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The Comparative Analysis of Indigenous Soil Bacteria and Their Oil-Degrading Potential in Certain Areas of the "Jarkurgan-Neft" Oil Fields

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Abstract: This article investigates the oil-degrading potential of indigenous bacterial strains isolated from various oil-contaminated soils and products in the Jarkurgan and Kumkurgan districts of Surkhandarya region. Based on the analysis of the quantity and quality of microorganisms present in collected samples of soil, oil sludge, crude oil, and water, 24 pure bacterial isolates—predominantly occurring species—were isolated. These strains were comparatively characterized in terms of their growth and oil-degrading activity in a minimal medium supplemented with crude oil as the sole carbon source. Additionally, nine bacterial isolates exhibiting high oil-degrading potential were studied under conditions of Raimondo synthetic medium containing 1.0–2.0% crude oil, focusing on their morphological characteristics and hydrocarbon utilization efficiency.

Keywords: Indigenous bacteria, oil-contaminated soil, bioremediation, crude oil, Raimondo nutrient medium, microbiota, bacteria, assimilation, growth rate, activity, oil degradation.

Introduction:

With population growth and industrial development, the demand for fossil fuels has increased, leading to the expansion of oil and gas industry activities. The processes of oil extraction, refining, and transportation generate waste that contains large amounts of toxic substances, which have a significant impact on the environment, particularly the soil ecosystem. Pollution can spread not only locally but also over long distances. As a result of such contamination, the stability of the soil biocenosis—especially the microbial communities—is disrupted, leading to ecological imbalance [1]. Pollution resulting from oil extraction can spread through soil and groundwater at various depths, thereby exacerbating environmental problems. Petroleum products are among the most significant chemical pollutants of essential natural components such as soil, water, and air. These substances contribute to the phytotoxicity of soil and hinder plant development [2].

Crude oil is a liquid fossil fuel composed of various high-molecular-weight hydrocarbons, and its presence in the soil environment leads to alterations in the microbiota. Although high concentrations of oil can cause the death of many microorganisms, over time, microbial communities specialized in hydrocarbon degradation may develop, thereby enhancing bioremediation processes [3]. Microorganisms capable of oxidatively degrading hydrocarbons can remain active for extended periods in oil-contaminated environments. Even when residual oil concentrations are below 10 g/kg, these microorganisms retain their biological activity. The presence of bacteria capable of utilizing hydrocarbons as the sole source of carbon and energy was first discovered approximately 80 ears ago [4]. However, high concentrations of oil (1–30 ml/kg) negatively affect the microbiological and ecological stability of the soil, disrupting its water-air balance. Microorganisms are ancient and widespread life

forms that play a crucial role in maintaining metabolic balance in natural environments. They regulate redox processes in the soil and, due to their sensitivity to environmental changes, are considered reliable bioindicators. According to research findings, when the concentration of petroleum hydrocarbons in soil exceeds 10%, it has a detrimental effect on the number of microorganisms [5].

However, low concentrations of oil can stimulate microbial growth, as oil-degrading microorganisms become activated. Oil also affects the activity of soil enzymes in different ways: in some cases, it enhances enzymatic activity, while in others, it suppresses it [6]. This effect depends on the type of substance and the properties of the soil. For instance, oil-contaminated soil often develops anaerobic conditions, which significantly alter the activity of soil enzymes—particularly oxidoreductases such as ferric reductase and catalase [7]. Once oil enters the soil, processes of fractionation and degradation begin. The efficiency of these processes depends on the enzymatic activity of the microbial communities present in the soil. Microorganisms capable of fully oxidizing hydrocarbons to CO_2 and water (mineralization) are widespread in natural ecosystems, and their activity is largely dependent on the presence of oxidase-group enzymes [8]. The sensitivity of enzyme activity can be ranked as follows: ferric reductase > catalase > urease > invertase [7]. The primary microorganisms responsible for hydrocarbon degradation in soil are bacteria and certain fungi. These include representatives of the genera Pseudomonas, Arthrobacter, Mycobacterium, Rhodococcus, Bacillus, Micrococcus, Klebsiella, among others [9].

This research is dedicated to studying the composition of the microflora present in the soils and certain products of the "Jarkurgan-Neft" area, isolating and purifying active strains, and investigating representatives with high oil-degrading potential.

