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STUDY OF SOIL MICROBIOTA RESPONSE TO OIL POLLUTION

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Abstract

Oil and petroleum products are one of the most dangerous types of pollution, which can manifest itself at all stages of technogenesis. Understanding the changes that occur in the soil when various amounts of oil enter it, and the reactions of living organisms to pollution, make it possible to correctly determine the state of the soil ecosystem and select effective remedial measures in specific conditions of oil contamination of the soil. Therefore, the aim of this work was to study the reaction of different parts of the soil microbiota to oil pollution.

To determine the response of soil microorganisms to oil pollution, we studied the dynamics of the number of the main physiological groups of bacteria and microscopic fungi in soils with a certain oil content. The following oil concentrations were used in this study: 1, 5, 10, 50, 100, 200, 300, 500 cm³/kg.

A study of the dynamics of microflora in oil-contaminated soils showed a significant linear increase in the number of microorganisms of various ecological and physiological groups in the concentration range of 10÷100 cm^3/kg. It has been shown that the basis of the microcenosis of the studied soil at high oil concentrations is spore-forming and hydrocarbon-oxidizing bacteria.

Unlike to bacteria, microscopic fungi showed a sharp decrease in the number of colony-forming units (CFU) at oil concentrations in the soil at a level of 10-100 cm^3/kg. However, in the range of high oil concentrations, their number remained at an almost constant level and at an oil level of 500 cm^3/kg was almost twice as high as the number of bacteria.

Keywords: *oil, pollution, soil, microbiota, toxicity, standardization.*

Introduction

In today's world, environmental pollution from technogenic waste is intensifying. One of the most serious ecological threats is the contamination of water bodies and soils by oil and petroleum products [1-4]. Oil pollutants are resistant to degradation due to their complex structure and high stability under environmental conditions (temperature, solar radiation, humidity, etc.). Among terrestrial ecosystem components, soils are the first to suffer from oil contamination. Due to the soil's high adsorption capacity, oil can persist for long periods, causing the loss of soil value and spreading pollutants into the air, water, and food chains [1].

Regulation and Assessment of Soil Contaminated by Petroleum Products

Effective environmental protection requires accurate information about the state of contaminated soils. Pollution levels exceeding the soil's self-purification potential are considered dangerous. Therefore, defining acceptable or safe levels of oil contamination is essential. However, there are no universally accepted standards. In Europe, a threshold of 1–3 g/kg is considered safe, and 20 g/kg marks serious ecological damage. In Ukraine, there is only a tentative permissible concentration (TPC) of 0.2 g/kg. Another regulatory document defines TPC as 4 g/kg, commonly used for oil contamination assessments.

Changes in Soil Properties Due to Oil Polution

Oil-contaminated sites develop a specific microclimate due to altered substrate composition, disrupted water and temperature regimes, and characteristic odor. Color changes (dark hues) enhance solar absorption and overheating [5-7]. If the pollutant includes salts, salinization also occurs (Fig. 1).

