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ABSTRACT

of the dissertation presented for the degree of Doctor of Science

MICROBIOLOGICAL ASSESSMENT OF TECHNOGENICALLY CONTAMINATED SOILS AND NATURAL LAKES OF ABSHERON AND DEVELOPMENT OF BIOTECHNOLOGICAL REMEDIES

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INTRODUCTION

Relevance and degree of development of the topic. In recent years, environmental problems have become a "constant companion" of modern society in the process of its development, and, therefore, the main object of contemporary research is the environment changing under the influence of anthropogenic factors. The escalating impact of anthropogenic activity is causing alarming global changes, particularly evident in the degradation of soil and water, the fundamental components of the biosphere. Therefore, researchers face several critical environmental challenges requiring urgent resolution: it is necessary to develop scientific methods for the protection and restoration of soil cover and water systems, which are irreplaceable resources that ensure the genetic diversity and habitats of plants, animals, and microorganisms, and are essential for agriculture, forestry, water management, and human life. A thorough analysis of these processes requires regional studies integrating scientific observations to evaluate the evolution and functional dynamics of landscapes and ecosystems.

"*Although the oil industry is economically beneficial, it gives rise to numerous environmental problems"*[1]. In the modern era, these problems have become evident across nearly all aspects of the environment. The extraction, transportation, refining, and consumption of petroleum "*generate hazardous technogenic waste, contaminating fragile ecosystems - soil, water, and air - while disrupting biocenoses"*[2]. Hydrocarbon contamination disrupts the structure and function of soil microbiocenosis, reducing microbial abundance and diversity, and ultimately causing partial or complete soil degradation.

Petroleum is a primary energy resource and serves as a driving force in industrial development both in our country and globally. While

[1] Наджафова, С.И., Кейсерухская, Ф.Х., Исмаилов, Н.М. Оценка биогенных ресурсов ассимиляционного потенциала основных типов почв Азербайджана в отношении органических загрязнений //Агрохимия, – 2022, № 5, – с. 87–932

[2] Двадненко, М.В., Маджигатов, Р.В., Ракитянский, Н.А. Воздействие нефти на окружающую среду // Международный журнал экспериментального образования, – 2017, № 3-1. – с. 89-90;

oil production is economically significant, it also has negative consequences that exacerbate environmental problems. Therefore, although prioritizing alternative energy sources is considered necessary to prevent the environmental problems caused by oil production, oil extraction remains at a significant level. In Azerbaijan as well, the scale and the impact of the oil industry are evident in the Absheron region. Even now, *"the Absheron Peninsula and Caspian Sea region of Azerbaijan continue to experience intensive natural resource extraction, with significant environmental consequences"*[3].

Azerbaijan's petroleum fields in the Caspian Sea *"have yielded over 2.1 billion tons of oil and 900 billion m³ of natural gas"* [4].

The degree and scale of technogenic pressure caused by petroleum pollutants are incredibly high, and this is reflected in the ecological condition of both the soil cover and water bodies.

For this reason, the Absheron Peninsula faces urgent environmental challenges, requiring comprehensive remediation of hydrocarbon-contaminated soils and restoration of degraded aquatic ecosystems. Numerous studies over the years have explored land restoration in regions affected by technogenic pollution, yielding significant findings for ecological recovery.

Scientific research in the Absheron Peninsula has yielded scientific insights into oil-contaminated soil remediation, including: *"ecological evaluation of contaminated soils"*[5], *"the challenge of treating and repairing the productivity of contaminated soils"*[6], *"ecological assessment criteria"*[7], *"the role and importance of oil-oxidizing*

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[3] https://www.civilnet.am/ru/news/785163/

[4] Гасумов, Э.Р. Использование и добыча природного газа в Азербайджане // American Scientific Journal, – 2020. – № 42-2(42). – с. 24-28

[5] Агакишибекова С.Ю. Современное состояние нефтезагрязненных почв Апшеронского полуострова. Почвоведение-продовольственной и экологической безопасности страны, – 2016, – с. 51-52

[6] Исмаилов Н.М. Научные основы практической экобиотехнологии. М.: ИНФРА-М., 2020, – 414 с.