METHODOLOGY

Soil samples were collected for microbiological analysis from oil-contaminated areas of the “Petromaruz-Uzbekistan” LLC site in Jarkurgan district and the “Lalmikor Neft” oil field in Kumkurgan district of Surkhandarya region. Sampling was carried out using a grid method from a depth of 0–20 cm in accordance with GOST 17.4.4.02–84 and R 54039–2010 standards. Additionally, samples of oil-separated water, oil sludge, and crude oil were selected as research objects.

Under laboratory conditions, the soil samples were cleaned and passed through a 2 mm mesh sieve. From each sample, 10 g of soil was taken and serially diluted in 90 ml of sterile water, then inoculated onto sterilized selective nutrient media. The oil sludge samples were processed in the same way, while the water samples were inoculated directly. As nutrient media, meat-peptone broth was used for bacteria, and Czapek medium was used for microscopic fungi. The diluted samples were inoculated using the deep plating method and incubated in a thermostat at 25–30 °C for 3–8 days [10]. Based on growth rate, morphological characteristics, quantity, and quality indicators, a total of 24 pure bacterial isolates were obtained from the studied samples. To assess the viability, growth activity, and hydrocarbon assimilation and degradation abilities of the isolated strains in an oil-containing environment—and to select promising strains for bioremediation—a solid artificial Raimondo nutrient medium was prepared. The medium consisted of the following components (g/L): Na_2CO_3 – 0.1; $CaCl_2$ – 0.1; $MnSO_4$ – 0.02; $FeSO_4$ – 0.01; $MgSO_4$ – 0.2; NH_4Cl – 0.2; NaCl – 3.0; Na_2HPO_4 – 1.5; KH_2PO_4 – 1.0. To this, 1% crude oil and 2% agar were added to prepare a solid medium. The medium was sterilized in an autoclave at 120 °C under 1 atmosphere of pressure for 30 minutes. After sterilization, the medium was poured into Petri dishes and allowed to solidify. Four bacterial strains were inoculated into each dish, which were then placed in a thermostat at 37 °C to begin the incubation process. The growth rate of the strains and their colony-forming activity in the presence of crude oil were observed.

A qualitative analysis was then conducted on the strains, and based on growth indicators, 14 out of the 24 isolated strains were selected. Subsequently, a new solid Raimondo nutrient medium was prepared by adding 2% crude oil and 2% agar. The medium was sterilized in an autoclave at 120 °C under 1 atmosphere of pressure for 30 minutes. After sterilization, it was poured into Petri dishes. Four bacterial strains were inoculated into each dish, which were then placed in a thermostat at 37 °C, and monitoring was carried out.

RESULTS AND DISCUSSION

According to the results of the study, 24 bacterial strains were incubated on solid nutrient medium containing 1.0% crude oil over varying time intervals (24–360 hours), and their growth was evaluated. Fourteen strains were individually inoculated into sectors of Petri dishes. After 5 days of incubation at 37 °C, the growth intensity and colony-forming characteristics of the strains were visually assessed. Based on the size of the growth and assimilation zones in the 1.0% oil-containing medium, the activity

levels of the isolates obtained from soils in different zones of the “Jarkurgan-Neft” site varied.

