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EXPERIMENTAL ARTICLES

Effect of Oil on the Population, Biomass, and Viability of Fungi in Highmoor Peats

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Abstract—Some microbiological parameters, such as the fungal mycelium length, the number of fungal spores, the distribution profiles of micromycetes, the viability of fungal propagules, the length distribution of micromycete hyphae, and the ratio between fungal spores and yeastlike cells, can be used to determine the degree of soil contamination with oil and the concentration that is inhibitory to micromycete complexes of highmoor peats.

Key words: oil, highmoor peats, direct method, microbial biomass, micromycete viability.

Oil and its products are abundant and recalcitrant pollutants of the environment. The self-cleaning of soils, waters, and other environments from oil contamination takes as long as several tens of years, depending on the surrounding conditions [1, 2].

An important role in the self-cleaning of oil-contaminated environments is played by microorganisms, which promote the degradation of oil hydrocarbons [3–5].

The response of a microorganism to any toxic compound depends on the origin of this compound, its concentration, the time of contact, the physiological state, and other properties of the microorganism.

The light fraction of oil partially inhibits heterotrophic microorganisms, but they can be metabolized by hydrocarbon-oxidizing bacteria. On the other hand, heavy oil fractions are less inhibitory to microorganisms, but they are poorly metabolizable. Moreover, heavy oil fractions drastically increase the hydrophobicity of soils and thus strongly suppress soil biota [6]. Along with the deterioration of the physical properties of soil, oil hydrocarbons also affect its chemical, agronomic, and other characteristics.

The response of soil biota to oil contamination is not uniform. General changes in the taxonomic composition of the microbial communities of oil-contaminated soil can be summarized as follows [7–12]:

(1) an increase occurs in the population densities of the specialized microorganisms capable of oxidizing gaseous hydrocarbons, solid paraffins, and aromatic hydrocarbons (bacteria of the genera *Arthrobacter, Bacillus, Brevibacterium, Nocardia, Pseudomonas, Rhodococcus*, and the asporogenous yeasts of the genera *Rhodotorula, Rhodosporidium, Sporobolomyces, Torulopsis*, and *Trichosporon*);

(2) the section diversity of soil actinomycetes declines;

(3) the species diversity of indigenous microscopic fungi declines, but new species of microscopic fungi simultaneously appear;

(4) the degree of domination of various indigenous amylolytic species changes, and new oil-resistant, amylolytic species appear.

It should be noted that data available in the literature on the effect of oil contamination on the abundance of bacteria, actinomycetes, and microscopic fungi in soil microbial communities are very contradictory [7–9, 11, 13]. The abundance of various soil microorganisms is usually determined by their plating on solid media. However, it is known that the enumeration of microorganisms on plates gives incomplete and often ambiguous results, since a restricted number of nutrient media are used for the growth of microorganisms that possess very different nutritional requirements. Therefore, data on the total population of soil microorganisms obtained by the plating method are not reliable and cannot be used for estimating the degree of oil contamination and its detrimental effect on the soil biota.

This calls for a direct count of microorganisms for the aforementioned purposes. Due to their metabolic diversity, soil bacteria are the main degraders of oil hydrocarbons. On the other hand, fungi dominate in soil microbial communities; therefore, their abundance in soil is a more reliable index of its contamination than the abundance of soil bacteria [14]. In view of this, the monitoring of fungal biomass seems to be a promising approach to controlling the restoration of the soil ecosystems altered by oil contamination. The similarity between the total and viable cell count procedures

