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Changes in the Number and Species Variety of Bacteria in Oil-Polluted Soils

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Abstract—Herein we have assessed the state of soil bacterial communities at different levels of pollution with oil. The dependences of the change in the total abundance of non-spore-forming bacteria and bacilli in sod-podzolic soil and typical chernozem have been obtained. The species composition of microorganisms in polluted soils has been assessed. The dynamics of the changes in the structure of bacterial communities with time after single oil pollution has been traced. Several microbiological parameters which can be used as diagnostic tools in case of oil pollution of soil have been found.

Keywords: soil covering, pollution by oil and oil products, microbiocenosis, non-spore-forming bacteria, bacilli, abundance and species composition of microorganisms

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INTRODUCTION

Soil microbial community plays a significant role in ecological and ecosystem functions of soil. Soil microorganisms decompose compounds incoming to the soil, control the composition of soil air, and determine the content and availability of biogenic elements required for plants growth and development [1–3].

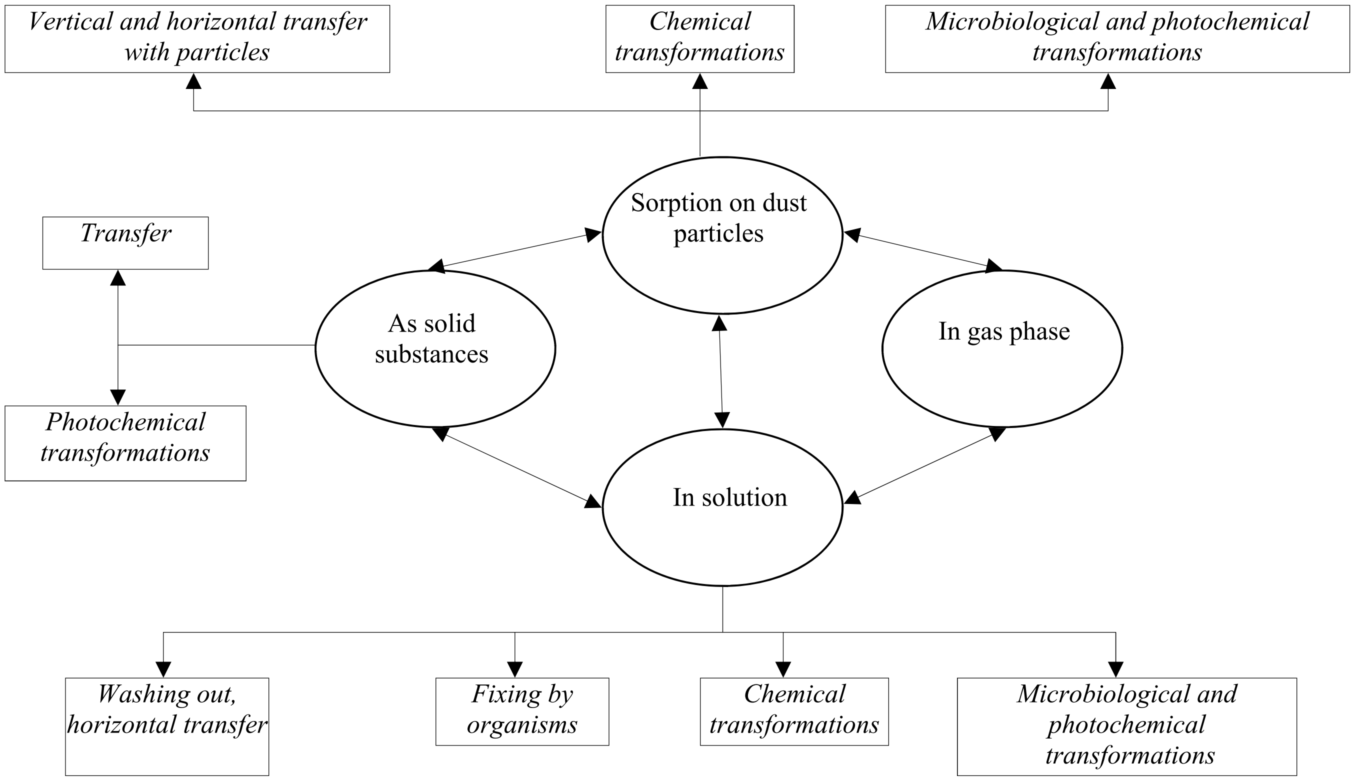
The structure of microbial communities significantly depends on the soil type, hydrothermal conditions, chemical composition, and contents as well as availability of incoming organic substances. For example, upper humus horizon is most populated by microbes; in lower soil layers, their abundance decreases with the decreasing nutrients and water content [4].

Species composition of soil microbiocenosis consists of bacteria, actinomycetes, fungi, protozoans, and viruses; generally, bacteria are predominating species. For instance, total amount of bacteria per 1 g of arable soil can achieve 2 billion, that of actinomycetes is up to 1 million, and that of mold fungi is up to 50 thousand [5].

