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Features of the functioning of microbiocenoses of cultivated soddy-podzolic soil under conditions of oil pollution

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Abstract. The study goal was to explore the characteristics of the functioning of microbiocenoses of well-cultivated soddy-podzolic soil, contaminated with doses of oil under the conditions of a field experiment. The level of initial pollution with petroleum products was in percentage: 0, 0.5, 1.0, 1.5 and 3.0. Sampling for laboratory research was carried out 14 days, 6 and 18 months after the oil spill. To assess the activity of the microbiota under conditions of soil self-purification from oil pollution, integral indicators were chosen. 1. The amount of carbon dioxide released from the soil, the so-called basal respiration, corresponds to the complete destruction of oil (to CO2 and H2O) by soil microorganisms. 2. The medium-regulating (regulatory) microorganisms' activity, which is defined as the biological response of the soil to a disruption of chemical balance, was assessed by the quantity of CO2 release after 1% glucose addition. The oil products content was defined in real time mode. Soil microbiocenoses were highly perseverant to the inhibitory effects of oil pollution. Disturbances in the normal functioning of the microbiota as a result of oil pollution were non-critical and reversible. Half-year after the oil spill, at all levels of pollution, there was a sharp increase in basal respiration. Its level exceeded the respiration rate of clean soil several-fold. This indicated the high biochemical activity of hydrocarbon-oxidizing resident microbiota and as a result an active self-cleaning of soil from oil pollution. The negative dynamics of the petroleum products content proved the microbial character of the oil destruction processes in the soil.

1 Introduction

One of the most significant indicators in soil biomonitoring is the biochemical activity of soil microorganisms. Soil microorganisms, carrying out the mineralization of organic residues and being the main link in the cycle of nutrients in the biosphere, represent the most important source of soil fertility [1, 2]. Typical indicators of soil microbiological activity are the number of microorganisms, their qualitative composition, biomass and enzymatic activity. However, by the amount of microbiota, its species composition and biomass, it is

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not always possible to determinate the intensity of the processes that it causes [3, 4]. When studying enzymatic activity, it is impossible to limit oneself to identifying only one or two enzymes; it is necessary to determine a significant number of enzymes of different classes. In addition, the soil enzymes' activity is influenced by multiple soil factors, some of which suppress, others, on the contrary, stimulate their action. Therefore, enzymatic activity often does not correlate with microbial functional activity and does not allow assessing the availability of nutrients to plants [5].

Thus, the most important and reliable indicator of the microbiological state of soils is the characteristics of the functional abilities of their microbiocenoses. The functional properties of the microbiome and their diversity support the stability of the ecological balance that has developed in the soil and determine the type of transformation of substances peculiar only to it [6]. The most important function of soil microorganisms is their environment-regulating activity, since microbiocenoses are not only products of the environmental situation, but also active agents in its regulation [7, 8]. In undisturbed natural soils, changes in the ecological situation are associated with seasonal and weather changes. These changes are cyclical and limited. Microorganisms are well adapted to them due to the presence of response mechanisms to external natural influences.

In anthropogenically disturbed soils, microorganisms find themselves in new unfavorable conditions, which can lead to a weakening of their biological reactivity, it means, to a failure of the mechanisms of microbial regulation of the soil environment. This, in turn, will inevitably cause a decrease in the ecological stability of microbe community, and, consequently, disruption of the normal functioning of the soil biogeocenose as a whole. Therefore, when monitoring anthropogenically disturbed soils, it is very important to assess the environment-regulating activity of the microbiota, with the help of which organic and mineral substances entering the soil are transformed and ecological balance is restored.

An integral indicator of the intensity of processes occurring in soils caused by microorganisms is the release of carbon dioxide (soil respiration). This indicator is especially important when studying the self-restoration of oil-contaminated soils. In this case, the destruction of petroleum hydrocarbons occurs solely due to the activity of native oil-oxidizing microorganisms and is always accompanied by the release of carbon dioxide [9].

The amount of CO2 characterizes the process of complete mineralization of oil by microorganisms [10]. Currently, the amount of CO2 produced by soil is considered one of the sensitive indicators of the intensity of oil biodegradation processes [11].