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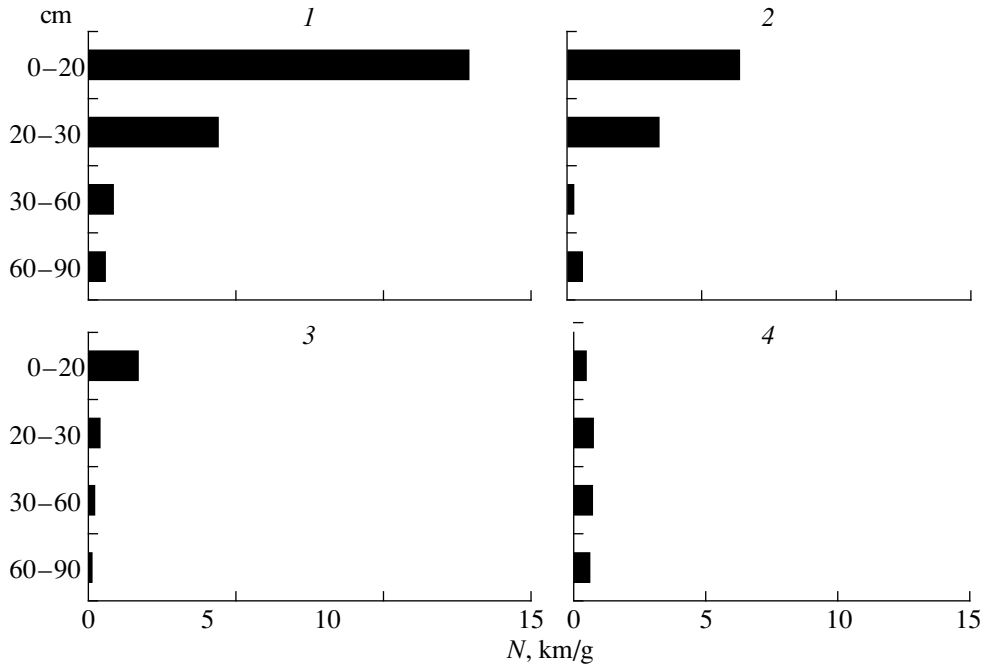

Fig. 1. Depth distribution of the fungal mycelium length in (*1*) control (unpolluted) peat deposit, (*2*) peat deposit situated on the periphery of the oil spill that took place in March 1999, (*3*) peat deposit situated close to the center of the same oil spill, (*4*) peat deposit situated close to the center of the oil spill that took place in 1994, and (*5*) peat deposit polluted as a result of the 1998 oil spill and recultivated by covering with a 70-cm layer of sand and 20-cm layer of imported peat.

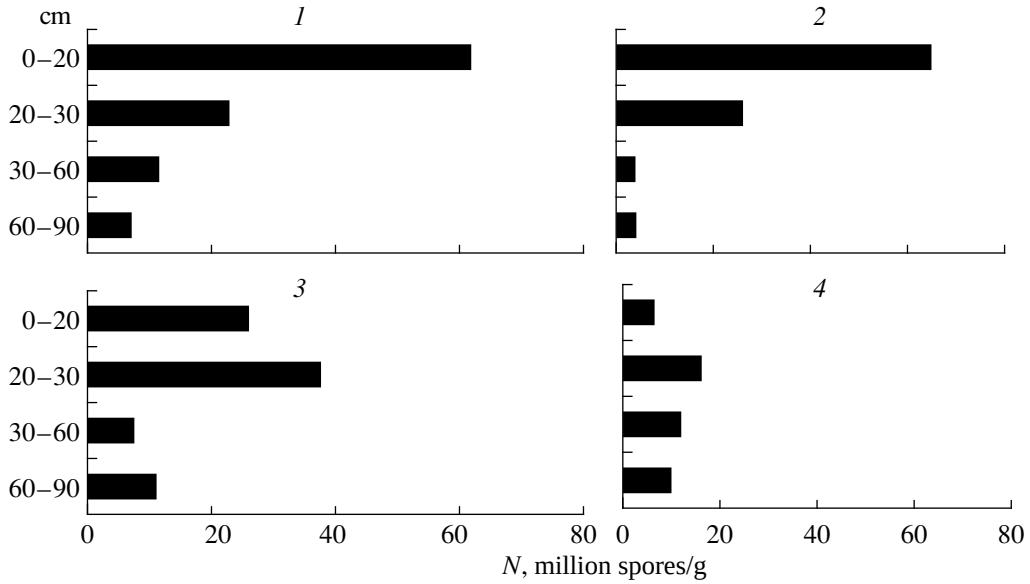

Fig. 2. Depth distribution of the total number of fungal spores and yeastlike cells in the peat deposits indicated in the legend to Fig. 1.

allows the representative data on the structure of fungal communities in unimpacted and oil-contaminated soils to be determined [15].

