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SOIL BIOLOGY

Changes in the Properties of Oily Gray Forest Soil during Biological Reclamation

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Abstract—Biological reclamation of gray forest soil severely contaminated by separator oil using Bacispecin was demonstrated. Agrophysical, agrochemical, physicochemical, and biological properties of the soil were restored in the plow layer during the reclamation.

Exploitation of oil deposits in the Republic of Bashkortostan for 20–60 years resulted in soil pollution and degradation; as a consequence, considerable areas of fertile lands were excluded from utilization, crop yield as well as forests and grassland productivity decreased, and subsoil water was polluted.

Presently, the level of oil industry development does not allow us to exclude its impact on the environment; hence, there is a need for development of techniques for reclamation of oil-polluted soil. Considering variable soil and climatic conditions, physicochemical properties of extracted oil, and the cost of reclamation, the search for optimal techniques adapted to particular conditions remains actual.

Biological reclamation of oil-polluted soil is finding wide use; it relies on application of various biological agents including microbial destructors of oil and stimulators of aboriginal hydrocarbon-oxidizing microflora (Dyadechko *et al*., 1990; Kireeva, 1994; Stabnikova *et al*., 1996; Sidorov *et al*., 1997; Ponomareva *et al*., 1998; Kapotina and Morshchakova, 1998; Alekseeva *et al*., 2000). These publications evaluate soil restoration by the degree and rate of petrochemical destruction, phytotoxicity, and crop yield; while complex studies on the changes in soil properties, that define its fertility and agroecological properties are not numerous (Abzalov *et al*., 1988; Demidienko and Demurdzhan, 1988; Ismailov *et al*., 1988).

The aim of this work was to study biological restoration of separator oil-polluted forest gray soil using a biological agent and nutrient additives stimulating aboriginal microflora as well as changes in agrophysical, agrichemical, physicochemical, and biological properties of the soil during reclamation in the field.

MATERIALS AND METHODS

Studies were conducted on forest gray soil. Experimental oil pollution was carried out using separator oil from the Arlanskoe Oilfield; the initial oil content was 18%. Since application of biological agents is inefficient for pollution levels exceeding 15–20% (Borzenkov and Pospelov, 2001), we added peat as an adsorbent and plowing was carried out. Then the soil was treated with biological agent Bacispecin (dry granular powder) at 100 g/m^2 (Andreson *et al*., 1994). At the same time, the process of biological restoration is often limited by unfavorable factors such as insufficient aeration and moistening or low concentration of the biogenic elements. Hence, we complemented Bacispecin with nutrient additive Biotrin containing up to 40% raw protein as well as micro- and macroelements, amino acids, and vitamins (20 g dry weight per m^2 soil) and watered oil-polluted soil at 10 l/m^2 with a solution of mineral fertilizer diammophos (0.5 g/l).

Population dynamics of the main microbial groups involved in biological transformation of petrochemicals was studied by inoculation of a soil suspension on solid media (Zvyagintsev, 1980). Abundance of hydrocarbon-oxidizing microorganisms (HOMs) on Tsukamura medium with sterile oil from the Arlanskoe Oilfield as a carbon source was recorded.

The content of petrochemicals was determined by gravimetric method after hydrocarbon extraction from soil with hot hexane using a Soxhlet apparatus (*Sovremennye metody*..., 1984).

Agrochemical and physicochemical indices of soil were determined as described elsewhere (Arinushkina, 1970; *Agrokhimicheskie metody*..., 1975). Total carbon in the soil was determined by the method of Tyurin; ammonia nitrogen, by the method of Bochkarev and