In the scientific literature, against the background of a large number of articles on the ecological state of various oil-contaminated soils, there is practically no information about the microbiological activity of podzolic soils formed in St. Petersburg region and artificially polluted in natural conditions

That's why, our investigation was aimed at finding the peculiarities of the microbial cenoses functioning in soddy-podzolic soil contaminated under natural conditions with various doses of crude oil.

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2 Materials and methods

The target of research was a well-cultivated loamy soddy-podzolic soil on moraine loam. This soil is widespread in the North of the Russian Federation and specifically in Leningradsky District (St. Petersburg region).

The most important properties of the studied soil are presented in the Table 1.

Table 1. Characteristics of cultivated soddy-podzolic soil (AY horizon).

pH_{H2O}	pH_{KCl}	C_{org},%	N_{total},%	C:N	P_2O_5, mg/kg	K_2O mg/kg,
6,8	6,2	2,0	0,16	12,5	11,0	16,1

To study the microbiological state of crude oil-contaminated soil, a microplot field test was carried out in quadruple repetition. Taking into account the relative levelness of the site in terms of soil conditions and in an effort to reduce environmental pollution, the accounting size of the plot was reduced to a minimum. It was 1 m^2.

The previous crop was perennial grasses. Vegetation was removed from the soil before oil contamination. The soil was dug to a depth of 0-20 cm and all roots and plant remains were carefully removed.

Doses of crude oil were poured onto the surface of the arable layer and then evenly distributed to a depth of 0-20 cm. The level of initial contamination with petroleum products for various experimental options was expressed as a percentage: 0; 0,5; 1,0; 1,5 and 3,0.

Soil samples for research were collected three times: at the start and at the end of the first vegetative period and at the end of the second vegetative period (14 days, 6 and 18 months after the oil spill).

To assess the degree of impact of oil doses on the functional activity of the microbiota of the studied soil, the following integral indicators were selected:

1. Basal soil respiration characterizes the total destruction of oil (to CO_2 and H_2O) by microorganisms. This indicator was determined by the CO_2 quantity coming from the soil in laboratory conditions using the adsorption method [12].

2. The medium-regulating activity of microorganisms limits the range of fluctuations in the chemical properties of the soil. To quantify it, we determined the intensity of the biological response of the soil to the addition of 1% glucose [13]. The intensity of the response was determined in laboratory conditions after the addition of energetic material by the quantity of CO_2 released, which was determined over time daily over several days using the adsorption method [12]. An indicator of the medium-regulating activity of microorganisms is the maximum daily level of carbon dioxide release by them after the addition of glucose. A statistically significant decrease in this indicator in oil-contaminated soil compared to the control sample will indicate a decrease in ecological flexibility and resistance to the stressful effects of soil microbiocenoses. Microbiological studies were carried out in freshly collected samples in 4 replicates.

The dynamics of the content of petroleum hydrocarbons in the studied soil was determined by infrared spectrometry.

The results of the studies were processed by mathematical statistics.

3 Results and discussion

The dynamics of the oil products content in soddy-podzolic soil is shown in the Figure 1. The studied soil was characterized by active processes of self-purification from oil. The content of petroleum products decreased especially intensively in variants with pollution doses in the range 1,0-3,0%. The concentration of petroleum hydrocarbons isolated from the soils of these experimental variants decreased on the average by over 30% during the 18 months of observation.

In soil with the minimum dose of contamination 0.5%, there is also a gradual decrease in the content of petroleum hydrocarbons. During the experiment it decreased by 15%.

