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Microbiocenoses of Oil-Polluted Soils

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Abstract—The state of soil microbiocenoses at different levels of oil pollution was assessed. The microbiological activity of two types of soils (sod-podzolic and typical black earth) under the conditions of stress caused by oil pollution was determined. The regularities in variation of the ratio and total number of the main groups of microorganisms in different soil types were reveled. The compositions of microorganism species in polluted soils were determined. The dynamics of changes in microbiocenoses after one-time oil spiking was traced. A number of microbiological parameters, which can serve as markers of oil pollution in soil, were proposed.

Keywords: soil cover; pollution by oil and oil products; microbiocenosis; microbiological activity of soils; abundance and species composition of microorganisms

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INTRODUCTION

The extremely tense environmental situation caused by soil pollution by oil and oil products in many regions of the world is a serious problem that requires a comprehensive study and development of an algorithm to solve it, taking into account regional climatic and socioeconomic features.

When oil enters the soil cover, its dispersion is determined by the type of soil and depends on the entire complex of soil properties. The depth of oil infiltration in small-grain soils is much larger than in clay soils. The accumulation of oil in the soil cover occurs mainly on geochemical barriers, and the rate and direction of its migration depends on the nature of the geochemical barriers. Penetrating into the soil, oil primarily accumulates in upper humus (or peat) horizons. Gley, permafrost, clay-illuvial, and other soil horizons can act as vertical geochemical and mechanical barriers [1].

In soils of the permafrost tundra and taiga landscape-geochemical regions, oil migration is controlled by biogeochemical barriers (peat and humus horizons), as well as by the permafrost barrier. In the taiga forest zone, oil migration depends on a combination of several geochemical barriers. Thus, in sod-podzolic soils, humus and illuvial horizons play a great role in the vertical oil dispersion. In the forest steppe zone, oil accumulates within a thick accumulative humus horizon, which has a negative impact on the soil itself, but prevents pollution of adjacent media. In semidesert, desert, and dry subtropical soils, almost all oil dwells in the upper part of the profile, and then actively degrades under the action of high temperatures and elevated levels of UV radiation. Intensive mineralization of oil and leaching of residual products occur in humid subtropical soils [2–4].

Oil pollution causes deep and sometimes irreversible changes in the morphological, physicochemical, and microbiological properties of soil. Oil penetrates pores and covers structural units and aggregates with a film, rendering the soil mass hydrophobic and thus prevents normal heat and gas exchange and isolates nutrients.

Kutanogenesis enhances, the color of coil changes and its structure deteriorates, the soil solution becomes more alkaline, the solubility of most trace elements decreases, the total carbon content increases, the carbon-to-nitrogen ratio is violated, etc. [6, 7]. As a result, soil microorganism communities undergo significant changes.

Table 1. Relative damage degrees of soil containing different amounts of oil

Degree of damage	Oil concentration in soil, mg/kg dry soil
Low –to-moderate: slight decrease in vegetation growth if no action is taken; temporary damage	5000–20000
Moderate-to-high: only some plants develop normally; the soil can recover within three years; without remediation, recovery will take 2-3 times longer time	20000–50000
High-to-very high: oil permeates the soil to a depth of 10 cm; only very few plants survive; with proper remediation, the soil can be restored in 3-5 years; without remediation, recovery will take 20 years or more	>50000

Table 2. Chemical characteristic of soils (depth 0–25 cm)

Soil	pH		Humus, %	mg equiv /100 g			Particles <0.01 mm, %
				acidity		Cation exchange capacity (CEC)	
	H_2O	KCl		exchange	hydrolytic		
Sod-podzolic	4.2	3.8	1.3	0.92	5.21	9.8	32.5
Typical black earth	6.4	–	8.7	0.19	–	52.7	44.9

The aim of this study was to study the effect of oil on the microorganism communities of soils of various natural climate zones of the European part of Russia, specifically sod-podzolic and typical black earth soils.

EXPERIMENTAL

The objects for study were natural soils of two types: sod-podzolic soil from the southern part of the Moscow Region (Stupinsky District) and a typical black soil from the central part of the Kursk Region (Kursky District). The chemical characteristics of these soils are presented in Table. 2. The effect of oil on the biological activity of different soils was studied in a model experiment with commercial oil (desalted, contains no formation fluids), performed in triplicate. The following oil spikes were used (L/m^2): 0 (control); 2.5; 5; and 10. Soil samples were taken 2, 4 and 8 weeks after one-time oil spiking from a depth of 0–25 cm.