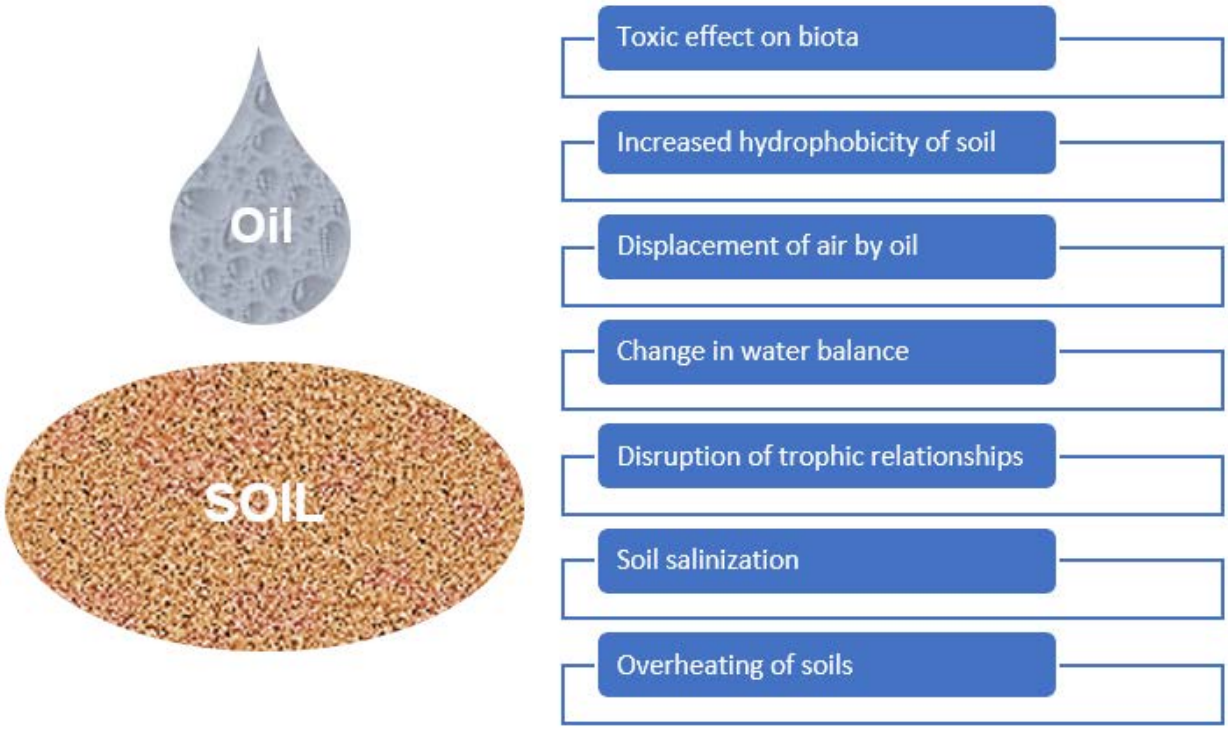

Fig. 1. *Effects of petroleum product contamination on soils*

Impact of Oil on Living Organisms

Soil biological properties drastically change upon oil contamination, particularly with decreased enzyme activity. Enzyme sensitivity to oil follows this order: dehydrogenase > catalase > urease > invertase [3].

Hydrocarbons alter soil physicochemical properties, reduce nutrient availability, and exert direct toxicity, particularly from volatile aromatics (benzene, toluene, xylene), naphthalene, and other water-soluble compounds [8]. Oil affects soil microorganisms differently—some are suppressed, others stimulated—depending on contaminant concentration and composition.

Actinomycetes, nitrifiers, and cellulolytic microbes are highly sensitive, while hydrocarbon-oxidizing bacteria and micromycetes thrive, using hydrocarbons as nutrients. Phytopathogenic and allergenic fungi can increase in number [9-10].

Soil algae respond similarly: low oil concentrations (0.01%) stimulate green algae growth (Chlorella homosphaera, Chlorella vulgaris), while higher concentrations (0.3%) reduce it. Cyanobacteria are most resilient and may utilize oil hydrocarbons [3].

Understanding these changes helps assess soil ecosystem health and determine effective remediation strategies.

Research Objective

To investigate the response of various components of soil microbiota to oil contamination.

Materials and Methods

Typical clay loam chernozem was used, sampled from a depth of 5–20 cm. Air-dried soil was sieved (1 mm mesh), moistened to 60 % field capacity, and mixed with crude oil from the Dnipro-Donetsk region (sulfur content: 0.2 %; density: 0.85 g/cm³).

Oil concentrations used: 1, 5, 10, 50, 100, 200, 300, 500 cm³/kg. Microbial groups studied: saprophytic bacteria (meat-peptone agar), spore-forming bacteria (Gromyko medium), hydrocarbon-oxidizing bacteria (Tauson's mineral medium with oil), and micromycetes (starch-ammonia agar, pH 6.5–7.8).

Results and Discussion

Impact on Saprophytic Microflora

A linear increase in saprophyte numbers was observed at 10–100 cm³/kg, followed by a sharp decline at higher concentrations (Fig. 2). At 500 cm³/kg, microbial activity was nearly suppressed.