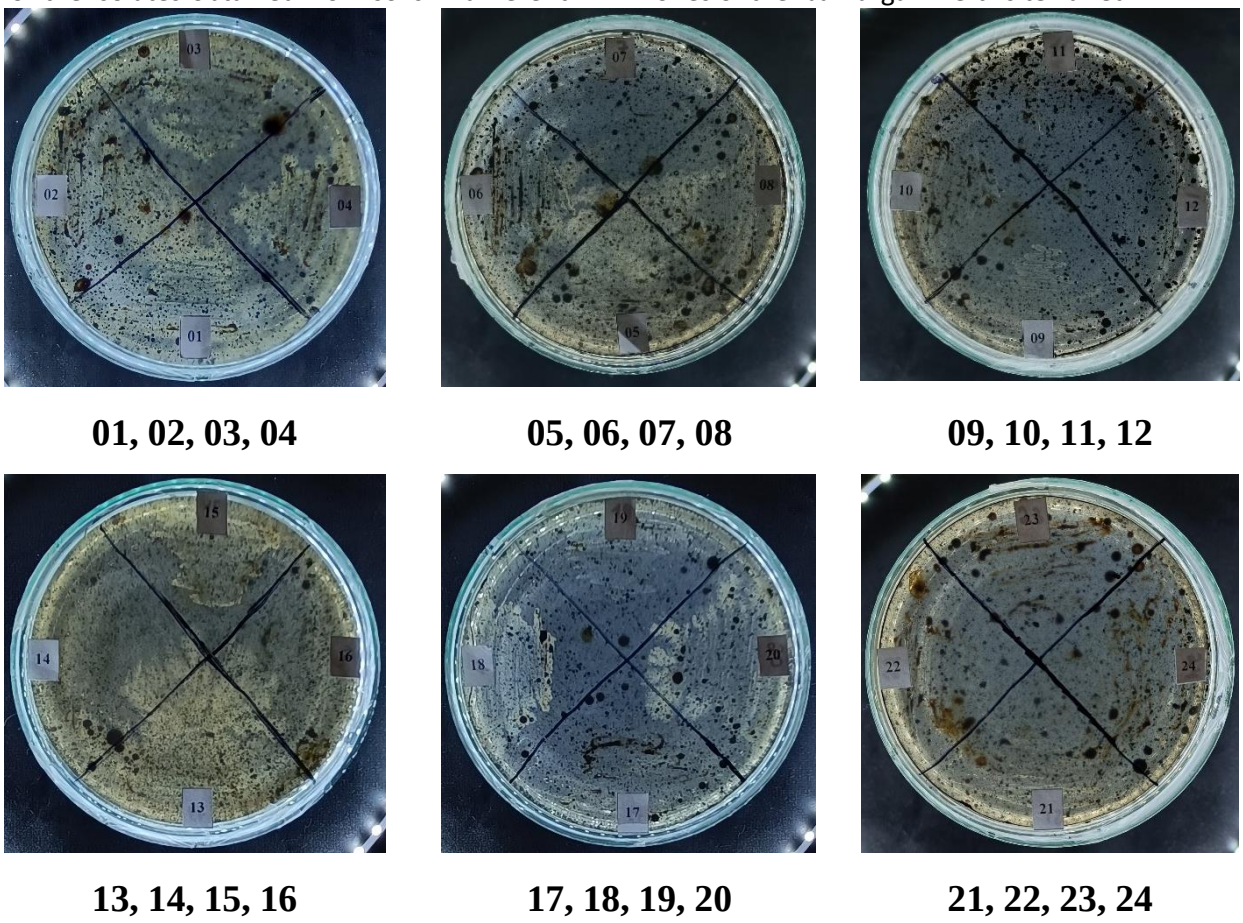


Figure 1. Oil assimilation levels of selected bacterial isolates obtained from the Jarkurgan oil fields on Raimondo nutrient medium supplemented with 1.0% crude oil. *Note: 01–24 refer to isolate numbers.

Early growth and oil assimilation were observed at 48–72 hours post-inoculation in the following isolates: JN-01, JN-04, JN-05, JN-06, JN-07, JN-13, JN-15, JN-18, JN-19, and JN-20. These isolates demonstrated good adaptability to the hydrocarbon-enriched medium, along with high activity and relatively strong oil-degrading capacity. Moderate-phase growth (96–168 hours) was observed in strains JN-08, JN-09, and JN-10. Although these strains became active more slowly, they still exhibited stable growth. Strains showing high growth activity (indicated in blue and dark blue): JN-04, JN-06, JN-07, JN-13, JN-18, and JN-20 demonstrated the highest growth intensity (0.7–0.8) and were identified as having significant potential for bioremediation applications.

