Influence of light fractions of petroleum hydrocarbons on the biological activity of soils

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Abstract. Experimental studies were conducted to assess the effect of oil's light and medium fractions on the soil's biological activity. Enzymes at different stages of petroleum's hydrocarbons biodegradation are considered. The results of the research were the biological activity of soils (soil microbiocenoses and enzymatic activity) contaminated with oil's light and medium fractions. The results can be used when choosing the direction of oil-contaminated soil's recultivation. The data can also be used as an indicator of the degree of soil contamination with oil products and its duration.

1 Introduction

Environmental pollution by oil and related substances is an urgent environmental problem for many regions of Russia. Contamination of the soil cover with oil hydrocarbons occurs at almost all stages of the technological process of oil and gas production. The negative impact of oil and gas production and oil and gas processing facilities is due to both the direct effect on the soils at the sites of oil and oil products spill, and the impact on the components of adjacent environments. As a result of such an impact, transformation products of oil and petroleum products are found in various objects of the biosphere.

Soils are capable of sorbing and accumulating oil products, while the rate of accumulation of oil hydrocarbons is significantly higher than the rate of biodegradation naturally [1]. The main role in the processes of self-purification of soils from oil and petroleum products is played by soil biocenosis [2]. The richness of the soil is expressed in the number and variety of microorganisms contained – bacteria, actinomycetes, algae, yeast, microscopic fungi and others. Natural ecosystems are characterized by their optimal species and quantitative composition of microorganisms, high fluctuations in the direction of decrease or increase have certain effects that are not always useful. Microorganisms play a major role in the processes of self-healing of soil ecosystems from oil and petroleum products.

The biodegradation of oil components in natural conditions depends on environmental factors – the natural conditions of the region, the humidity of the substrate, the temperature and the activity of the soil biota. Therefore, there is a need to search for criteria indicating the degree and duration of contamination. These criteria could be used not only to assess the degree of pollution, but also to assess damage to soil resources.

Today, there are no established criteria that could indicate a safe level of soil contamination with oil and petroleum products, due to the complexity of assessing the

impact of oil and petroleum products on facilities, the environment, as well as the multicomponent composition of oil and petroleum products. Therefore, the question of how to assess the impact of oil pollution on environmental objects is relevant.

When oil products enter the natural environment, xenobiotics undergo biodegradation. The following types of bio-purification are distinguished depending on the final result: biodestruction (mineralization), transformation, binding of pollutants with other substances [3, 4].

During mineralization, the components of oil hydrocarbons can be used by microorganisms as an energy source. Complete biodestruction takes place only with the combined action of microbiocenosis and abiotic factors.

Incomplete degradation of pollutants consists in simplifying the structure of organic substances under the action of microbial enzymes, while their biodegradation doesn't occur [5]. Soils on which complete biodegradation of oil hydrocarbons has occurred are characteristically different in the structure of microbocenoses from the natural long time [6].

The processes of self-purification of soils can last for many years, therefore, criteria are needed that would allow assessing the degree of self-purification. One of these criteria is the microbiological and enzymatic activity of soils. Microbiological activity is characterized by the intensity of growth of microorganisms. Enzymatic activity is assessed by enzymes characterizing important processes of material and energy metabolism [7, 8].

The biological activity of the soil, including enzymatic, is affected by a number of environmental parameters [9, 10]. Substances of petroleum origin can negatively affect the biochemical processes of the soil and change the fertility of the soil, which is crucial for soil reclamation [11-14]. The pollutants contained in these substances reduce microbial activity. However, since these substances are mixtures of various hydrocarbons, they also serve as a carbon source for some microorganisms [15]. Numerous studies have shown that the determination of the enzymatic activity of the soil can be used to assess soil contamination with hydrocarbons of petroleum origin, which in recent years have been one of the main pollutants of the natural environment [16-20].

The destruction of oil and petroleum products occurs during the active vital activity of microorganisms and the enzymes they secrete, one of such enzymes is catalase. The level of catalase activity is a criterion for self-purification of the soil from oil. Catalase is an enzyme due to which oxygen appears in the soil as a result of the decomposition of hydrogen peroxide and the aerobic regime improves [21].