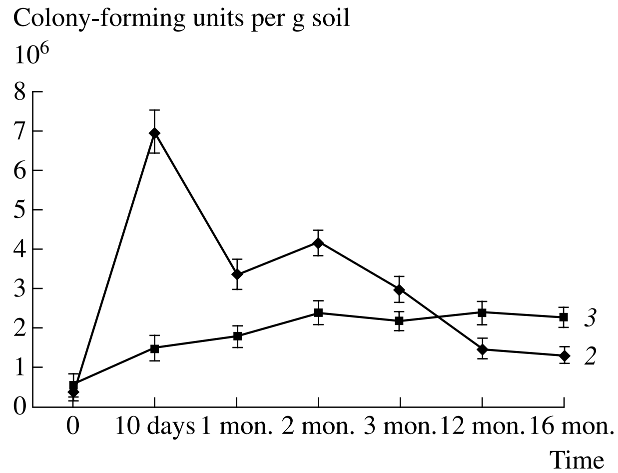

Fig. 1. Changes in the abundance of hydrocarbon-oxidizing microorganisms during reclamation of oily forest gray soil (horizon A_p 0–20 cm). Curve numbers corresponds to experimental variants (see the *Materials and Methods*; for Figs. 1–4).

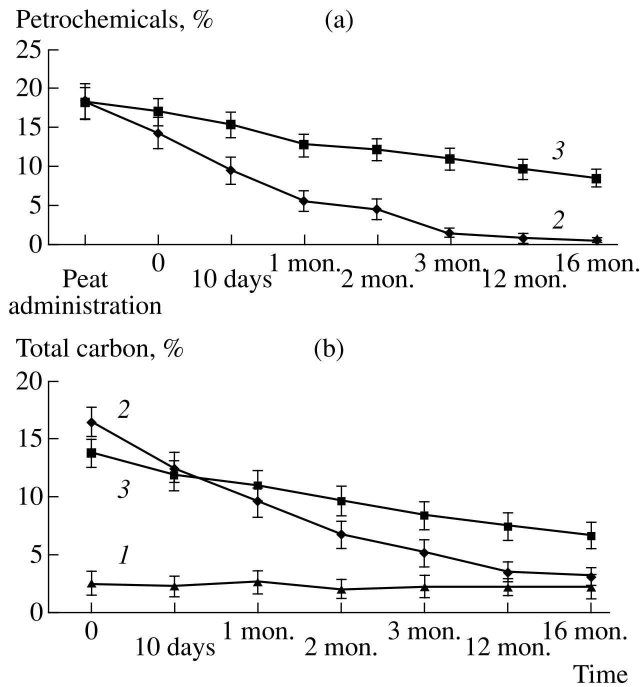

Fig. 2. Changes in the content of petrochemicals (a) and total carbon (b) during reclamation of oily forest gray soil.

Kudeyarov; available phosphorus, by the method of Chirikov; pH of aqueous and saline suspensions, by potentiometry; exchangeable Ca and Mg, by complexometry.

Humidity of capillary bond opening, water absorption capacity, and maximum capillary capacity were calculated from water retention curves as described by Voronin (1986). Soil aggregate size distribution was determined by the method of Savvinov modified by Baksheev; soil moisture content, absolute and capillary moisture capacity, and bulk density were determined by the standard methods (Vadyunina and Korchagina, 1986). Specific surface area was calculated from hygroscopic capacity as described by Pakshina (1997).

Enzyme activities were assayed according to Galstyan (urease and dehydrogenase) and Khaziev (invertase) (Khaziev, 1990).

Experimental variants: (1) background forest gray soil; (2) oily forest gray soil + Bacispecin + Biotrin + diammophos; and (3) oily forest gray soil.

Experiments were conducted in four replicates. The obtained data were statistically processed (Dmitriev, 1995), tables and plots present average values.

RESULTS AND DISCUSSION

Our experiments demonstrated that plowing oily forest gray soil with introduced peat decreased oil content by 5% and established conditions for successful application of the biological agent.

HOMs are an important ecotrophic group of microorganisms involving fully reduced hydrocarbon molecules into matter circulation in various biotopes. In particular, they play an important role in degradation of petrochemicals in soil and aquatic ecosystems. That is why, increasing functional activity of HOMs in various biotopes is of considerable practical importance and is directly linked to restoring natural fertility of oily soil (Kireeva, 1994).

Addition of the biological agent and nutrient additives sharply increased the abundance of HOMs. After 10 days, the abundance of HOMs in the oily soil increased 2.7 times, while that in the Bacispecin variant increased 19 times. In the following months, microbial abundance significantly varied but never exceeded the explosion level after biological agent application. A considerable decrease in the abundance of HOMs was observed by the end of the experiment, which can be attributed to a significantly decreased concentration of the hydrocarbon substrate (Fig. 1).