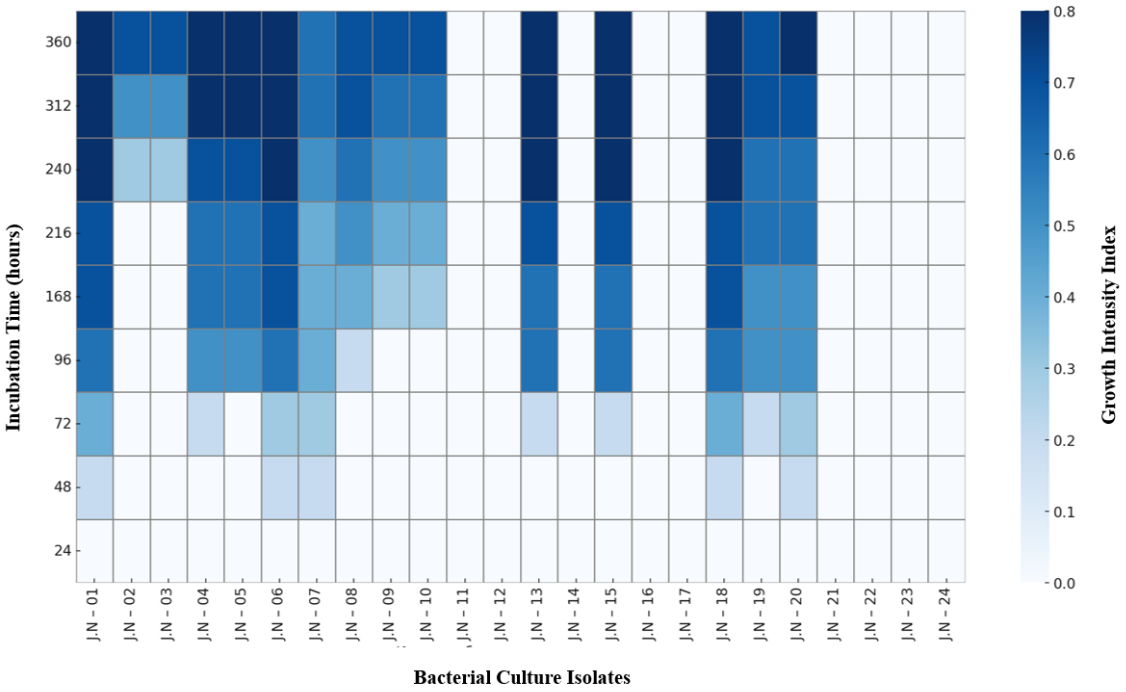
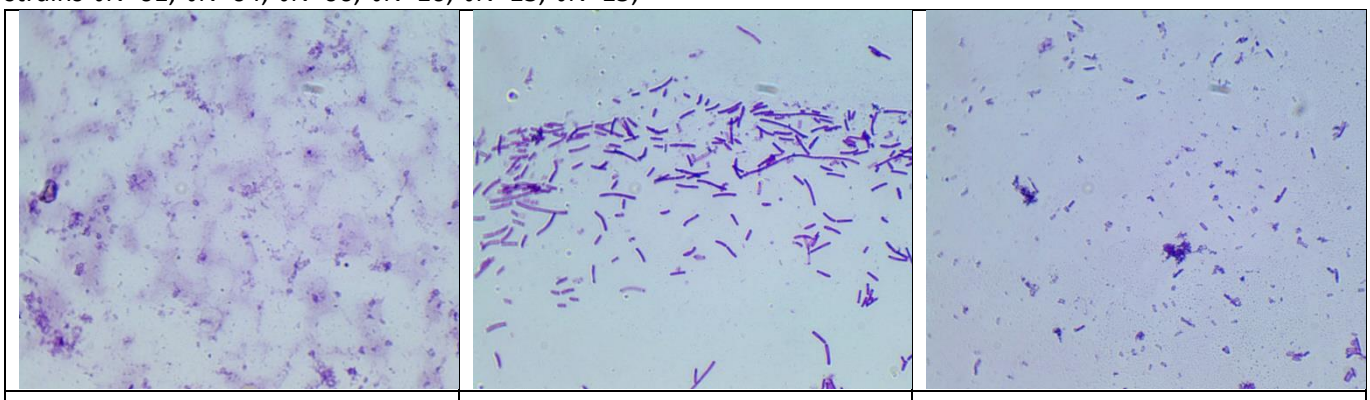


Figure 2. Growth and comparative oil assimilation indicators of 20 bacterial isolates obtained from soils of various zones of the "Jarkurgan-Neft" (JN) oil fields on oil-containing nutrient medium.

For each strain, the onset time of growth and growth intensity were monitored, and a total of 14 active strains were selected. These strains were re-incubated on Raimondo nutrient medium supplemented with 2% crude oil, and growth was assessed in a second phase. After 48 hours of incubation, clear signs of growth were observed in strains JN–01, JN–04, JN–06, JN–10, JN–13, JN–15, JN–18, and JN–20. Additionally, growth was recorded in strain JN–05 after 144 hours of incubation. Based on growth intensity and stability at the end of the incubation period, a total of 9 bacterial strains were identified as highly active. The morphological characteristics of these strains were then examined using microscopy.



