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Bioremediation of oil product contaminated soils in conditions of North Near-Polar Area

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Abstract. The impact of oil products (gasoline, diesel fuel, stable gas condensate, fuel oil) in concentration from 1 to 10 % on the properties of the soil, plants and soil microbiota has been studied under field test experiments. Purification of cultivated podzolic soils from light hydrocarbons occurs within one vegetation period. For three months of the growing season gas condensate has been completely removed from the soil, diesel fuel – almost entirely (90 %); heavy hydrocarbons have been taken out of the soil at the rate of 70-85 %. We have selected a range of plants for bioremediation of soils at high latitudes. The following plants being highly resistant to the oil product contamination have been recommended for phytoremediation: *Phalaroides arundinace*, *Festuca pratensis*, *Phleum pretense*, *Leymus arenarius*. It is important to note that soil contamination with oil products has increased the proportion of opportunistic species of fungi compared with the control soil. In clean soil a fraction of opportunistic fungi accounted for 30 %, in the oil contaminated – 50-60 % of the total number of species.

Аннотация. Изучено воздействие нефтепродуктов (бензин, дизельное топливо, стабильный газовый конденсат, топочный мазут) в концентрации от 1 до 10 % на свойства почвы, растения и почвенное микронаселение в условиях полевых модельных опытов. Очищение окультуренной подзолистой почвы от легких углеводородов происходит в течение одного вегетационного периода. За три месяца вегетационного периода газовый конденсат был полностью удален из почвы, дизельное топливо – практически полностью (более 90 %); смесь нефтепродуктов, содержащих мазут, удалена за сезон на 70-85 %. Подобран ассортимент растений для биоремедиации почв высоких широт. Рекомендованы следующие растения, обладающие высокой устойчивостью к загрязнению нефтепродуктами: двукисточник тростниковидный, овсяница луговая, тимофеевка луговая, волоснец песчаный. Выявлена закономерность возрастания доли потенциально-патогенных грибов в почвах, загрязненных нефтепродуктами. В чистой почве доля этих грибов составила 30 %, в загрязненной нефтепродуктами – 50-60 % от общего числа видов.

Key words: oil products, microorganisms, phyto-meliorate, bioremediation
Ключевые слова: нефтепродукты, микроорганизмы, фитомелиоранты, биоремедиация

1. Introduction

Extraction and transportation of oil and gas in our country is moving into the northern regions characterized by relatively low self-purification capacity of the environment from pollution due to the slowness of the processes of energy and mass transfer. One of the main ways to intensify cleansing of soils from pollution by oil and petroleum products (PP) is bioremediation. Planting in contaminated soils of plants, the rhizosphere of which is inhabited by microorganisms transforming pollutants is a promising and cost-effective technique to improve the quality of contaminated soils. Thus, phytoremediation of 1 m^3 of oil-contaminated soil, according to the BP Amoco (*Molotkov, Kasjanenko*, 2005), costs 3 USD per year, whereas in the use of other engineering methods of treatment cost is much higher – between 10 and 1000 USD. Besides, the green plants have positive aesthetic impact on a person in a technologically impaired environment.

Today there is a large amount of material on the importance of microbiological processes in purifing the environment from oil hydrocarbons accumulated. However, it concerns mostly water areas for oil spills and to a lesser extent – the soil. Our studies were performed in a region characterized by a unique combination of natural and anthropogenic factors – in the harsh climatic conditions and intensive industrial development. The studies are advanced in nature, since massive oil spills on land ecosystems of the Kola Peninsula have fortunately not been observed. Development of the Barents Sea shelf, transportation of petroleum products may lead to contamination, as it is confirmed by national and international practice on the explored oil and gas fields.

Maximum permissible concentrations (MPC) on crude oil and petroleum products for soil are not finalized up to the present time, and it is unlikely this process will ever be completed taking into account the variety of soil types with their characteristic features and diversity of bioclimatic zones in our country. In addition, oil is a mixture of about 1000 individual substances and the composition of the various oil fields may differ significantly from each other.

There are proposed approximate permissible concentrations (APC) of crude oil and PP in soils (*Accessibly…*, 2000), according to them APC of oil and heavy PP in soils with low biogenic activity (low rate of self-purification) is equal to 700 mg/kg, for soils with medium biogenic activity – 2000 mg/kg, for soils with high biogenic activity – 4000 mg/kg (0.4 %). Most soils of the Kola Peninsula are characterized by low and medium biogenic activity (*Evdokimova, Mozgova*, 2001).