The purpose of this work was to identify the influence of petroleum hydrocarbons on the microbiological and enzymatic activity of soils contaminated with light and medium fractions of hydrocarbons.

2 Materials and Methods

To study the influence of oil hydrocarbon processes on the state of soil biocenoses, we conducted experimental studies. To exclude the influence of native microflora of different types of soil, artificial soil prepared in accordance with GOST 32632-2014 "Testing of chemicals of environmental hazard" was used in the experiment. To prepare the soil, sand, peat and kaolin were taken in a ratio of 6:3:1. A mixture of heptane, dodecane and tridecane, and naphthalene and toluene in ratios of 10:1:1 and 3:1, respectively, was used as oil hydrocarbons. The choice of these compounds characterizes light and medium fractions of oil. The control samples were artificial soil without contamination, peat and sod-podzolic sandy loam soil.

To study the microbiological activity, the following indicators were selected: the number of saprophytes, hydrocarbon-oxidizing microorganisms (HOM), actinomyces, as these groups play a major role in the biodegradation of oil hydrocarbons.

The preparation of samples for microbiological analysis, technical seeding, cultivation, colony counting and statistical data processing was carried out according to the methods given in the special literature. Methods of direct microscopy of cells on fixed stained smears were used to identify and account for the total number of microorganisms in soil samples. When studying the group and generic composition of microorganisms in soil samples, the method of sowing on elective nutrient media was used: meat-peptone agar, Towson solution, starch-ammonia agar.

Catalase was selected as a control enzyme for the study. Based on previous experiments, it was found that this enzyme reacts to contamination at the initial stage and with prolonged contamination.

The catalase activity was determined by the method of R. S. Katsnelson, V. V. Ershov. The technique is based on measuring the amount of hydrogen peroxide when cleaved by an enzyme. Determination of the amount of H_2O_2 was carried out by titration with a solution of potassium permanganate. Statistical processing of catalase activity data was carried out using standard statistical methods [22, 23].

3 Results and Discussions

The study of microbiocenoses of soils contaminated with light and medium fractions of oil will allow us to assess the degree and duration of contamination. The selected groups of microorganisms are capable of decomposing organic substances entering the soil and are resistant to environmental factors. The results of the quantitative analysis are presented in Table 1.

Table 1. Quantitative characteristics of microorganisms

Group of microorganisms	Concentration of oil products, g/kg	Fraction of oil		Peat	Control
		Light	Medium		
Saprophytes, CFU/g	1.0	—	$(1.0\pm0.1)\cdot10^3$	$(1.9\pm0.3)\cdot10^3$	$(1.9\pm0.3)\cdot10^3$
	2.5-2.8	$(2.2\pm0.4)\cdot10^3$	$(1.35\pm0.2)\cdot10^3$		
	5.0-5.3	$(11.9\pm2.1)\cdot10^3$	$(2.1\pm0.3)\cdot10^3$		
	10.0-10.7	$(2.5\pm0.4)\cdot10^3$	$(3.8\pm0.5)\cdot10^3$		
	15.0	—	$(6.0\pm0.8)\cdot10^3$		
	50.0	$(1.9\pm0.3)\cdot10^3$	—		
Hydrocarbon-oxidizing microorganisms, CFU/g	1.0	—	$(1.2\pm1.6)\cdot10^3$	$(3.0\pm0.5)\cdot10^2$	$(0.6\pm0.1)\cdot10^2$
	2.5-2.8	$(7.5\pm1.3)\cdot10^2$	$(5.5\pm0.8)\cdot10^3$		
	5.0-5.3	$(4.4\pm0.8)\cdot10^2$	$(6.1\pm0.9)\cdot10^3$		
	10.0-10.7	$(3.9\pm0.7)\cdot10^2$	$(8.2\pm1.1)\cdot10^3$		
	15.0	—	$(19.5\pm2.7)\cdot10^3$		
	50.0	$(4.0\pm0.7)\cdot10^2$	—		
Actinomyces, CFU/g	1.0	—	$(7.1\pm1.0)\cdot10^3$	$(1.0\pm0.2)\cdot10^2$	$(0.1\pm0.01)\cdot10^2$
	2.5-2.8	$(0.4\pm0.1)\cdot10^2$	$(4.0\pm0.5)\cdot10^3$		
	5.0-5.3	$(0.9\pm0.2)\cdot10^2$	$(11.8\pm1.6)\cdot10^3$		
	10.0-10.7	$(1.2\pm0.2)\cdot10^2$	$(11.0\pm1.5)\cdot10^3$		
	15.0	—	$(22.8\pm3.2)\cdot10^3$		
	50.0	$(1.2\pm0.2)\cdot10^2$	—		

