al., 2021; Revina et al., 2024).

Microbial indicators also reflected the biochar's impact. The sorbent enhanced bacterial growth in oil-contaminated Luvic and Greyic Phaeozems Albic; however, in unpolluted soils, only Gleyic Albeluvisols showed excessive microbial growth. This growth is likely caused by biochar's ability to detoxify oils via sorption and simultaneously supply key biogenic elements preserved after pyrolysis (Dike et al., 2021; Ding et al., 2016; Gorovtsov et al., 2020; Zhu et al., 2017).

Despite being effective in initiating biological activity in all soil types, the changes were most important in Gleyic Albeluvisols. Its performance is attributed to improved aeration, provision of microbial habitats, and nutrient input, favoring the oil-degrading microbial communities (Zhang et al., 2019). Nonetheless, other variables such as biochar feedstock, pyrolysis conditions, climate, and soil type must be accorded proper consideration to avoid hindering the degradation process (Mukome et al., 2020). Based on these results, birch biochar is recommended to be utilized for oil spill remediation in natural systems.

5. CONCLUSION

This work highlights the positive impact of biochar as a soil amendment, particularly in the rehabilitation of oil-polluted soils. The study found that biochar not only improved the overall health and ecological status of different soils, but it also lowered petroleum hydrocarbon content drastically, particularly in Gleyic Albeluvisols. Biological parameters used in the research can be utilized as useful indicators to evaluate the efficacy of such treatment for rehabilitation processes. This reinforces the use of biochar in sustainable soil management and environmental restoration. Therefore, future research aspects must be focused on optimizing biochar application to utilize with a broader range of soil types, pollutants, and in various climatic conditions.

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Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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