Following climatic and soil features of the Kola region influence on the processes of soil contamination and self-purification: the low temperature and short period of self-purification of natural environments; relatively low diversity of soil biota species and, therefore, limited opportunities in recycling and transformation of pollutants; acid reaction of soil solution for the dominant in the Kola North podzolic and peat soils, which increases the mobility of contaminants and their toxicity; leaching of soil water regime contributing to leaching of elements in the soil profile, that can lead to contamination of groundwater; high sunshine duration in the summer months, almost equal to the equatorial values, that is positive for self-purification of soils by photochemical reactions.

Specific soil and climatic features of the Kola region, in conjunction with specific features of the microbiota of soils of high latitudes (psihrotolerance, oligotrophic, low biodiversity) create the need to develop recommendations for soil bioremediation of petroleum for the Kola subarctic region.

The purpose of the work is to determine the period of purification of soil for North-Western Russia on various petroleum products to determine their effects on soil biota and pick assortment of plants resistant to pollution.

Oil products getting into the soil are exposed to biological transformation, redox processes, physical influences. Light PP (gasoline, kerosene, diesel and gas condensate) are largely decomposed and evaporated already on the soil surface, and are easily washed away by water currents. Up to 40 % of light oil fractions are removed from the soil by evaporation. The greatest efficiency of evaporation is achieved during the first four days. Heavy PP (fuel oil, lubricants and bitumen) have toxic effects on organisms, significantly alter the physical and chemical properties of soil. Covering the roots of plants, they dramatically reduce the inflow of moisture. The process of their destruction by microorganisms is slow, sometimes for decades of years.

There are three basic methods of bioremediation of soils contaminated with oil hydrocarbons:

1 – the activation of the biodestructive activity of indigenous microorganisms by providing additional sources of supply in the form of mineral and organic fertilizers, lime, extra aeration, changes in the acid-alkali treatment of the soil. The use of light correcting films as a covering material should be noted as one of the recent developments. It leads to activation of native oil-oxidizing bacteria vital functions.

2 – the introduction of biological products based on active genetically modified strains of oil-oxidizing bacteria and fungi. Biological products should be comprehensive, containing several strains of oil-oxidizing microorganisms, able to utilize hydrocarbons with different carbon chain lengths. There are a lot of such chemicals developed and offered on the market: Devoroil, Destroil, Mikrobak, Mikrozim, Petro Treat, Universal, biologically active composition (BAC), Novozymes Biologicals, Roder, Naftox, Psevdomin. The disadvantage of many commercial products is fairly rapid decline in titer oxidizing microorganisms during storage and transportation of drugs and problems with the adaptability of introduced microorganism species in the soil.

In order to prevent leaching of alien cells introduced with biosubstances, and increasing their resistance to the competitive exclusion by the indigenous microbiota sorbents (ekolan, severat, sorbonaft) are used. They contribute to the immobilization of oil-oxidizing microorganisms and prolong the duration of their being in the soil (*Sharapova et al*., 2009).

3 – the phytoremediation – growing resistant plant species in contaminated soils. Plants stimulate the development of rhizosphere bacteria and fungi-destructors of oil and PP, exo-enzymes of plants can put down, bind and degrade organic pollutants. Plants immobilize contaminants adsorbing them on their own roots and carrying the process of phytostabilization (*Bioremediation…*, 2008).

2. Materials and methods

We studied the impact of oil products (gasoline, diesel fuel, stable gas condensate, fuel oil) on the properties of the soil, plants and soil microbiota under field test experiments. The concentration of PP in the experiments was at the level of the weak and moderate pollution (from 1 to 10 %). Land-improving plants (16 species) used in the experiments are from different families (grasses, crucifers, legumes), annuals and perennials, native and introduced. Chemical ameliorant used to reduce the toxicity of contaminated soils are mineral and organic fertilizers. Bacterial treatment was based on our own collection of microorganisms. As sorbents we used C-Verad made on the basis of vermiculite. Oil products content was determined by the infrared spectrometry method.

3. Results and discussion

Purification of cultivated podzolic soil from light hydrocarbons occurs within one growing season. During three months of the growing season gas condensate (GC) was completely removed from the soil, diesel fuel – almost entirely (90 %) (Fig. 1).