The content of organic matter including petrochemicals and total carbon decreased according to the microbial abundance and activity. The content of petrochemicals in the experimental variant gradually decreased and 87.9% of oil was degraded by the end of the vegetation season. Soil purification to the admissible levels occurred by the end of next vegetation season, when total carbon content was similar to the background. Within this period, 52.2% petrochemicals was degraded in the untreated soil and total carbon content was by 4.5% higher than the background (Fig. 2).

Table 1. Structural composition of forest gray soil after reclamation, %

Horizon, depth, cm		Fraction size, mm								Coefficient of structuring	Water-stable aggregates >0.25 mm
		>10	10–7	7–5	5–3	3–1	1–0.5	0.5–0.25	<0.25		
1. Background of forest gray soil											
Ap	0–20	0.6	3.6	6.8	12.6	17.5	11.8	27.7	19.4	4.0	46.3
C	70–80	2.1	9.3	12.0	18.4	19.6	8.4	13.4	16.8	4.3	50.3
2. Oily forest gray soil + Bacispecin + Biotrin + diammophos											
Ap	0–20	8.1	6.1	6.7	10.4	14.1	20.3	21.8	12.5	3.9	76.4
C	70–80	53.0	12.5	9.2	10.0	8.2	2.8	2.4	1.9	0.8	81.2
Oily forest gray soil											
Ap	0–20	45.1	12.7	8.9	9.8	8.0	4.0	6.2	5.3	1.0	86.0
C	70–80	39.0	13.6	9.0	9.3	7.3	5.0	10.2	6.6	1.2	84.4

Table 2. Aquatic physical properties of forest gray soil after reclamation

Horizon, depth, cm		Bulk density, g/cm^3	Soil humidity at the moment of bulk density determination, %	Moisture content, %		Coefficient of filtration, mm/min
				capillary	total	
1. Background of forest gray soil						
Ap	0–20	1.31	16.2	29.8	33.9	0.02
C	70–80	1.49	19.1	23.6	25.9	0.21
2. Oily forest gray soil + Bacispecin + Biotrin + diammophos						
Ap	0–20	1.17	41.6	42.4	44.9	0.06
C	70–80	1.55	18.1	20.7	25.5	<0.001
3. Oily forest gray soil						
Ap	0–20	1.50	22.1	24.2	24.8	<0.001
C	70–80	1.36	22.2	23.9	26.2	<0.001

Morphological analysis of oily forest gray soil demonstrated the color (from dark gray to black) and strong smell of soil horizons typical for oil. Lumpy–powdery structure of the plow layer with fresh oil pollution was transformed to loose granular one. The bulk density of the soil increased. The maximum depth of oil penetration was 125 cm, which covered illuvial horizons and the transition to the soil-forming ones.

Eventually, agrophysical properties of the soil sharply changed. The proportion of the globular fraction increased up to 75 times (Table 1). The coefficient of soil structuring decreased up to four times, while the coefficient of water stability increased almost twice. The proportion of water-stable aggregates reached 90% in the polluted soil, the bulk density increased, while the moisture opacity and water permeability decreased (Table 2).

Table 3. Pore diameter and ratio (according to the water retention curves) in forest gray soil after reclamation

Horizon, depth, cm		Pores, %		Diameter of drainage pores, mm
		Water retention	drainage	
1. Background of forest gray soil				
Ap	0–20	44.8	55.2	0.0027
C	70–80	44.1	55.9	0.0022
2. Oily forest gray soil + Bacispecin + Biotrin + diammophos				
Ap	0–20	41.7	58.3	0.0023
C	70–80	43.1	56.9	0.0019
3. Oily forest gray soil				
Ap	0–20	48.0	52.0	0.0017
C	70–80	48.5	51.5	0.0018

