The effect of sulfurous oil on the biological activity of soils

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Abstract. The influence of oil hydrocarbons on soils with short-term and long-term pollution is considered. The regularity of the change in the number of microbiological community depending on the duration of oil hydrocarbon pollution has been established. The dependence of catalase activity on the time of contamination in the soil is shown.

1 Introduction

The soil is a valuable natural resource and a habitat for various living organisms, and also performs protective functions. Various processes of creation and destruction of organic and mineral chemicals occur in the soil. Soil contamination with various chemicals, especially those that are difficult to oxidize, disrupts the biological balance and leads to a decrease in fertility and land degradation.

As a result of the work of industrial enterprises, as well as in areas of oil production and processing of hydrocarbon raw materials, contaminated with various organic and inorganic substances.

Oil hydrocarbons are common pollutants found in the soil as a result of past and current industrial activities. Onshore oil spills affect entire ecosystems by altering vegetation, wildlife, microbial processes, soil characteristics and the general condition of the soil. The environmental impact of oil on the functioning of soils is most clearly manifested in changes in the activity of soil microorganisms and enzymes [1, 2], which makes the microbial activity of the soil a sensitive biological and biochemical indicator of soil quality. At the same time, the ability of microorganisms to metabolize oil hydrocarbons is successfully used for disinfection of oil-contaminated areas as one of the most environmentally friendly and universal approaches.

Oil hydrocarbons pollute the soil and disrupt its biochemical processes, which leads to a change in the structure of soil ecosystems. The number and diversity of microorganisms, such as bacteria, actinomyces, algae, yeast and microscopic fungi, are indicators of soil richness [3]. The decrease in the number of microorganisms in the soil is the primary process of the receipt and distribution of xenobiotics [4].

Oil hydrocarbons are difficult-to-decompose organic pollutants that can seriously affect soil productivity and cause varying degrees of harm to microorganisms, plants and animals in the ecosystem around the source of pollution [5, 6]. The intake of petroleum-based substances into the soil caused a sharp increase in the organic carbon content in the soil, which led to an imbalance between the carbon and nitrogen content in the soil [7-9]. This imbalance destroys the habitat of soil microbiocenoses and changes the number and diversity of their populations [10, 11]. Microorganisms play a very important role in maintaining the functions of the soil ecosystem; they can effectively decompose plant and animal residues; contribute to the cycles of carbon, nitrogen, sulfur, phosphorus and other nutrients in the soil; and regulate the circulation of soil material and energy [12-15]. Current studies of microorganisms of oil-contaminated soil are mainly focused on the recultivation effect of soil microorganisms (screening of effective degradation bacteria, degradation characteristics, etc.), while the structural diversity of local microbial communities of oil–contaminated soil in the northern forest areas is less studied [16-18].

The effect of prolonged exposure to oil on the soil can manifest itself in a change in its microbiological properties. In the first weeks and months after pollution, mainly abiotic processes of oil changes in the soil occur. There is a stabilization of the flow, partial scattering, a decrease in concentration, which allows microorganisms to adapt, rebuild their functional structure and begin active activities for the oxidation of hydrocarbons. The processes of self-purification of soils from oil and petroleum products can last several years. At the same time, the structure of microbocenoses of soils on which complete biodegradation of petroleum hydrocarbons has occurred characteristically differs from the natural one for a long time [19].

Numerous studies have demonstrated the high efficiency of bioremediation in the purification of oil–contaminated soils [20-22]. Since remediation technologies affect not only the oil concentration, but also the enzymatic reaction [23, 24], enzyme activity is a sensitive indicator used to evaluate and monitor the bioremediation process [23, 25]. Enzymes that have been found useful for monitoring the removal of hydrocarbons include soil dehydrogenases, catalases and ureases [25-28].