The scarce relevant data available in the literature were obtained from the luminescence microscopic studies of the upper horizons of intrusive soils contaminated with oil and residual fuel oil. These studies showed a reduction of the length of fungal mycelium and an increase in the number of fungal spores and yeastlike cells with respect to the control (unpolluted) soil sample [16].

The aim of the present work was to estimate the effect of oil contamination on the fungal complex of highmoor peats using the direct microbial count method.

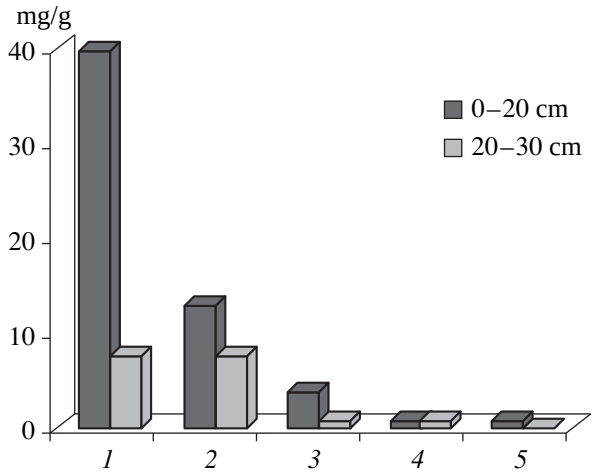

Fig. 3. Fungal biomass in the upper layers of the peat deposits indicated in the legend to Fig. 1.

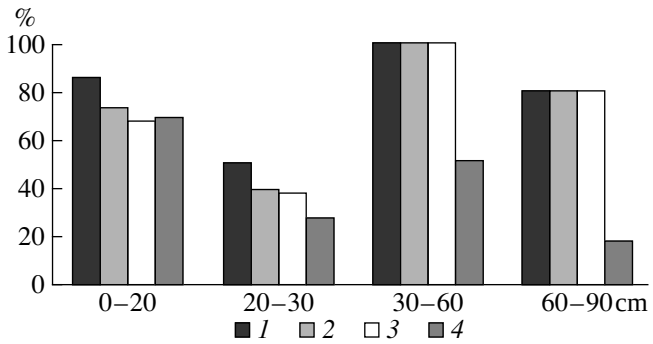

Fig. 4. Percentage of viable micromycete hyphae in the various layers of the peat deposits indicated in the legend to Fig. 1.

MATERIALS AND METHODS

Experiments were performed on peat samples with different degrees of oil contamination. The samples were taken from five highmoor peat deposits situated in the Bystrinskoe oil field (Tyumen oblast, Surgut region, Russia). Sample descriptions are as follows:

(1) control (uncontaminated) peat deposit;

(2) peat deposit situated on the periphery of the oil spill that occurred in March 1999;

(3) peat deposit situated close to the center of the March 1999 spill;

(4) peat deposit situated close to the center of the oil spill that occurred in 1994;

(5) peat deposit polluted as a result of the 1998 oil spill and subjected to recultivation by covering it with a 70-cm layer of sand and 20-cm layer of imported peat.

The peat deposits were sampled in September 1999. Peat samples were taken from the upper 90-cm layers of 2 to 2.5 m thick peat deposits and stored in a refrigerator until analysis. The total number and the biomass of fungi were determined using the method of luminescence microscopy [17]. The samples were preliminarily treated with ultrasound using a UZDN-1 disintegrator (Russia). The fungal mycelium and spores were stained with calcofluor white [18]. The biomasses of fungal spores and mycelium in the peat samples were calculated using the following formulas that express, respectively, the masses of one spore and 1 m of mycelium: $0.083r^3 \times 10^{-11}$ g and $0.628\ 0.628r^2 \times 10^{-6}$ g. Here, r is the mean radius of spores or mycelial hyphae [19].

The viability of spores and hyphae were determined as described earlier [15].