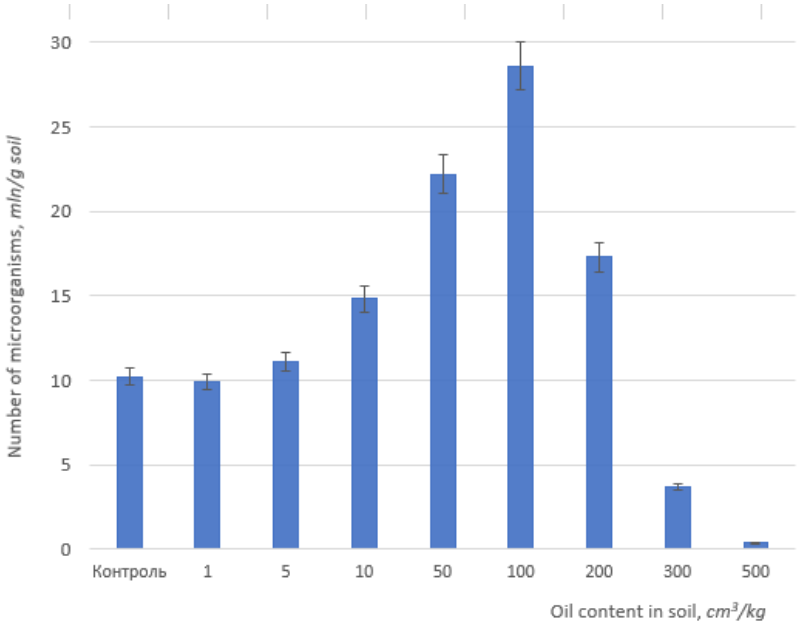

Fig. 2. *Impact of oil pollution on saprophytic microflora*

Hydrocarbon-Oxidizing Bacteria (HOB)

When soil is contaminated with oil, the ecological group of hydrocarbon-oxidizing bacteria (HOBs) is of particular importance, as they are able to use oil and petroleum products as the sole source of carbon and energy. Hydrocarbon-oxidizing microorganisms are part of the heterotrophic community and are present in both oil-polluted and unpolluted ecosystems. They differ from other microorganisms in the presence of a complex of enzymes that oxidize hydrocarbons and the ability to absorb hydrophobic substrates. Soil contamination with oil and petroleum products introduces an additional source of carbon into the ecosystem, which stimulates the development of this group of bacteria (Fig. 3). A decrease in the number of microorganisms of this physiological group was observed only at the highest of the studied concentrations.

Spore-Forming Bacteria

As for spore-forming bacteria, they demonstrated high resistance to oil contamination of soils, which is facilitated by the high resistance of spores to many adverse environmental factors (Fig. 4). The concentration range from 10 to 100 cm^3/kg is noteworthy, at which the number of bacteria of this group reaches a maximum and almost does not change. The number of spore-forming bacteria is slightly lower at an oil concentration of 200 cm^3/kg. At a concentration of 500 cm^3/kg, the number of representatives of spore-forming bacteria is almost equal to the number of saprotrophic bacteria at a given concentration, which indicates that the basis of the microcenosis of the studied soil at high oil concentrations is spore-forming bacteria.