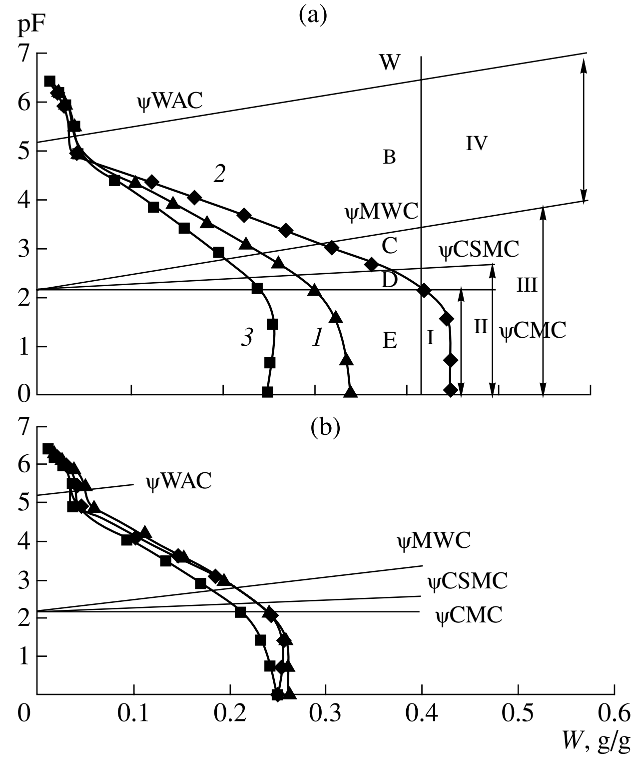

Fig. 3. Soil moisture opacity in the plow (a) and illuvial (b) horizons of oily forest gray soil after reclamation; WAC, water absorption capacity; MWC, molecular water capacity; CSMC, capillary–sorption moisture capacity; CMC, capillary moisture capacity; A, firmly absorbed pellicular water; B, loosely pellicular water; C, pellicular–capillary water; D, capillary water; E, capillary–gravitational water; I, infiltration pores; II, aeration pores; III, drainage pores; IV, water retention pores; Ψ, soil water potential.

The water retention curves demonstrate that oil pollution decreased soil capacity to retain all water categories (Fig. 3). In the plow layer, the area of water transition from firmly absorbed to loosely pellicular (pF = 5) decreased to a lower extent (by 11%), while the areas of water transition from loosely pellicular to pellicular–capillary decreased more (by 23.5%); the potential of capillary water (by 26.8%) and capillary–gravitational water (pF < 2.18) decreased most. This indicates considerable disturbances in accessibility of drainage pores, particularly, infiltration and aeration pores, for water. Decreased volume of drainage pores is also confirmed by a decrease in their diameter and proportion calculated from water retention curves (Table 3).

In the illuvial horizons, the finest water retention pores in the transition areas of firmly absorbed and loosely pellicular water are disturbed most (by almost 30%); the ranges of pellicular–capillary and capillary water decreased by 20 and 10%, respectively. This horizon also features a less pronounced decrease in the diameter of drainage pores and in their proportion in total pore space. These data indicate that heavy oil fractions are largely retained in the plow layer filling the largest infiltration and aeration pores and drainage pores in general. Light oil fractions penetrate to the underlying soil profiles and are largely retained in the illuvial horizon and basically fill fine water retention pores. This illustrates a chromatographic effect of the soil profile so that the plow and illuvial layers serve as geochemical barriers for heavy and light oil fractions, respectively.

Reclamation decreased the content of petrochemicals in the plow layer to 0.4%. The aggregate size distribution of the plow layer also changed: the content of the globular separates decreased by 23.8% while the content of the 3–1 mm fraction increased by 4.8%.

This soil also demonstrated a decrease in bulk density, a considerable increase in all moisture capacity indices, and an increase in soil moisture potential. The plow layer became water-permeable, while water permeability was nearly absent in the illuvial layer (70–80 cm) where anaerobic conditions inhibited the microbial activity and the content of petrochemicals remained high. Optimization of aquatic physical conditions in the upper layer of reclaimed soil is clearly due both to the destruction of petrochemicals and mixing of the plow layer with the remains of peat used for free oil absorption immediately after the pollution.