There is a tendency of more rapid removal of diesel fuel from soil under reed canary grass (I) and using a single introduction of a bacterial treatment that should be put at least twice a season in the soil (II). Commercial bacterial treatments, as recommended by the developers, should be introduced into the soil up to three times per a season.

Rate of PP loss from the forest soil is slower than from the cultivated one, mainly due to slowness of processes of evaporation and photochemical reactions under the canopy compared to open spaces. During the first 5 days after gas condensate putting in its quantity decreased by 70 % in the cultivated soil, and in the forest – by 8-10 % (IV).

Heavy hydrocarbons are fixed in the upper soil layers, adversely affecting their water-physical properties. During three months of the growing season the mixture of petroleum-containing fuel oil was taken out of the soil at the rate of 70-85 %. Residues of petroleum products were traced in 15 months (III). In the case of introduction of Mikrozim together with the sorbent "C-Verad" the process of mixture decrease of petroleum products was going a little intensely. During first days, this difference was 10 %, in a month – 3-4 %.

Plants being higher eukaryotic organisms are more sensitive to oil hydrocarbons than organisms possessing the function of the degradation of many organic compounds. We have selected a range of plants for bioremediation of soils at high latitudes. We recommend the following plants with high resistance to oil pollution: reed canary grass (*Phalaroides arundinacea* (L.) Rauschert.), meadow fescue (*Festuca pratensis* Huds.), timothy grass (*Phleum pratense* L.), *Leymus arenarius* (L.) Hochst. We should especially mention the reed canary grass giving high vegetative biomass and mature seeds in the conditions of the polar region. Application of representatives of leguminous plants such as *Lotus corniculatus* L.s.l. characterized by medium resistance to oil pollution will improve soil fertility due to biological nitrogen enrichment.