JN - 1	JN - 4	JN - 5

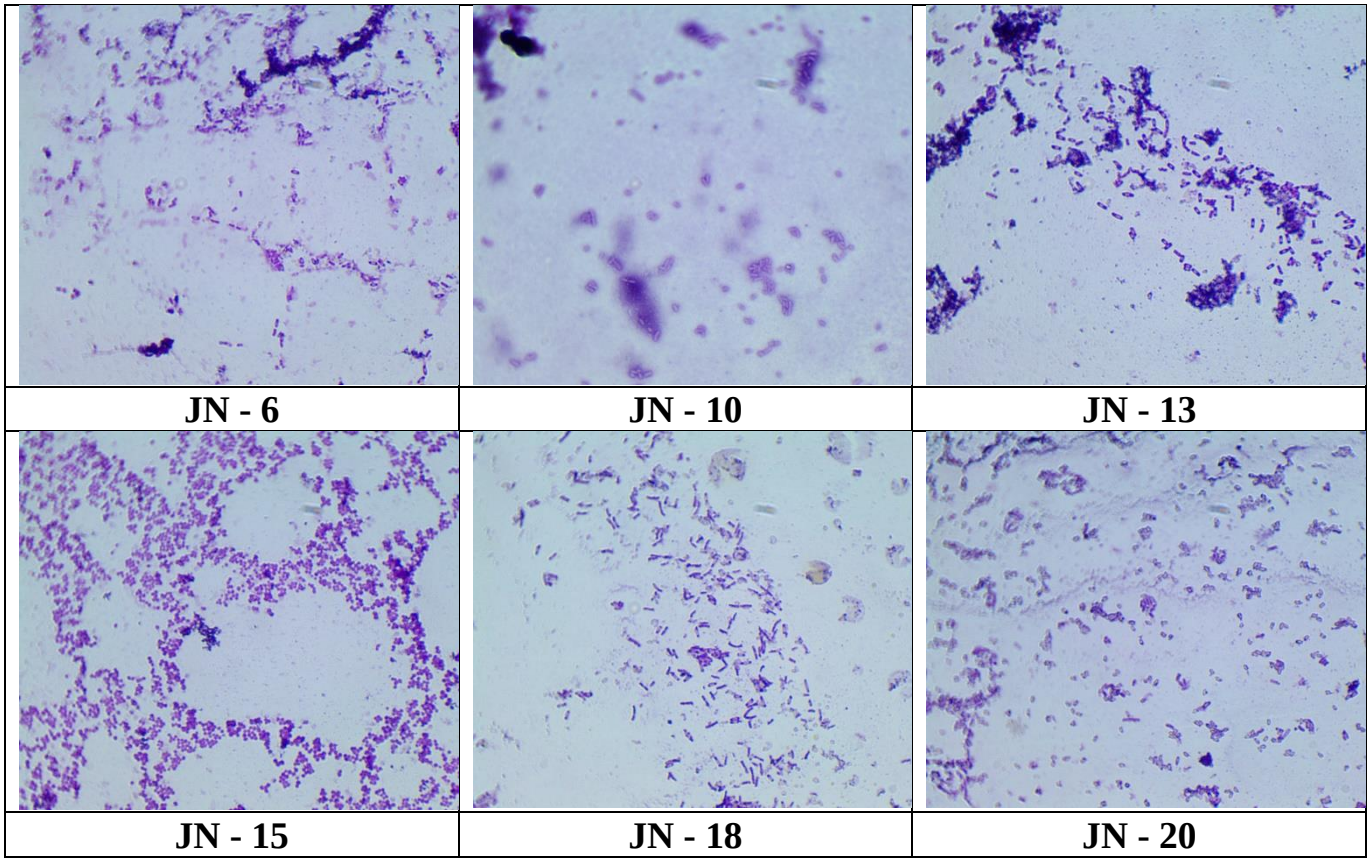


Figure 3. Microscopic images of the most active bacterial isolates obtained from oil-contaminated soils. JN – Jarkurgan-Neft.

Microscopic examination of the isolates was performed using Gram staining (1×1000). The strains exhibited morphological diversity, with the majority identified as Gram-negative rods and coccus-shaped forms. Some strains formed densely clustered colonies in the oil-containing medium, indicating their strong growth and assimilation activity in the presence of crude oil.

Table 1. – Protein synthesis dynamics by bacterial isolates in oil-containing liquid nutrient medium (mg/ml).