In the plow layer, the potential corresponding to the capillary–sorption moisture capacity was restored most, i.e., oil destruction was most active in the aeration pores. In the illuvial layer, at the background of insignificantly decreased concentration of petrochemicals, molecular water capacity and capillary–sorption moisture capacity remained constant, while the water absorption capacity considerably increased and was even higher than the background. This suggests going of two main processes, accelerated destruction of light oil fractions on the one hand, and their downward movement under gravity on the other hand.

The quality of oil provided for an insignificant change in pH of the polluted soil (Table 4). Application of the biological agent and fertilizer slightly decreased the pH but it was neutralized and approached the background level with time. Table 4 demonstrates that oil pollution decreased the content of exchangeable bases in the soil, particularly, calcium, which was due to encapsulation of soil colloids in an oil film. Their content increased along with oil degradation and corresponded to the unpolluted soil in the Bacispecin variant by autumn of the second year.

Pollution had a considerable impact on the nutrient status of forest gray soil. The content of available nitrogen forms decreased 3.5–5 times larger the at the expense of nitrate. Ismailov (1988) proposed that a fraction of ammonia formed by ammonification bypassing nitrification is readily absorbed by the soil

Table 4. Dynamics of physicochemical and agrichemical properties of forest gray soil during reclamation (horizon Ap 0–20 cm)

Time, months	pH		Ca^{2+}	Mg^{2+}	Σ	N–NH_4	N–NO_3	Σ	Available P_2O_5, mg/100 g soil
	H_2O	KCl	mgEq/100 g soil			mg/kg soil			
1. Background of forest gray soil									
0	6.8	5.6	22.0	7.0	29.0	11.6	19.7	31.3	9.5
10 days	6.5	5.7	19.0	10.0	29.0	30.9	48.9	79.8	11.6
1	6.9	5.9	21.0	9.0	30.0	11.7	16.3	28.0	8.7
2	6.6	5.8	20.0	8.0	28.0	11.9	13.1	25.0	9.5
3	6.9	5.9	22.0	8.0	30.0	4.3	20.5	24.8	11.8
12	6.8	5.8	20.0	10.0	30.0	4.5	22.2	26.7	6.3
16	6.7	5.7	19.0	8.0	27.0	1.3	5.7	7.0	4.3
2. Oily forest gray soil + Bacispecin + Biotrin + diammophos									
0	6.7	5.6	9.0	8.0	17.0	8.0	0.8	8.8	4.3
10 days	5.4	4.7	8.0	6.0	14.0	92.0	13.1	105.1	1.8
1	5.2	4.2	7.0	5.0	12.0	41.5	2.3	43.8	1.5
2	6.2	5.2	10.0	5.0	15.0	14.5	3.2	17.7	5.7
3	6.6	5.3	14.0	5.0	19.0	6.5	1.0	7.5	10.0
12	6.7	5.7	11.0	7.0	18.0	7.5	0	7.5	5.9
16	6.8	5.8	18.0	10.0	28.0	11.2	1.1	12.3	6.9
3. Oily forest gray soil									
0	6.7	5.7	9.0	6.0	15.0	5.2	0.7	5.9	3.1
10 days	6.6	5.6	8.0	5.0	13.0	6.5	1.1	7.6	3.2
1	6.6	5.6	9.0	6.0	15.0	8.4	2.2	10.6	3.7
2	6.5	5.8	8.0	5.0	13.0	14.3	1.6	15.9	6.2
3	6.4	5.8	11.0	5.0	16.0	13.3	2.5	15.8	12.6
12	6.5	5.7	9.0	6.0	15.0	2.9	0	2.9	7.1
16	6.6	5.7	11.0	8.0	19.0	16.4	1.6	18	6.3

absorption complex and, in contrast to nitrates, is not washed out, which explains the predominance of the ammonia nitrogen in oily soils. Application of nitrogen with diammophos together with the biological agent has an absolute significance for soil supply with available nitrogen and for the proportion between nitrogen forms.