RESULTS AND DISCUSSION

The effect of oil on the fungal mycelium density was most pronounced in the upper horizons (0–20 cm deep) of the peat deposits studied. The content of fungal mycelium in oil-contaminated peats declined with increasing oil contamination in the peat deposits situated on the periphery of the oil spills (the decline was twofold with respect to the control peat). The decline was four- to eightfold in the peat deposits situated close to the centers of the oil spills. The amount of fungal mycelium also decreased with increasing time of contact with the pollutant: the decline was two- to fourfold in the recently polluted peat deposits and eightfold in the peat deposit contaminated in 1994.

In the lower layers (20–30 cm deep), the growth of fungal mycelium was inhibited only in the peat deposits situated close to the oil spill centers (Fig. 1). The depth distribution of fungal mycelium in the recently contaminated peat deposits was almost the same as in the control peat deposit. There was a two- to fourfold reduction in the mycelium length at depths of 20–30 cm and a tenfold reduction at depths of 30–90 cm. In the peat deposit contaminated in 1994, the content of fungal mycelium was low (0.3–0.6 km/g) in both the upper and lower horizons, suggesting that the detrimental effect of oil contamination is not mitigated over the course of time.

Fungal spores showed similar depth distribution patterns and high sensitivity to oil contamination in the upper horizons of peat deposits 3 and 4 (Fig. 2). The content of fungal spores between 20- and 30-cm depths was high. As noted above, this layer is characterized by the low content of mycelium. This is obviously a specific response of micromycetes to varying environmental conditions caused by the contamination of the upper horizons of peat deposits by oil.

Thus, oil contamination greatly affected the micromycete population of the upper horizons of oligotrophic peat deposits. The decline in the micromycete population of these horizons was almost proportional to the degree of contamination. A tolerable degree of contamination probably exists on the periphery of the 1999 oil spill, since the content and depth distribution of the

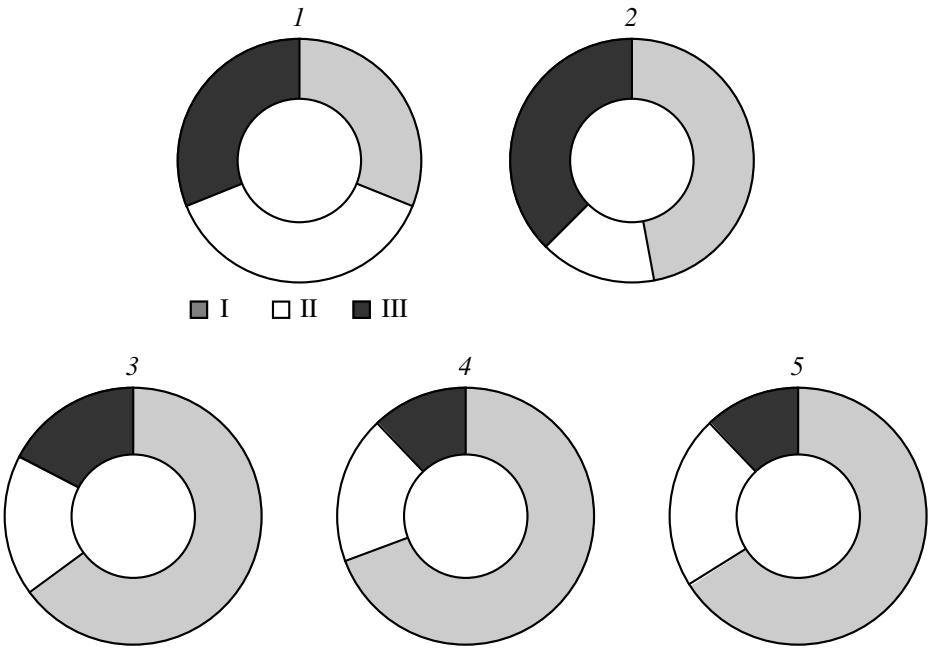

Fig. 5. The proportion between different classes of micromycete hyphae in the upper layers of the peat deposits indicated in the legend to Fig. 1. The classes are listed in the legend to Fig. 6.

micromycete mycelium and spores there are close to those in the control peat deposit.