Isolated strains	Protein production dynamics (mg/ml) during growth over time (hours)			
	48	**72**	**96**	**120**
JN - 01	4,0	3,0	3.5	2.5
JN - 04	2.3	4,0	4.3	4,0
JN - 05	2,0	3.9	4.3	2.8
JN - 06	1.8	4,0	4,0	3.5
JN - 10	1.3	3.6	2.8	2.3
JN - 13	5.5	4.5	4,0	2.8
JN - 15	2.3	3.8	3.3	3.5
JN - 18	3.3	3.9	4	4.3
JN - 20	2.8	3,0	2.8	2.8

The growth activity of bacterial strains in liquid nutrient medium supplemented with 1% crude oil

was evaluated at different incubation times. According to the results, some strains began active growth as early as 48 hours (e.g., JN–13 — 5.5 mg/ml), while others reached their maximum growth levels at 72–96 hours (e.g., JN–04, J.N–18). Notably, strain JN–18 exhibited a growth level of 4.3 mg/ml at 120 hours. These findings indicate differences in the strains' adaptability to crude oil and their hydrocarbon assimilation activity. During incubation, most strains showed an initial increase in growth, followed by stabilization or a slight decline. This trend indicates that the substrate in the nutrient medium gradually approached saturation, leading to limitations in microbial activity.

CONCLUSION

The conducted research confirmed the presence of indigenous bacteria in oil-contaminated soils surrounding the "Jarkurgan-Neft" oil field and revealed their high adaptability to petroleum-polluted environments. A total of 24 bacterial isolates were obtained, of which 14 demonstrated active growth in media containing 1–2% crude oil. Among them, 9 strains stood out with high growth intensity and strong oil-degrading capabilities. Their growth dynamics on Raimondo nutrient medium and visible colony formation indicated not only efficient hydrocarbon assimilation but also stable survival in oil-rich environments.

Furthermore, experiments conducted in oil-containing liquid media recorded protein synthesis dynamics over various incubation periods. Notably, strains such as JN–13 and JN–18 exhibited the highest protein production levels, reflecting their elevated metabolic activity and relevance for bioremediation processes.

These results confirm the potential to isolate effective oil-degrading strains from local microflora and develop environmentally friendly biotechnological methods and biopreparations based on them. In the future, it is advisable to investigate the genomic structure, enzymatic activity, and interactions of these strains with plants.

REFERENCES

Parkhomenko, A. N. (2011). Impact of petroleum product and sulfur pollution on the soil microflora of the arid zone: Author's abstract of the PhD thesis in biological sciences (03.02.08). Ufa, 26 p.

Majona, M., & Vasanthi, P. (2021). Bioremediation of petroleum-contaminated soils – A review. Materials Today: Proceedings, 45, 7117–7122.

Shamraev, A. V., & Shorina, T. S. (2009). Influence of oil and petroleum products on various components of the environment. Bulletin of the Orenburg State University, (6).

Sivaraman, C., Ganguly, A., & Mutnuri, S. (2012). Cloning and expression of catechol 2,3-dioxygenase and cyclodextrin glucanotransferase genes in E. coli and their role in biodegradation. Vol. 2, No. 3.

Kireeva, N. A., & Yamaletdinova, G. F. (2000). Phenoloxidase activity of oil-contaminated soils. Bulletin of Bashkir State University, (1), 48–51.

Aliev, S. A., Gvozdenko, D. V., Babaev, M. P., & Gadzhiev, D. A. (1981). Recommendations for the reclamation of oil-contaminated lands. Baku: Elm, 26 p.

Bocharnikova, E. D. (1990). The impact of oil pollution on the properties of serozem soils of Absheron and gray forest soils of Bashkiria. Moscow: Nauka, 160 p.

Murzakova, G. B., Biteeva, M. B., & Morshchakova, G. N. (1992). Biotechnology for the remediation of oil-contaminated areas: Review information. Moscow: NIISENTI.

Kvasnikov, E. I., & Krivisky, I. P. (1968). Some regularities in the distribution of hydrocarbon-assimilating microorganisms in soils of oil fields. Microbiology, 37(2), 321–326.

Abdullayeva, Y. A., & Akhmedova, Z. R. (2024). Microbiological monitoring of soils contaminated with oil and petroleum products at the "Petromaruz Uzbekistan" LLC site. In International Scientific-Practical Conference on "The Current State and Prospects for the Development of Fundamental and Applied Microbiology: A View of Young Scientists" (pp. 474–478).