Unpolluted forest gray soil has a medium supply with available phosphorus, while it is low or very low in the oily soil. Application of phosphorus with fertilizers did not significantly change phosphorus supply. Phosphorus can be reduced and escape as hydrogen phosphide under disturbed water–air conditions (Demidienko and Demurdzhan, 1988). An increase in phosphorus to the medium level was observed only after degradation of 87.9% oil (Table 4).

Enzyme activity is a sensitive marker of soil pollution level. Oil pollution inhibited invertase and dehydrogenase activities but these activities relatively quickly restored after reclamation (Fig. 4). At the same time, dehydrogenase activity decreased by the end of the experiment; Ismailov (1988) attributed such decrease to toxic effect of the products of hydrocarbon oxidation.

In contrast to these activities, urease activity increased after oil pollution and its dynamics was largely determined by the content of ammonia nitrogen in the soil. High content of this nitrogen form after application of the biological agent and diammophos inhibited urease activity; but it increased by the second month and remained higher than the background to the end of experiment.

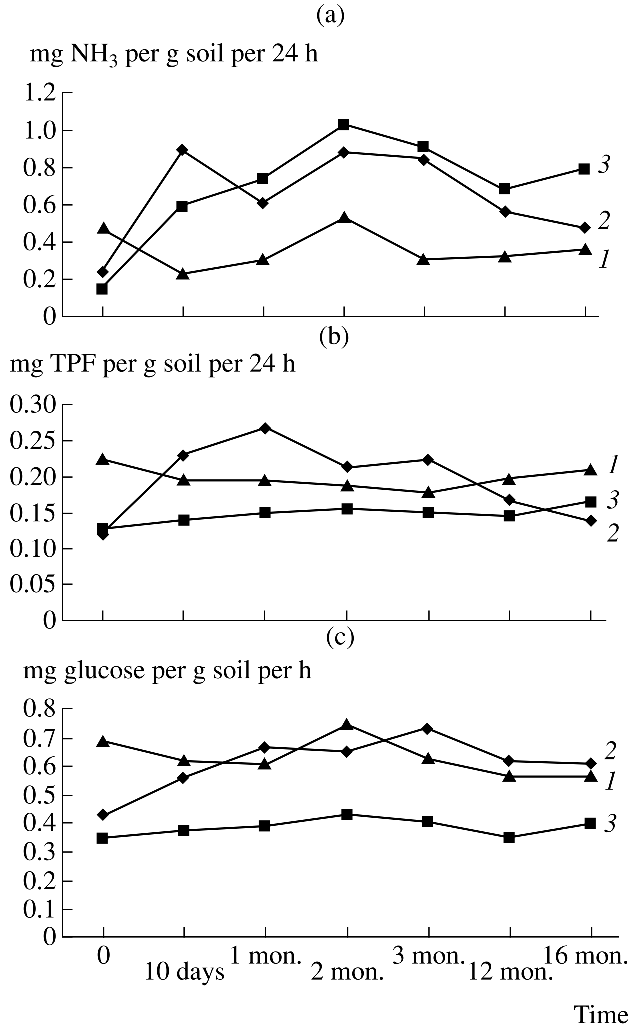

Fig. 4. Changes in enzyme activities of urease (a), dehydrogenase (b), and invertase (c) during reclamation of oily forest gray soil.

Active oil degradation and improving soil quality during biological reclamation favored growth and development of crops. The proportion of the reclaimed field overgrowth with maize and winter rye was 75–80%.

Thus, reclamation treatment including plowing with added peat and application of biological agent Bacispecin with a nutrient additive and mineral fertilizer decreased the concentration of petrochemicals from 18 to 0.4% in the plow layer of forest gray soil. The affected soil properties gradually improved along with oil degradation. Soil structure, bulk density, total and capillary moisture capacities, and soil moisture potential were restored. Excessive drainage of the aggregates decreased. The content of exchangeable bases increases to the level of unpolluted soil. The quantities of nutrient elements, available nitrogen and phosphorus, increased. Reclamation had an ambiguous effect on soil enzyme activities. Dehydrogenase activity decreased, urease activity increased, while invertase activity restored to the level of unpolluted soil.

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