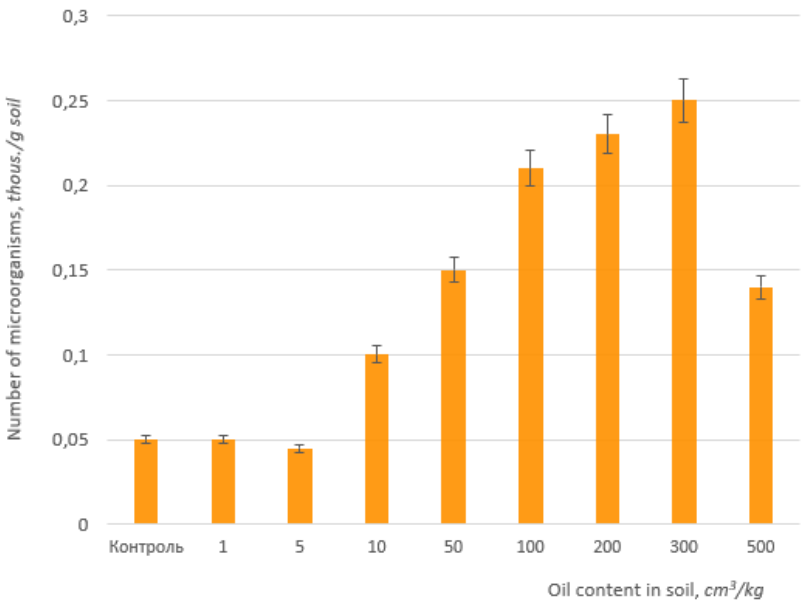

Fig. 3. *Impact of oil pollution on hydrocarbon-oxidizing bacteria*

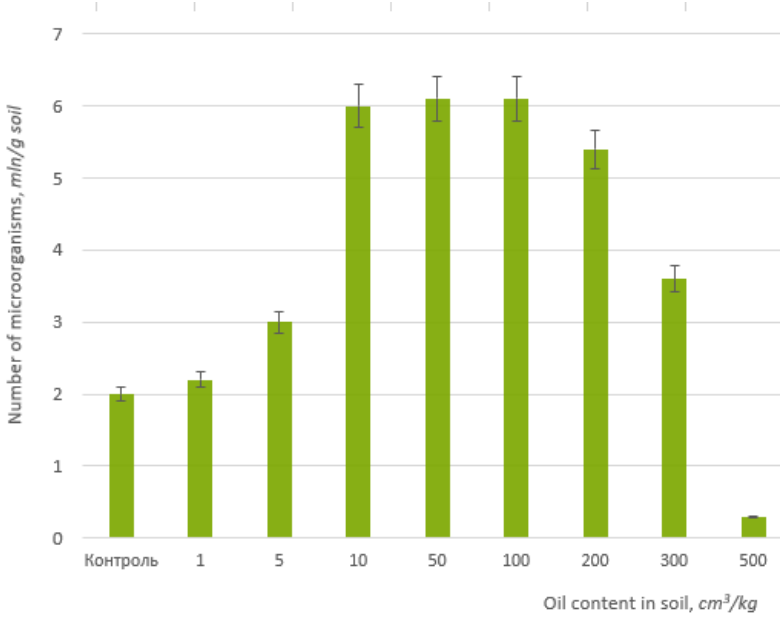

Fig. 4. *Impact of oil pollution on spore-forming bacteria*

Micromycetes

Unlike bacteria, which at an oil concentration in the soil at a level of 10-100 cm^3/kg showed an increase in the number of all physiological groups compared to the control, microscopic fungi demonstrated a sharp decrease in the number of colony-forming units (CFU) in this concentration range (Fig. 5). The obtained result somewhat contradicts the established idea of high resistance of microscopic fungi to contamination by oil products. However, in the range of high oil concentrations, their number remained at an almost constant level and at an oil load of 500 cm^3/kg was almost twice as high as the number of bacteria.