Chemical analysis of soil samples was performed by conventional procedures [8, 9]. Characteristics of microorganism communities (populations of different microorganism species) were determined by direct microscopy [10–12].

RESULTS AND DISCUSSION

In the model experiment we obtained data on changes in the populations of microorganisms (fungi, non- spore-forming bacteria, bacilli, and actinomycetes) in sod-podzolic and typical black soils depending on the oil spike. In the same experiment we also studied the dynamics of changes in micro-biocenoses in time (8 weeks) after one-time oil spiking (Tables 3 and 4).

In the sod-podzolic soil (Table 3), a tendency of the total population of fungi to steeply increase as the oil dose was increased to 10 L/m^2 was revealed. The maximum growth (4.5 times) was observed at a dose of 5 L/m^2 in 4 weeks after spiking. The number of actinomycetes and bacteria increased to the contamination level of 5 L/m^2, and then dropped sharply. Moreover, at the maximum oil dose (10 L/m^2) the population of all the microorganism species increased with time.

The typical black earth showed generally the same tendencies as the sod-podzolic soil (Table 4). However, the maximum increase in the fungal population (4.8 times) was observed at an oil dose of 10 L/ m^2 in 4 weeks after spiking. At the same time, the

Table 3. Microorganism populations in the sod-podzolic soil (depth 0–25 cm)

Oil dose, L/m^2	Microorganism populations, mln/g soil											
	Fungi						Actinomycetes					
	2 weeks		4 weeks		8 weeks		2 weeks		4 weeks		8 weeks	
		%[a]		%		%		%		%		%
0 (control)	0.31	100	0.28	100	0.34	100	0.30	100	0.32	100	0.29	100
2.5	0.38	123	0.94	336	0.88	259	0.37	123	0.48	150	0.57	115
5	0.46	148	1.26	450	1.05	309	0.35	117	0.40	125	0.65	151
10	0.71	229	1.10	393	1.42	418	0.08	27	0.13	41	0.34	118
$HCP_{0.95}$	0.03	–	0.04	–	0.04	–	0.03	–	0.02	–	0.03	–
	Nonspore-forming bacterial						Bacilli					
0 (control)	2.7	100	2.6	100	2.6	100	0.76	100	0.81	100	0.78	100
2.5	2.4	89	3.4	131	4.5	173	0.88	116	0.78	96	0.97	124
5	2.6	96	3.9	150	5.0	192	0.75	99	0.97	120	1.12	144
10	1.6	59	2.2	85	2.7	104	0.30	40	0.43	53	0.82	105
$HCP_{0.95}$	0.05	–	0.1	–	0.1	–	0.05	–	0.06	–	0.04	–

[a] % of control.

Table 4. Microorganism populations in the typical black earth soil (depth 0–25 cm)

Oil dose, L/m^2	Microorganism populations, mln/g soil											
	Fungi						Actinomycetes					
	2 weeks		4 weeks		8 weeks		2 weeks		4 weeks		8 weeks	
		%[a]		%		%		%		%		%
0 (control)	0.36	100	0.35	100	0.38	100	1.77	100	1.75	100	1.70	100
2.5	0.45	125	0.56	160	0.97	255	1.90	107	1.94	111	1.95	115
5	0.90	250	0.91	260	1.32	347	1.88	106	2.25	129	2.56	151
10	0.63	175	1.68	480	1.24	326	1.51	85	1.20	69	2.00	118
$HCP_{0.95}$	0.02	–	0.03	–	0.05	–	0.04	–	0.05	–	0.04	–
	Nonspore-forming bacterial						Bacilli					
0 (control)	5.7	100	5.6	100	5.4	100	2.7	100	2.9	100	2.9	100
2.5	6.1	107	5.9	105	6.8	126	3.3	122	3.1	107	3.8	131
5	7.1	125	8.8	157	9.3	172	3.7	137	3.9	135	4.7	162
10	5.6	98	6.2	111	9.1	169	1.8	67	2.2	76	3.7	128
$HCP_{0.95}$	0.1	–	0.12	–	0.1	–	0.07	–	0.08	–	0.06	–

[a] % of control.