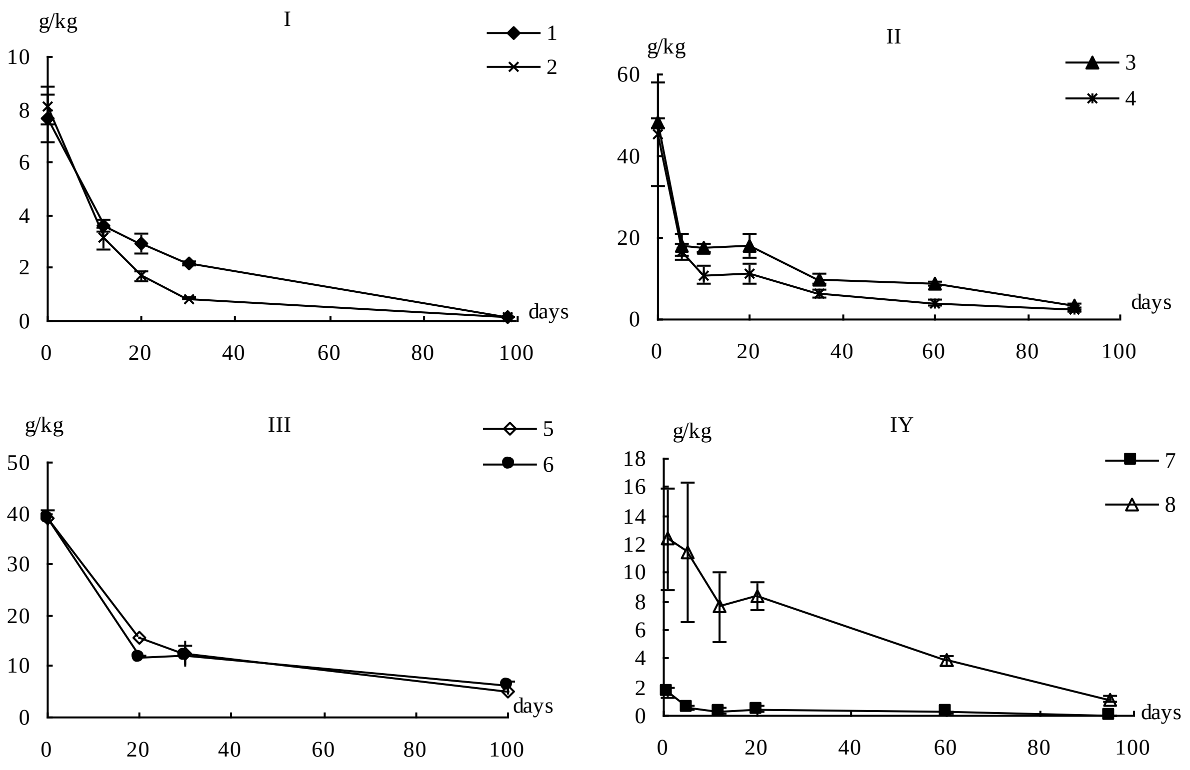

Fig. 1. Dynamics of oil products decrease from the soil: 1 % DT (I), 10 % DT (II), 4 % mix "DT + gasoline + crude oil" (III), gas concentrate, 3 liter per м^2 (IV). I: 1 – steam, DT; 2 – with *Phalaroides arundinace* plant; II: 3 – DT; 4 – DT + bacterial treatment; III: 5 – DT + gasoline + crude oil; 6 – DT + gasoline + crude oil + Mikrozim + S-Verad sorbent. IV: 7 – GK, cultivated soil; 8 – GK, forest soil

The main destructors of oil and PP are hemogeterotrophic aerobic microorganisms. Active biodestructors of petroleum hydrocarbons are found among the following genera of soil bacteria: *Arthrobacter*, *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Nocardia*, and microscopic fungi: gg. *Penicillium*, *Fusarium*, *Trichoderma*, *Cladosporium*, *Aureobasidium*. All of them are widely spread in soil at high latitudes. It has been shown that soil bacteria are resistant to soil contamination with diesel fuel at its contents in the range of 1-10 % (Fig. 2). This applies generally to both saprotrophic bacteria and to a specialized group of hydrocarbon-oxidizing bacteria. Diesel fuel stimulation of breeding hemoorganotrophic soil bacteria is a positive factor in the bioremediation of soils contaminated with PP. We have found tolerant species of soil fungi, typical for podzols contaminated with PP. The most resistant to all investigated types of petroleum products (fuel oil, gasoline, diesel fuel, GA) is *Penicillium miczynskii*. *P. restrictum*, *P. simplicissimum*, *P. miczynskii*, *Trichoderma koningii*, *T. viride*, *Aspergillus fumigatus* also show high resistance to oil and PP.

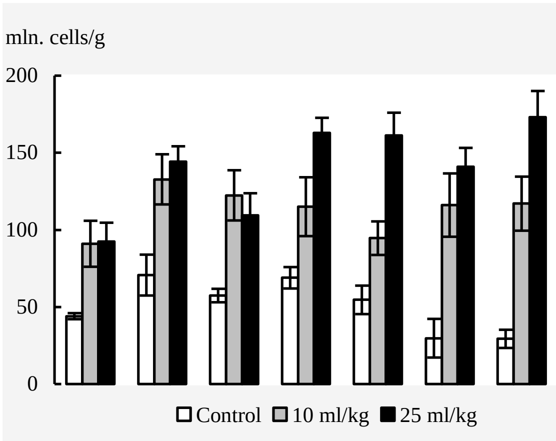

Fig. 2. Number of saprotrophic bacteria, mln.cells/g of soil under various plants in the experiment with diesel fuel. 1 – *steam*, 2 – *Lolium perenne*, 3 – *Phalaroide arundinacea*, 4 – *Phleum pratense*, 5 – *Festuca pratensis*, 6 – *Lotus corniculatus*, 7 – *Galega orientalis*

It is important to note that soil contamination with oil products has increased the proportion of opportunistic species of fungi as compared with the control soil that can cause various allergic reactions and respiratory diseases. In clean soil a fraction of opportunistic fungi accounted for 30 % of the total number of species, in the oil contaminated – 50-60 % depending on the type of PP.

The degree of soil pollution by oil and PP and its ability to heal itself depends on soil properties such as organic matter and nutrients, cation exchange capacity, acid-alkali treatment of the soil, the characteristics of hydrothermal regime, biomass and activity of soil biota and its biochemical heterogeneity providing a variety of channels transfer of matter and energy.

4. Conclusion

Hence, purification of cultivated podzolic soil from light hydrocarbons occurs within one vegetation period. For the three months of vegetation season gas condensate has been completely removed from the soil, diesel fuel – almost entirely (90 %); heavy hydrocarbons have been taken out of the soil at the rate of 70-85 %. We have selected a range of plants for bioremediation of soils at high latitudes. The following plants which are highly resistant to oil product contamination have been recommended for phytoremediation: *Phalaroides arundinace*, *Festuca pratensis*, *Phleum pretense*, *Leymus arenarius.* It is important to note that soil contamination with oil products has increased the proportion of opportunistic species of fungi as compared with the control soil.

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