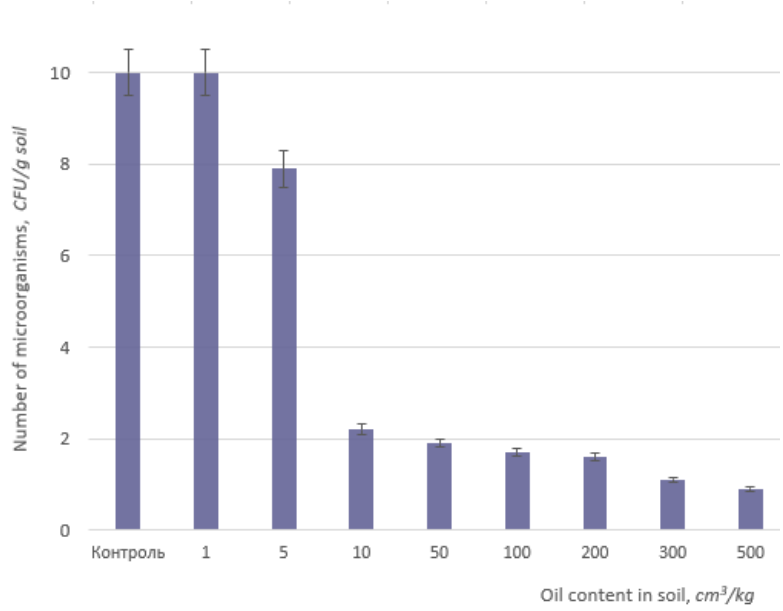

Fig. 5. *Impact of oil pollution on micromycetes*

Conclusions

The oil industry, playing an important role in the global economy, is one of the most intensive sources of environmental pollution. The production, transportation, storage and sale of oil and petroleum products significantly affect the state of the environment, leading to profound changes in all its components. In general, in oil-contaminated soils, there is a violation of the ecological balance in the soil system; a change in the morphological, physical, physicochemical and chemical characteristics of soil horizons and the structure of the soil profile; a violation of the natural ratio between individual groups and fractions of soil organic matter; penetration of oil and oil products into groundwater; reduction in soil fertility and the emergence of toxicologically dangerous situations. The absence of established MPC standards for most petroleum products makes it impossible to take into account the environmental hazard of the combined action of hydrocarbons. An integrated environmental assessment, which links the level of oil contamination of soils with the impact on plant test organisms, provides a quantitative determination of toxicity, pollutant content in the soil and the level of contamination and is the first attempt at a comprehensive assessment of such complex pollution as oil [11-12].

Microorganisms, due to their physiological and genetic characteristics, respond quickly to changes in environmental quality and the effects of stress factors. In this regard, they can be used to assess the degree and nature of environmental pollution. Indicators of biological pollution include microorganisms that secrete hydrolytic enzymes into the environment that can break down organic compounds. Microorganisms are very important for the restoration and purification of our environment. Through destruction, microorganisms are able to degrade and transform a huge number of synthetic and organic compounds and other chemicals that have toxic properties.

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ДОСЛІДЖЕННЯ РЕАКЦІЇ ҐРУНТОВОЇ МІКРОБІОТИ НА НАФТОВЕ ЗАБРУДНЕННЯ

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Анотація

Нафта та нафтопродукти є одними з найбільш небезпечних видів забруднення, яке може проявлятися на всіх етапах техногенезу. Розуміння змін, які відбуваються з ґрунтом при потраплянні в нього різних кількостей нафти, реакції живих організмів на забруднення дають можливість правильно визначати стан ґрунтової екосистеми та підбирати ефективні ремедіаційні заходи в конкретних умовах нафтового забруднення ґрунту. Тому метою даної роботи стало дослідження реакції різних ланок ґрунтової мікробіоти на нафтове забруднення.

Для визначення реакції ґрунтових мікроорганізмів на нафтове забруднення досліджували динаміку чисельності основних фізіологічних груп бактерій та мікроскопічних грибів у ґрунтах із визначеним вмістом нафти. У даному дослідженні використовували наступні концентрації нафти: 1; 5; 10; 50; 100; 200; 300 та 500 $см^3$/кг.

Дослідження динаміки мікрофлори у забруднених нафтою ґрунтах показало достовірне лінійне зростання чисельності мікроорганізмів різних екологічних та фізіологічних груп у концентраційному діапазоні 10÷100 $см^3$/кг. Подальше підвищення концентрації нафти призводило до зниження чисельності усіх груп бактерій окрім вуглеводнеокиснювальних. Концентрація нафти на рівні 500 $см^3$/кг майже повністю придушувала розвиток ґрунтової мікрофлори. Показано, що основу мікроценозу дослідженого ґрунту при високих концентраціях нафти складають спороутворюючі та вуглеводнеокиснювальні бактерії.

На відміну від бактерій мікроскопічні гриби продемонстрували різке зниження чисельності колонієутворюючих одиниць (КУО) при концентрації нафти у ґрунті на рівні 10÷100 $см^3$/кг. Проте в діапазоні високих концентрацій нафти їхня кількість зберігалася на майже постійному рівні і при нафтовому навантаженні на рівні 500 $см^3$/кг була майже вдвічі вищою за кількість бактерій.

Ключові слова: нафта, забруднення, ґрунти, мікробіота, токсичність, нормування.