Data on the fungal biomass content of the upper horizons of peat deposits are presented in Fig. 3. In the uppermost 20-cm layer, the content of fungal biomass declined with increasing oil contamination and with increasing time of contact with the pollutant. The decline was threefold (with respect to the control peat) in the peat deposit situated on the oil spill periphery, 10- to 40-fold in the peat deposits situated close to the oil spill center, three- to tenfold in the peat deposit polluted in 1998, and 40-fold in the peat deposit polluted in 1994. The lowest fungal biomass (0.5 mg/g) was revealed in the imported peat used to recultivate the oil-contaminated peat deposits. Conditions in this layer were obviously far from being favorable for fungal growth.

In a deeper layer located 20 to 30 cm from the surface, the fungal biomass responded to only severe oil contamination and did not depend on the time of contact with the pollutant (Fig. 3). The fungal biomass in the peat deposits 3 and 4 decreased eightfold as compared with the control.

Of great interest is the effect of oil contamination on the viability of fungal propagules, which determines, in particular, the rate of fungal implication in the recultivation of polluted peat deposits. The fraction of viable fungal hyphae in the uppermost 20-cm layer of oil-contaminated peat deposits was 12–20% lower than in the control peat deposit. In the lower layers of recently contaminated peat deposits, the viability of micromycete hyphae only slightly decreased. However, the viability of fungal hyphae in the 5-year contaminated peat deposits decreased by 50% in the layer between 30- and 60-cm depths, and by 17% between 60- and 90-cm depths (Fig. 4).

The distribution of micromycete hyphae over five classes, I–V, characterized by hyphal lengths 0–30, 30–60, 60–90, 90–120, and >120 μm, respectively, is shown in Fig. 5. In the upper layer of the control peat deposit, the hyphae of classes II, III, and V are equally abundant. In the upper layers of oil-polluted peat deposits, there is a tendency for an increased amount of class II hyphae. The lowest content was observed for the class IV hyphae in the peat deposit polluted in 1994 and in the imported peat of the recultivated peat deposit (Fig. 5).

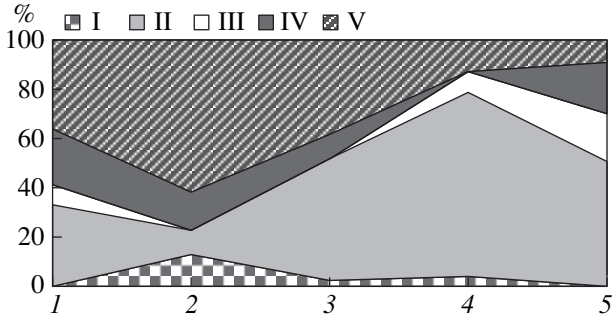

Fig. 6. The proportion between different classes of micromycete hyphae as determined in germination experiments in the upper layers of the peat deposits indicated in the legend to Fig. 1. The hyphae are divided into five classes, I, II, III, IV, and V, corresponding to hyphal lengths 0–30, 30–60, 60–90, 90–120, and >120 μm, respectively.