The results of experimental studies have shown that light fractions of oil in concentrations of more than 10 g/kg have a toxic effect for saprophytic microorganisms, whereas medium fractions of oil, on the contrary, had a stimulating effect on this group of microorganisms. For hydrocarbon-oxidizing microorganisms, light fractions of oil had a toxic effect with an increase in the concentration of xenobiotic, while medium fractions had

a stimulating effect on this group. Actinomyces reacted negatively to light fractions of oil, although in general there was a slight increase in this group. The average fractions had a favorable effect on the growth of actinomycetes, and with a direct dependence of the growth of this group on the concentration of xenobiotic was observed. With an increase in the concentration of the average fraction of oil, an increase in the number of actinomyces was observed. Consequently, medium fractions of oil have the least toxic effect for soil microbiocenoses. The results obtained confirm the literature data that the second stage of biodegradation of oil, which occurs in the second year after the spill of petroleum products, accounts for the maximum number of microorganisms. The light fractions of oil have the most toxic effect, which affect the biocenosis of soils in the first year after the oil spill.

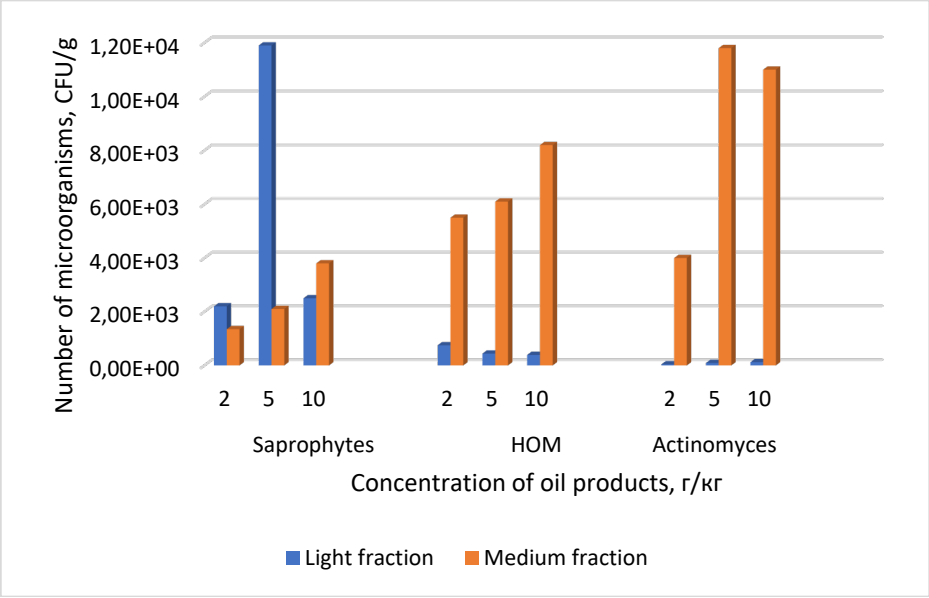

Fig. 1. The number of microorganisms at different concentrations of oil hydrocarbons

In addition to studying microbiocenoses during biodegradation of oil hydrocarbons, it is necessary to evaluate enzymatic activity, which is the most important indicator of soil biological activity and characterizes the ability of the soil ecosystem to maintain homeostasis. According to the results of early studies, one enzyme was isolated that can be used to assess the degree and duration of contamination – catalase.

Catalase belongs to the class of oxidoreductases and accelerates the decomposition reaction of hydrogen peroxide into water and molecular oxygen. The results for the determination of catalase activity are presented in Table 2.