Furthermore, the number of bacteria is significantly different depending on the soil type. According to P.A. Bulanov and O.I. Koleshko (1969), the largest number of bacteria is found in gray earths (125–200 million/g of humus), chestnut soils (110–200), and podzolic soils (up to 20–30 million/g) [6].

Significant changes in the structure of soil bacterial community are observed under anthropogenic soil pollution in particular by raw oil and petroleum products. In this case, the reaction of soil microorganisms depends on concentration and individual features of microorganisms as well as the oil composition.

Raw oil is a complex mixture of aliphatic, alicyclic, aromatic, and other hydrocarbons, containing small amounts of nitrogen and sulfur compounds. More than 1000 individual organic compounds have been observed in oils, containing 83–87% of carbon, 12–14% of hydrogen, 0.5–6% of sulfur, 0.02–1.7% of nitrogen, 0.005–3.6% of oxygen, and traces of mineral compounds [7]. Aromatic and alicyclic hydrocarbons are the most toxic for microorganisms; alkenes and alkanes are less toxic [8].



The behavior of oil hydrocarbons in the soil.

After getting to the Earth surface, oil is subject to novel conditions: the anaerobic environment featuring very slow geochemical processes are changed into the environment with good aeration; under these conditions, biochemical factors come into the play, firstly, the action of microorganisms. Ultraviolet part of solar radiation significantly accelerates decomposition of oil components; however, this process may be dangerous because of the formation of highly toxic products. Oil decomposition is slowed down because of very low rate of biochemical oil decomposition after evaporation of highly volatile components and dissolution of low-molecular fractions. The action of microorganisms on oil components is highly selective, for instance, different species of microorganisms are required for complete decomposition of oil. The decomposition of alkanes is the fastest, cycloalkanes are more stable, and aromatic hydrocarbons remain intact for even longer; high fractions of oil decompose very slowly, forming resinous clots and affecting the entire set of soil properties [9, 10]. Figure schematically demonstrates the possible processes of transformations of hydrocarbons in soil [11].

The changes in the set of soil properties are divided into four types, depending on the degree of oil pollution [12, 13]:

Homeostasis zone corresponds to oil concentration range of 0–0.7 mL/kg of soil. Significant changes in soil microbial community do not occurs at these concentrations.

Stress zone corresponds to concentration range of 0.7–50 mL/kg of soil. Quantitative changes are observed in soil microbial complex. The relative amount of the microorganisms in the soil is changed.

Resistance zone corresponds to concentration range of 50–300 mL/kg of soil. Drastic qualitative changes are observed in the structure of the microbial community. The microorganisms resistant to high concentrations of the pollutants start to dominate.

Repress zone corresponds to oil concentration higher than 300 mL/kg of soil. Growth and development of soil microorganisms are significantly suppressed.

Table 1. Chemical parameters of soils (depth 0–25 cm)

Soil	pH		Humus, %	μg×eq/100g			Particles < 0.01mm, %
	H_2O	KCl		acidity		cation exchange capacity (CEC)	
				exchangeable	hydrolytic		
Sod-podzolic	4.2	3.8	1.3	0.92	5.21	9.8	32.5
Typical black soil	6.4	–	8.7	0.19	–	52.7	44.9

Table 2. The number of microorganisms in sod-podzolic black and typical chernozem (depth 0–25 cm)[a]

Oil dose L/m^2	The number of microorganisms, million per 1 g of soil											
	non-spore-forming bacteria						bacilli					
	2 weeks		4 weeks		8 weeks		2 weeks		4 weeks		8 weeks	
Sod-podzolic soil												
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	–
Typical chernozem												
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] %, relative to the control sample.

At low concentrations, oil usually exhibits stimulating action to biota, being energetic substrate for a vast variety of microorganisms. In this case, microorganisms such as *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Nocardia*, *Pseudomonas*, and *Rhodococcus* predominate in the structure of bacterial community [14]. On the other hand, high concentrations of oil hydrocarbons exhibit acute toxic effect depending on the content of different oil fractions on the soil and are fatal to microbiocenosis.

The aim of our study was to elucidate the features of the change in the structure of bacterial communities of sod-podzolic soil and chernozem under typical oil pollution.

EXPERIMENTAL

The objects of the investigation were two types of natural soils: sod-podzolic soil from the south part of the Moscow region (Stupino subregion) and typical chernozem from central part of the Kursk region (Kursk subregion). Chemical characteristics of the soils are given in Table 1. The model experiments on oil influence on bacterial communities of different soils were carried out in triplicate. The commercially available oil was used (desalt, without stratum fluids) in the dose of (L/m^2): 0 (control), 2.5, 5, and 10. The samples of soils were collected 2, 4, and 8 weeks after the pollution from 0–25 cm depth.