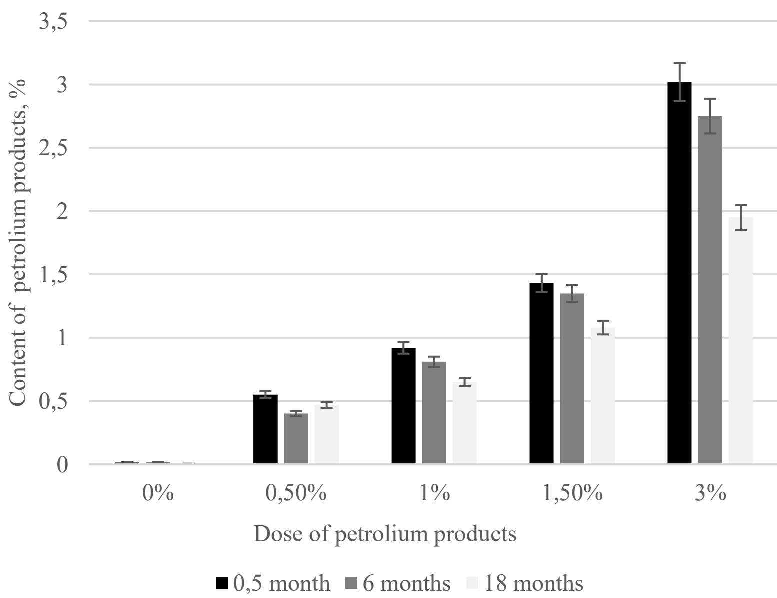

Fig. 1. Oil products’ content n cultivated soddy-podzolic soil.

The results of the study of basal respiration of the studied soil are presented in the Figure 2. Soil microbiota were characterized by high resistance to the inhibitory effects of oil pollution. Concentrations of petroleum products in the range of 0.5-1.5% did not have a toxic effect on soil microorganisms. Microbial respiration values in contaminated samples did not differ from those in clean soil. A decrease in microbial respiratory activity by 1.4 times compared to the control was found in the first observation period only in the variant with the maximum dose of oil (3%). However, it is known that this level of inhibition of biological activity does not have a negative impact on the functioning of the soil ecosystem [14]. In addition, the decrease in respiration in the most contaminated soil was reversible and after six months respiration not only completely recovered, but significantly exceeded the control level.

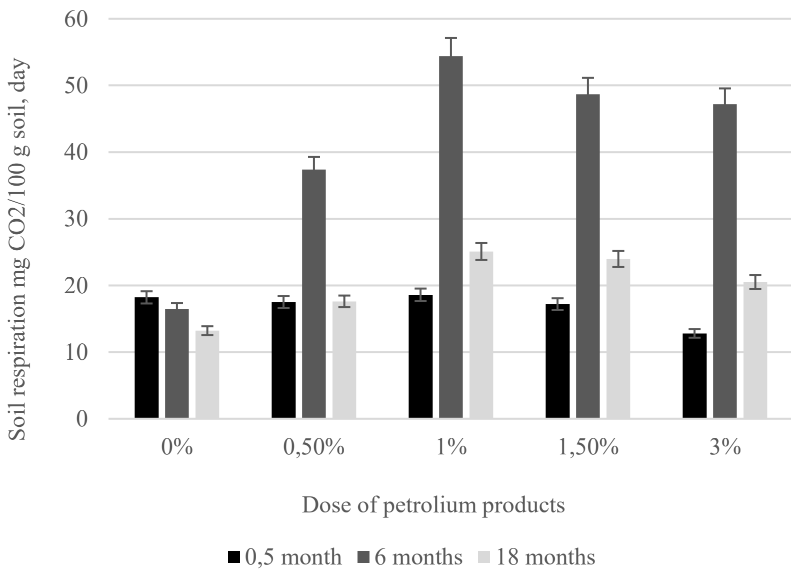

Fig. 2. Basal respiration of cultivated soddy-podzolic soil.

Half-year after the oil spill, at all levels of pollution, there was a sharp increase in basal respiration. Thus, the quantity of CO_2 coming from the soils with doses of oil of 1.0-3.0% exceeded the level of respiration of clean soil on the average of 3.3 times. This indicated the high biochemical activity of hydrocarbon-oxidizing resident microbiota and as a result an active soil self-cleaning from oil pollution. The negative dynamics of the petroleum products content proved the microbial character of the oil destruction processes in the soil (Fig. 1).

Carbon dioxide emissions from the soil with a minimum dose of oil (0.5%), as well as the soils' respiration of the other options, increased sharply six months after contamination. However, the excess over the control level was noticeably less than in more contaminated soils (Fig. 2).

Perhaps this phenomenon is associated with greater resistance of soil microorganisms to low doses of oil than to high doses [15, 16].

At the end of the field test, the basal respiration of the soils of all pollution levels decreased, but remained significantly higher than in the control variant.

The dynamics of the medium-regulating activity of microbiocenoses in soddy-podzolic soil is shown in the Figure 3. Minimum doses of oil products (0.5%) during the first half-year of the field test did not have any effect on the medium-regulating microbe activity in the studied soil. On the contrary, higher doses (1.0-3.0%) inhibited it by an average of 28% as contrasted with the control. It must be emphasized that at the same time, namely 6 months after the oil spill, a sharp increase in the oil-oxidizing activity of the microbiota was recorded (Fig. 2). Against this background, inhibition of environment-regulating activity indicates that all the "forces" of microbiocenosis were aimed at the destruction of petroleum hydrocarbons, while other microbiological processes associated with oxygen consumption were suppressed.