Table 2. Catalase activity in experimental samples

Enzyme	Concentration of oil products, g/kg	Fraction of oil		Peat	Control
		Light	Medium		
Catalase activity, ml O_2/g soil in min.	1.0	—	0.22±0.013	0.5±0.027	0.8±0.04
	2.5-2.8	0.4±0.02	0.25±0.015		
	5.0-5.3	0.5±0.027	0.28±0.017		
	10.0-10.7	0.6±0.03	0.32±0.019		
	15.0	—	0.43±0.026		
	50.0	0.8±0.04	—		

The results of studies on the determination of catalase activity have shown that when any fraction of oil enters the soil, there is a sharp decrease in catalase activity. Figure 2 shows the dependence of catalase activity on the concentration of contamination.

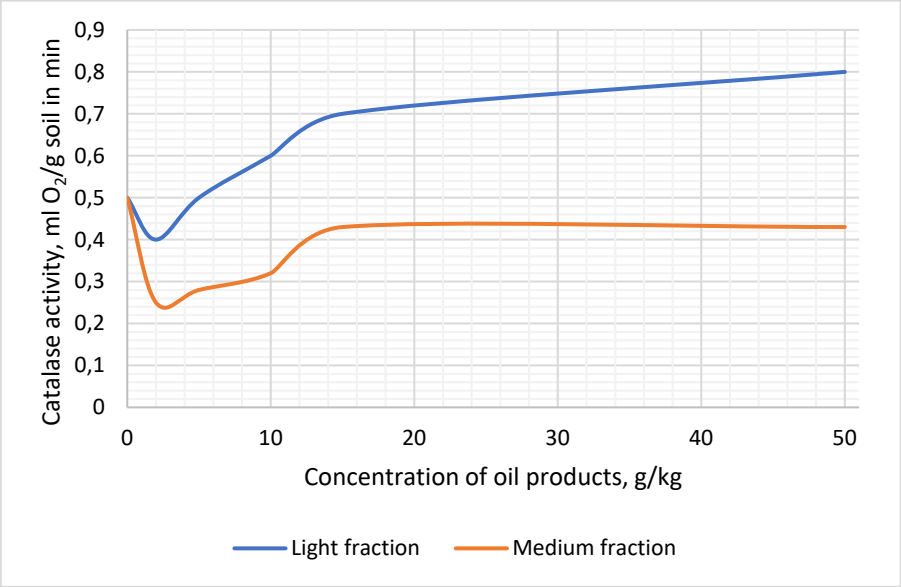

Fig. 2. Dependence of catalase activity on the concentration of oil hydrocarbons

Catalase activity in the studied soil changes as the concentration of pollutants increases. The observed increase in catalase activity during soil contamination with light and medium oil fractions may be due to the fact that soil microorganisms begin to show higher activity in response to stressful conditions caused by pollution. This may be due to an increase in the number of microbial cells capable of catalyzing the decomposition of hydrogen peroxide, or to the activation of more resistant strains of microorganisms that can cope with the toxic effects of petroleum products. However, this increase in activity may be temporary and may not lead to an improvement in the overall condition of the soil in the long term. It can be said that active redox processes are underway, which may be associated with an increase in the toxicity of petroleum products with a high content of the pollutant.

4 Conclusions

One of the important indicators of the flow of biochemical processes in the soil is their enzymatic activity. When the soil is contaminated with oil and petroleum products, the activity of the released enzymes changes.

Based on the experimental studies conducted to study the effect of light and medium-sized oil hydrocarbons on the microbiological and catalase activity of soils, the following conclusions can be drawn:

1. Microbiological activity changes when soil is contaminated with oil hydrocarbons. Medium fractions of oil have the least toxic effect for soil microbiocenoses. The results obtained confirm the literature data that the second stage of biodegradation of oil, which occurs in the second year after the spill of petroleum products, accounts for the maximum number of microorganisms. The light fractions of oil have the most toxic effect, which affect the biocenosis of soils in the first year after the oil spill

2. For light and medium fractions of oil hydrocarbons, there is a pattern in the change in enzymatic activity. The results of studies on the determination of catalase activity have shown that when any fraction of oil enters the soil, a sharp decrease in enzyme activity occurs first. However, when contaminated with light and medium fractions of oil, with an increase in the concentration of xenobiotic, catalase increases, which is associated with an increase in redox processes in soils.

3. The obtained results of the assessment of enzymatic activity can be used as an indicator of the degree and duration of soil contamination with oil hydrocarbons.

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