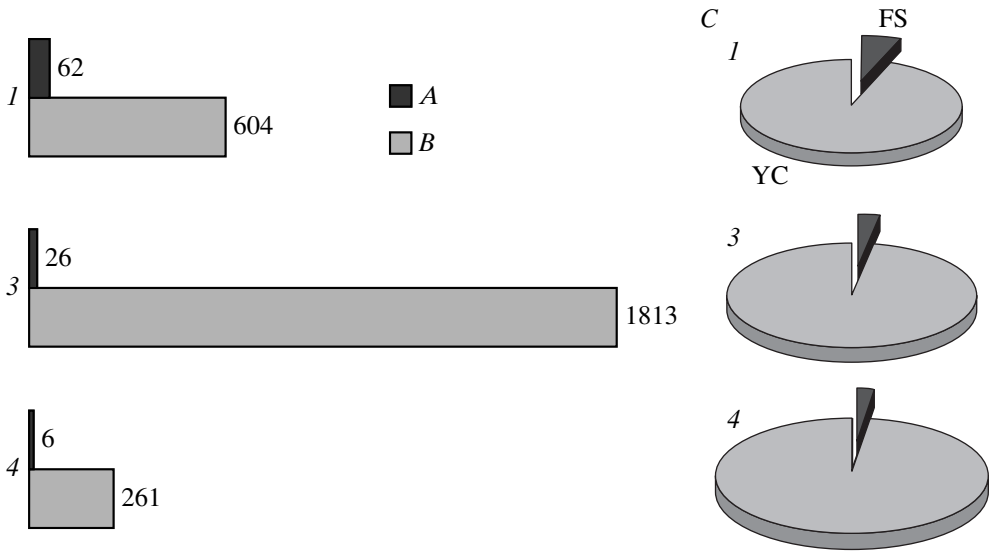

Fig. 7. The number of fungal spores (FS) and yeastlike cells (YC) (the data are expressed as millions/g) in (*A*) control peat samples, (*B*) peat samples subjected to germination, and (*C*) proportion between the spores and yeasts in the upper horizons of the peat deposits indicated in the legend to Fig. 1.

The viability of mycelial fragments was confirmed in germination experiments, which showed that the germination of mycelial fragments present in the control peat deposit and on the oil spill periphery led to an increase in the proportion of the long hyphae of classes IV and V. At the same time, class II hyphae dominated in all samples taken from the oil-contaminated peat deposits located close to the oil spill centers (Fig. 6). Therefore, fungal propagules grew poorly in samples taken from these peat deposits.

Thus, oil contamination primarily affected the germination rate of the medium-length fungal propagules of class II. The detection of the hyphal fragments of class II (the germ tubes of spores) and of classes IV and V (the germinated short fragments of fungal mycelium) in the peat deposits situated close to the oil spill centers shows that micromycetes remain viable even there.

The viability of fungal spores was high in all peat deposits, irrespective of the degree of contamination. In our experiments, we distinguished germinating fungal spores (which possess germ tubes) and germinating yeastlike cells (which possess buds). As a result, a great number of yeastlike cells and a small number of fungal spores were revealed in all of the samples of highmoor peats. This was not surprising, since the excess moisture and low values of pH, temperature, and degree of degradation, which are typical of highmoor peats, promote the growth of yeasts [20].

The increase in the total number of germinated fungal spores and yeastlike cells in peats samples taken from the upper layers of the control peat deposit (10-fold increase), from the peat deposit contaminated with oil in 1994 (40-fold increase), and from the peat deposit contaminated in 1999 (70-fold increase), was due to the reproduction of yeastlike cells rather than fungi (Fig. 7). The same tendency was observed in the lower layers of the last two peat deposits. Thus, oil contamination beneficially influences the population of zymogenous microorganisms and decreases the number of micromycete spores in highmoor peats.

Analysis of some relevant microbiological parameters, such as the fungal mycelium length, the number of fungal spores, the depth distribution of micromycetes, the viability of fungal propagules, the length distribution of micromycete hyphae, and the proportion between fungal spores and yeastlike cells, can be used to estimate the inhibitory effect of oil on the microbial communities of oligotrophic peat deposits.

The severe contamination of such peat deposits with oil is associated with:

(1) a more than tenfold decline in the fungal mycelium length, in the number of fungal spores, and in the fungal biomass of the upper layers of the peat deposits;

(2) a decrease in the content of viable fungal hyphae in the upper and lower layers;

(3) an increase in the proportion of short hyphae (30–60 μm in length) in the upper layers;

(4) growth of the zymogenous microorganism population in all layers;

(5) characteristic changes in the depth profile of micromycetes.

Peat deposit 4, situated close to the center of the oil spill that occurred five years before the study, was affected the most. Peat deposit 2, situated on the periphery of the oil spill that occurred 0.5 years before, was impacted the least.

In our opinion, the state of the micromycete population in oil-contaminated peats serves as a reliable indication of ecological disorder. Earlier, we arrived at the

same conclusion when studying the effects of recultivation [15]. For estimating the tolerable concentration of oil in soils, various chemical, physical, and microbiological parameters of soils with respect to the degree of oil contamination should be studied.

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