Among the various oxidoreductase enzymes, catalase is the best for determining the microbial activity of the soil. It reflects the level of physiologically active microorganisms in the soil and thus provides correlative information about the biological activity and microbial population of the soil. Catalase also reflects the degree of decomposition of organic matter and the presence of nutrients in the soil. Thus, a change in catalase activity can be a reliable indicator for determining changes in soil fertility as a result of biological oxidation of organic matter. Dehydrogenases play a role in the biological oxidation of organic matter in the soil, catalyzing the transfer of hydrogen from organic substrates to inorganic acceptors. Catalase activity may reflect the rate of transformations occurring in the soil [29 – 32].

The process of self-purification of soils from oil and petroleum products can last several years. Therefore, criteria are required that would allow us to assess the degree of self-purification or biodegradation of petroleum hydrocarbons. The main of such criteria is the microbiological and enzymatic activity of soils [33, 34].

There are inherent limitations associated with laboratory research. The duration of the experiment is one of the main differences between studies conducted in the laboratory and in the field. The former mainly focus on short-term effects observed after several weeks or even days of contamination, while the study period of several years is unusual [34, 35]. Soil properties change dramatically during prolonged incubation in the laboratory, which makes it difficult to interpret any observed effects. In addition, laboratory experiments cannot reproduce the changes that occur in natural conditions over time. Overall progress in developing effective bioremediation strategies depends on achieving the same good results in the field as in the laboratory, which in itself is a difficult problem [36].

In this study, an experiment was conducted to assess the impact of petroleum products on the biological activity of the soil during fresh and prolonged contamination.

2 Materials and methods

To study the influence of oil hydrocarbon processes on the state of soil biocenoses, we conducted experimental studies. The samples were contaminated soils collected in the Komi Republic. Sampling points were selected taking into account the level of contamination and soil characteristics after visual inspection. Three samples were taken at the weathered site and three samples at the recently polluted site. The control samples were peat and marsh-podzolic soil.

The characteristics of oil for pollution sites are the density of oil is 0.95 g / cm^3, the content of sulfur compounds is 0.45 – 1.89 %, resinous substances – 28 %.

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

The enzyme catalase was chosen to study the enzymatic activity. 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 [37, 38].

3 Results and discussions

The study of microbiocenoses in soil samples with fresh and long-term contamination will allow us to assess the degree and duration of exposure. 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	Soil samples		Peat	Control
	Fresh pollution	Long-term pollution		
Saprophytes, CUF/g	$(1.33 \pm 0.3) \cdot 10^5$	$(0.34 \pm 0.1) \cdot 10^4$	$(1.9 \pm 0.3) \cdot 10^3$	$(1.9 \pm 0.3) \cdot 10^3$
	$(1.46 \pm 0.3) \cdot 10^5$	$(0.41 \pm 0.1) \cdot 10^4$		
Hydrocarbon-oxidizing microorganisms, CUF/g	$(1.40 \pm 0.3) \cdot 10^4$	$(0.82 \pm 0.2) \cdot 10^4$	$(3.0 \pm 0.5) \cdot 10^2$	$(0.6 \pm 0.1) \cdot 10^2$
	$(1.39 \pm 0.3) \cdot 10^4$	$(0.94 \pm 0.2) \cdot 10^4$		

According to the results of experimental studies, it was found that fresh pollution can have both a toxic and stimulating effect on different groups of microorganisms of soil microbiocenosis.