Table 5. Numbers of sterile actinomycetes and phytopathogenic fungi in soil, fraction, % (depth 0–25 cm, 2 and 8 weeks after spiking)

Oil dose, L/m^2	Sterile actinomycetes		Phytopathogenic fungi, species			
			Fusarium		*Alternaria*	
	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks
Sod-podzolic soil						
0.0	8	6	–		–	
2.5	15	12	5	7	3	3
5.0	18	23	10	7	8	10
10.0	30	37	38	44	19	27
$HCP_{0.95}$	1.3	1.2	1.0	1.0	1.7	1.3
Typical black earth						
0.0	–		–		–	
2.5	–		–		–	
5.0	12	14	3	4	5	8
10.0	16	19	22	19	11	12
$HCP_{0.95}$	1.5	0.8	1.1	1.0	1.0	0.7

Table 6. Sporulation rates of *Penicillium* fulgi, % of 200 microscope fields (depth 0–25 cm, 2 and 8 weeks after spiking)

Oil dose, L/m^2	Fungal sporulation rate			
	sod-podzolic soil		typical black earth	
	2 weeks	8 weeks	2 weeks	8 weeks
0.0	7.5	7	4	5
2.5	39	30	19	25
5.0	73	69	34	37
10.0	61	72	47	40
$HCP_{0.95}$	1.7	2.0	1.0	1.3

populations of actinomycetes and bacteria not so dramatically dropped at an oil dose of 10 L/m^2 as in the sod-podzolic soil. At the maximum oil dose (10 L/m^2) the population of all the microorganism species increased with time.

The increase in the microorganisms populations in two different types of soil is explained by both an increase in the amount of nutrients and the pH shift to neutral values. Thus, the pH (H_2O) of the sod-podzolic soil at maximum oil dose increased from 4.2 to 5.7 and that of the typical black earth soil, 6.4 to 6.8. At the same time, numerous studies have shown that microbiological decomposition of hydrocarbons reaches a maximum intensity at pH values close to neutral, since these parameters are optimal for the activity of soil microflora [6, 7, 13–15].

Oil pollution impairs the reproductive function of actinomycetes, which increases the prportion of their sterile forms (Table 5). Thus, the proportion of sterile actinomycetes in the sod-podzolic soil at the oil dose of 10 L/m^2 increased 3.8 times within 2 weeks after spiking and 6.2 times within 8 weeks. In the typical balck oil soil, sterile actinomycetes appear at the spiking level of 5 L/m^2.

The phytopathogenic fungi of *Fusarium* and *Alternaria* families begin to be detected in the sod-podzolic soil at the oil dose of 2.5 L/m^2 and the typical black earth soil, at 5 L/m^2. The population of these fumgi increases with increasing oil dose (Table 5).

Oil pollution of soil drives formation of the sporulation organs of *Penicillium* fungi (Table 6). At the same time, in the sod-podzolic soil, the maximum increase in the fungal sporulation rate is observed 2 weeks after spiking at a dose of 5 L/m^2 (9.7 times) and 8 weeks after contamination at a dose of 10 L/m^2 (10 times). In the typical black earth soil, the maximum increase at the oil dose of 10 L/m^2 is observed both 2 and 8 weeks after spiking (11.8 and 8 times, respectively). However, in general, the number of *Penicillium* fungal spores in the black soil is much lower than in the sod-podzolic soil, whatever the level of soil pollution.

The sporulation rate of *Penicillium* fungi in the sod-podzolic soil begins to decrease at the oil dose of 10 L/m^2, whereas in the black soil at the same oil dose no decrease in sporulation rate is observed.

CONCLUSIONS

Oil pollution of soil produces profound changes in the structure and functions of the soil microorganism complex, which is manifested in a change in the number and species composition of microorganisms and, consequently, in the level of the microbiological activity of soils. Our study showed that oil pollution of soil causes preferential development of microorganisms resistant to pollutants and inhibits development of sensitive species.

The fungal population and the sporulation rate of *Penicillium* fungi increase, and phytopathogenic *Fusarium* and *Alternaria* fungi appear. At a high degree of pollution, the total population of actinomycetes decreases, while the proportion of sterile actinomycetes increases; the total bacterial population decreases, but the population of some individual species increases. These findings point to a violation of the functional integrity of microbiota ensuring stability of soil microbiocenosis, which eventually leads to its partial or complete degradation.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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