In our case, these are processes of microbial oxidation of glucose added to the soil in order to regulate the soil environment and restore chemical balance in it.

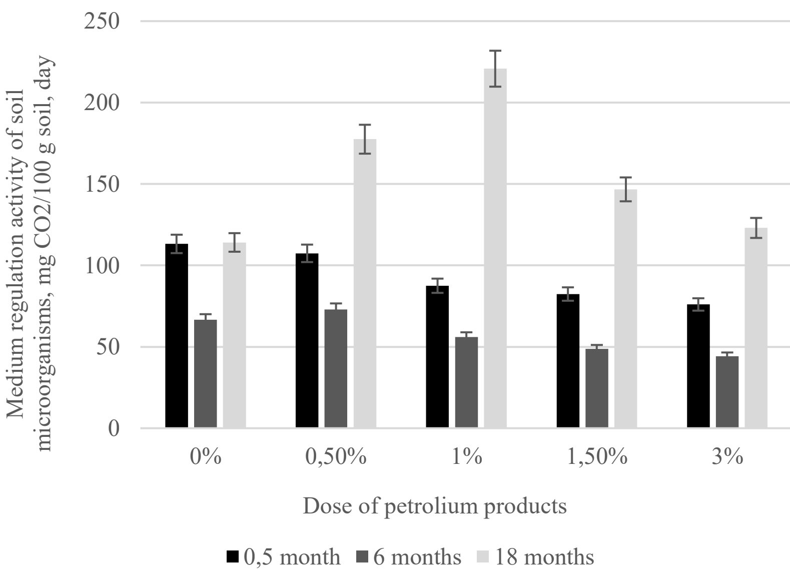

Fig. 3. Medium-regulating activity of microorganisms in cultivated soddy-podzolic soil.

So, medium-regulating activity turned out to be a more sensitive index of the inhibitory effect of oil on soil microorganisms than basal respiration. However, a 28% decrease in regulatory activity compared to control is considered acceptable and does not pose a significant threat to the stability of the system [14]. In addition, the disruption of regulatory functions was reversible. By the end of the second growing season, in the contaminated soils the medium-regulating microbe activity reached the level of clean soil and then essentially exceeded it (Fig. 3). This indicates a complete restoration of the regulatory functions of microbiocenoses in the studied oil-contaminated soddy-podzolic soil.

4 Conclusions

Microbiocenoses of the studied cultivated soddy-podzolic soil, contaminated with crude oil under the conditions of a field experiment, were characterized by high resistance to the inhibitory effects of petroleum hydrocarbons. Pollutant concentrations of 0.5, 1.5 and 1.5% did not have an inhibitory effect on the intensity of basal respiration. The decrease in respiration in the most contaminated soil, recorded in the first weeks of the experiment, was reversible. Half-year after the artificial oil spill, at all doses of contamination there was a sharp increase in basal respiration, several times higher than in the clean soil. This indicated the high biochemical activity of indigenous oil-oxidizing microorganisms, which underlies the processes of self-purification of the studied soil from oil products. Reduction in the

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content of petroleum products in soils during the field test proved the microbial character of the oil destruction processes in the soil.

Carbon dioxide emissions from the soil with a minimum dose of oil (0.5%), as well as the soils' respiration of the other options, increased sharply six months after contamination. However, the excess over the control level was noticeably less than in more contaminated soils.

Medium-regulating microbe activity was a more sensitive marker of the suppressive influence of oil on soil microorganisms than basal respiration. By the end of the first growing season, doses (1.0-3.0%) inhibited medium-regulating microbe activity by an average of 28% compared to the control. However, by the end of the second growing season, in the contaminated soils the medium-regulating microbe activity reached the level of clean soil and then essentially exceeded it. This indicates a complete restoration of the regulatory functions of microbiocenoses in the studied soddy-podzolic soil, disturbed as a result of oil pollution. Thus, after non-critical disturbances, the normal functioning of microbiocenoses was restored towards the end of the field test.

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