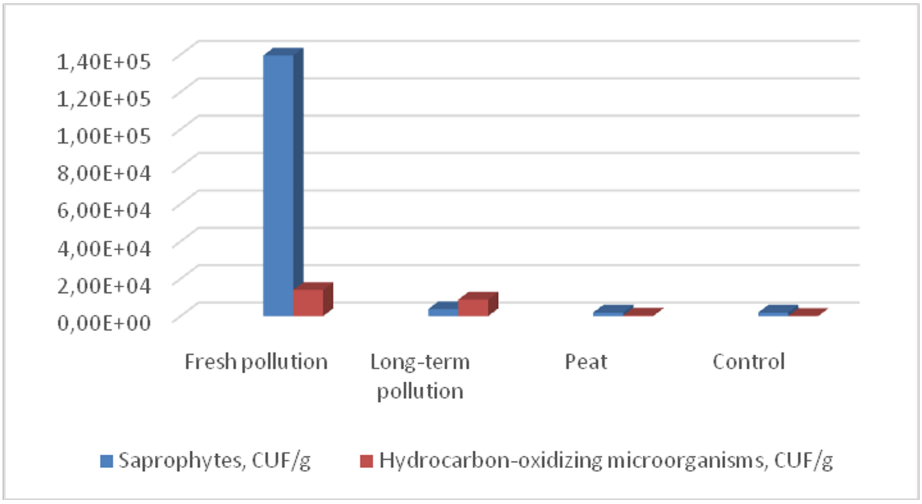

Fig. 1. The number of microorganisms in soil samples

Concentrations of hydrocarbons during fresh contamination have a stimulating effect on saprophytic microorganisms, but with the duration of contamination, the toxic effect of oil begins. The presence of a light fraction of oil in hydrocarbons has a small stimulating effect on groups of microorganisms compared to the control and peat, which may indicate that light fractions of oil that have not had time to break down are the main source of nutrition for these groups of microorganisms. The main representatives of saprophytes were the bacteria g. *Bacillus sp.* and g. *Pseudomonas sp.*, the main representatives of hydrocarbon-oxidizing microorganisms are the bacteria g. *Rhodococcus sp*. and g. *Pseudomonas sp*. Figure 2 shows colonies of hydrocarbon-oxidizing bacteria g. *Rhodococcus sp*.

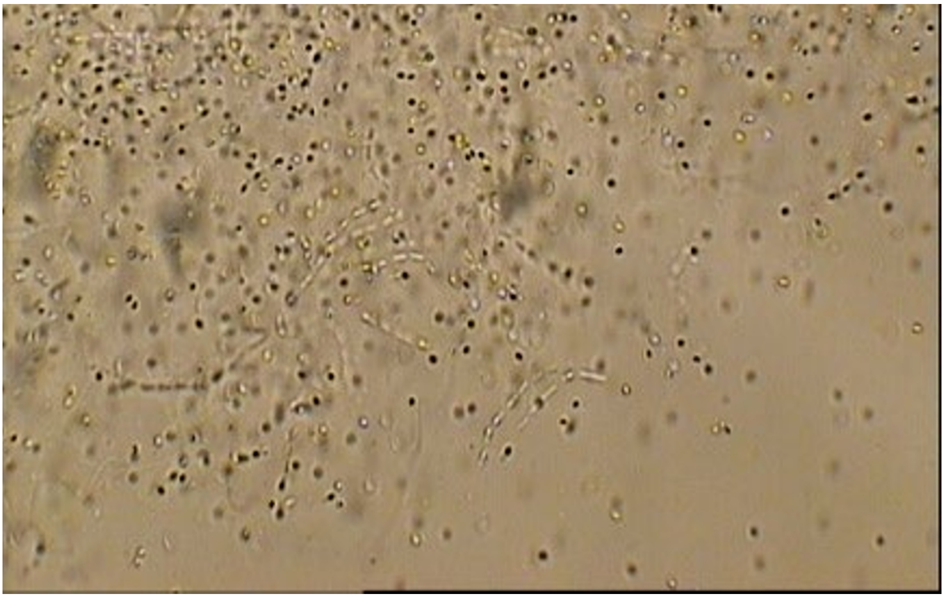

Fig. 2. Hydrocarbon-oxidizing bacteria of g. *Rhodococcus sp.*

With prolonged exposure to petroleum products, there is a sharp decrease in all studied groups of microorganisms, which indicates a toxic effect on this group of microorganisms.

However, in comparison with control samples with prolonged soil contamination, the values are higher, which means it has a stimulating effect on the groups of microorganisms considered. This phenomenon is explained by the fact that with prolonged exposure to petroleum products, heavy fractions of oil remain in the soils.