Table 3. The species composition of *Bacillus* genus bacteria in soils, thousand per 1 g of soil (depth 0–25 cm, 4 weeks after the pollution)

Species	Oil dose, L/m^2			
	0	2.5	5	10
Sod-podzolic soil				
Bac. megaterium	26.2±0.4	24.3±0.2	29.2±0.4	21.1±0.3
Bac. idosus	28.1±0.2	33.7±0.3	23.9±0.3	19.4±0.4
Bac. agglomeratus	7.7±0.1	9.8±0.2	13.5±0.2	7.5±0.1
Bac. cereus	7.1±0.2	8.5±0.2	10.2±0.1	7.0±0.2
Bac. asterosporus	4.1±0.1	4.3±0.1	3.2±0.2	3.3±0.2
Bac. virgulus	1.9±0.1	3.6±0.2	3.3±0.1	1.7±0.2
Bac. mesentericus trevisan	1.0±0.1	1.3±0.1	1.9±0.1	1.3±0.1
Bac. mesentericus niger	Not observed			
Typical chernozem				
Bac. megatrium	58.1±0.3	72.7±0.4	59.6±0.3	45.1±0.2
Bac. idosus	30.3±0.2	39.8±0.3	30.9±0.2	21.5±0.2
Bac. agglomeratus	1.1±0.1	1.8±0.2	4.0±0.2	1.7±0.1
Bac. cereus	7.9±0.2	13.5±0.3	16.6±0.3	10.4±0.2
Bac. asterosporus	3.1±0.1	4.0±0.1	4.8±0.1	3.3±0.1
Bac. virgulus	2.9±0.1	7.1±0.1	7.0±0.2	4.4±0.1
Bac. mesentericus trevisan	10.5±0.1	11.2±0.2	15.3±0.2	7.5±0.1
Bac. mesentericus niger	Not observed			

Soil analysis characterizing chemical properties were carried out using conventional methods [15, 16]. Microbial community was characterized using direct microscopic examination [17–19].

RESULTS AND DISCUSSION

The data on the change in the number of non-spore-forming bacteria and bacilli in sod-podzolic and typical chernozems depending on the oil dose were collected under the conditions of model experiment. Dynamics of the change of microbiocenosis after the oil pollution were monitored over 8 weeks**.**

The obtained results showed the increase in the total number of bacteria at the highest oil concentration (10 L/m^2) for both types of the soil. In the case of sod-podzolic soil, the number of bacteria was increased up to the oil dose of 5 L/m^2, and then sharply dropped (Table 2). In the case of typical chernozem, the same trend was observed. However, the decrease in the number of bacteria under the oil dose of 10 L/m^2 was not so prominent as in the former case (Table 2).

Such increase in the total number of bacteria in two different types of soils could be apparently explained by the increase in the amount of nutrients as well as the shift in the pH to neutral values [9, 10, 20–23]. For example, pH value (H_2O) in sod-podzolic soil at the highest oil dose was increased from 4.2 to 5.7; for typical chernozem, that value was increased from 6.4 to 6.8 (Table 1).

The transformation of the bacillar population in the soil accompanied the main trend of the change in the total number of bacteria under pollution with oil (increasing at low doses because of the appearance of nutrient medium, and then decreasing). The development of certain species and the suppression of other ones occurred. For example, *Bac. idosus* and

Bac. megaterium dominated in genus *Bacillus* in the investigated two types of soils.

Different species of bacteria genus *Bacillus* could be arranged in the following series according to the abundance in non-polluted sod-podzolic soil: *Bac. idosus* = *Bac. megaterium* > *Bac. agglomeratus* = *Bac. cereus* > *Bac. asterosporus* > *Bac. virgulus* = *Bac. mesentericus trevisan.*

The number of *Bac. megaterium* exceeded that of *Bac. idosus* in the polluted sod-pozolic soil (oil doses 5 and 10 L/m^2).

In the case of typical chernozem the following series were found:

(1) non-polluted soil: *Bac. megaterium* > *Bac. idosus* > *Bac. mesentericus trevisan* > *Bac. cereus* > *Bac. asterosporus* = *Bac. virgulus* > *Bac. agglomeratus;*

(2) polluted soil: *Bac. megaterium* > *Bac. idosus* > *Bac. cereus* > *Bac. mesentericus trevisan* > *Bac. virgulus* > *Bac. asterosporus* > *Bac. agglomeratus.*

The obtained dependences confirmed the previously published data that oil pollution which is a nutrient substrate led to the increase in the number of hydrocarbon-oxidative bacteria in the structure of soil microbial community, enhancing the domination of certain taxons [24–28]. Moreover, that effect became more prominent with the increasing oil concentration.

CONCLUSIONS

Soil pollutions with oil and oil products led to significant changes in the composition of soil biota: quantitative and qualitative changes were observed in the structure of bacterial communities. The presence of oil in the soil provided the dominant growth of hydrocarbon-oxidative bacteria including the ones of genus *Bacillus* and suppression of sensitive species. Moreover, the number of certain taxons was increased even among stable microorganisms under high pollution level on the background of the decrease in the total number of bacteria. Such heterogeneity in the structure of soil microbiocenosis might be a base for the diagnostics of pollution level and the development of rehabilitation methods for oil-polluted soils.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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