During the studies, data on changes in catalase activity were obtained. The results of the quantitative analysis are presented in Table 2.

Table 2. Catalase activity of samples

Soil samples	Catalase activity, ml O_2/g soil in min
Fresh pollution	0.11±0.02
	0.12±0.03
long-term pollution	0.26±0.05
	0.20±0.04
Control	0.5±0.027
Peat	0.8±0.04

With prolonged contamination of the soil with petroleum products, the activity of catalase increases. The dependence of catalase activity on the concentration of oil is shown in Figure 3.

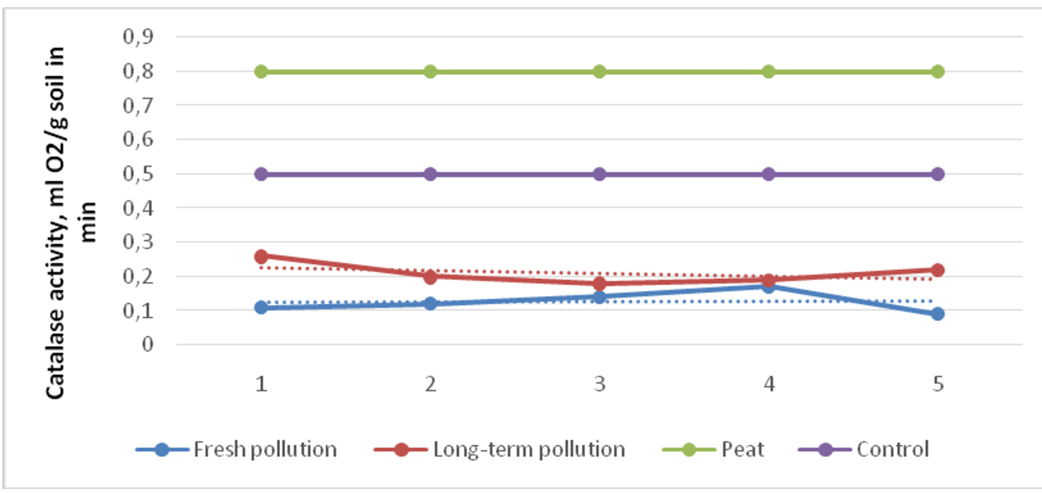

Fig. 3. Dependence of catalase activity on the concentration of petroleum hydrocarbons

With an increase in the duration of soil contamination with petroleum products, the activity of catalase increases. With an increase in the duration of exposure to petroleum hydrocarbons in the soil, activation of microorganisms occurs that can decompose these components of petroleum products. In addition, there is an increase in redox processes, which may be associated with an increase in the toxicity of petroleum products with a high content of the pollutant.

4 Conclusions

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

1. Soil contamination with hydrocarbons can cause changes in the physical condition and biological activity of the soil, and prior knowledge of these processes is considered necessary for the selection and development of the most appropriate methodology for the

restoration of contaminated soils. Over long periods of time, there is a restructuring of the microbial community, which leads to a decrease in the microbiological activity of the soil. Microorganisms living in oil-contaminated soils can be adapted to the use of petroleum products as a food source.

2. With fresh contamination, microbiological activity is higher than with long-term contamination, since hydrocarbons have not yet had time to degrade and retain their biological availability as a food source for microorganisms.

3. Catalase activity increases over long periods of time, which indicates that there will be active redox processes of self-purification. The reduced values of enzyme activity during fresh contamination are explained by a decrease in the activity of aerobic microorganisms as a result of a violation of moisture and gas exchange processes

4. The obtained results of the assessment of the biological activity of soils contaminated with hydrocarbon raw materials can be used as an indicator of the degree and duration of soil contamination with hydrocarbon raw materials. The data obtained can be included in the methods of calculating damage to soil resources in case of contamination with hydrocarbon raw materials, to assess the duration of contamination of territories.

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