[7] Наджафова, С. И., Гасанова, З. П., Исмайлов, Н. М. Биотехнологии очистки нефтезагрязненных почв Апшеронского полуострова и интегральный показатель их биологического состояния // «Живые и биокосные системы»., – 2019. – № 27, – с.77-81.

microorganisms"[8], *"optimized treatment methodologies, and practical bioremediation applications in the Absheron peninsula"*[9], and *"phytoremediation approaches for (oil) petroleum hydrocarbon contamination"*[10].

It's important to note that current knowledge of Absheron's contaminated ecosystems remains fragmented, with studies of microbiota and ecological assessments conducted in isolation. This research will address two unmet needs: (1) an integrated bioecological evaluation of soil-water systems, and (2) development of native microbe-based bioremediation technologies tailored to local conditions.

Currently, a significant concern is the restoration of the biological and ecological quality of the low-biogenic soils in the Absheron industrial region through the application of modern biotechnologies to ensure the prompt recovery of their biological functions and enhance their biological productivity.

The urgency of addressing this contamination grows increasingly critical each year, particularly in Baku, a major metropolitan area in need of valuable reserves of ecologically clean water, air, and land resources.

Studying the development of microbiological and biochemical patterns that reflect the direction and intensity of biological transformations in soil and water during contamination with petroleum products and heavy metals—two of the primary pollutants in the Absheron industrial region—is of great importance.

Therefore, considering the pivotal role of microorganisms in self-purification processes, analyzing the state of the microbiocenosis—as an indicator reflecting soil and water quality—remains consistently essential.

Biological restoration methods are increasingly prioritized in the

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[8] Вердиева Ф.Б, Алиева Т.Р, Исмайлова М.Э. Рекультивация нефтезагрязненных земель Апшеронского полуострова. Бюллетень науки и практики, – 2022;8(5), с.186-91.

[9] Наджафова С. И., Гасанова З. П., Исмайлов Н. М., Биотехнологии очистки нефтезагрязненных почв Апшеронского полуострова и интегральный показатель их биологического состояния // «Живые и биокосные системы», – 2019. – № 27; – с.186-191

[10] Султанова ГД, Абдуллаева М. Очистка нефтезагрязненных земель в климатических условиях Азербайджана. Бюллетень науки и практики, – 2021;7(7) – с.31-38.

modern era, given their advantages in terms of both environmental safety and economic efficiency.

The United Nations' designation of the 21st century as the 'Century of Biotechnology,' along with the attention given by the President of the Republic of Azerbaijan *to the remediation of contaminated soils and lakes"*[11,1213], once again underscores the relevance of researching scientifically and methodologically grounded projects aimed at improving their bioecological condition.

The object and subject of the study are various microorganisms distributed in the soils and some natural lakes of the Absheron Peninsula affected by technogenic pollution.

The purpose and objective of the research are to investigate the microbiological and biochemical characteristics of soil and water bodies exposed to technogenic impacts in the Absheron industrial zone and to explore approaches for restoring their biological activity and ecological functions. In line with this objective, the following research tasks have been formulated:

1. Investigation of the key microbiological and enzymatic processes in gray-brown soils and natural lakes of the Absheron Peninsula affected by technogenic pollution.
2. Assessment of the impact of various pollutants on selected microbiological and biochemical indicators of the studied soils and natural lakes.
3. Determination of the population dynamics of selected indicator groups of microorganisms in technogenically degraded soils and natural lakes of the Absheron Peninsula.
4. Isolation of hydrocarbon-degrading microorganisms and

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[11] "Azərbaycan Respublikasında ekoloji vəziyyətin yaxşılaşdırılmasına dair 2006-2010-cu illər üçün Kompleks Tədbirlər Planı" // Azərbaycan Respublikası Prezidentinin 2006-cı il 28 sentyabr tarixli № 1697 Sərəncamı ilə təsdiq edilmişdir- Bakı, – 2006

[12] "Azərbaycan Respublikası Prezidentinin "Böyükşor gölünün ekoloji vəziyyətinin yaxşılaşdırılması, mühafizəsi və istifadəsi sahəsində əlavə tədbirlər haqqında" 2013-cü il 26 dekabr tarixli 61 nömrəli Fərmanı Bakı, – 2013

[13] "Zığ gölünün ekoloji vəziyyətinin yaxşılaşdırılması, mühafizəsi və ondan istifadə sahəsində əlavə tədbirlər haqqında" 2014-cü il 15 oktyabr tarixli 774 nömrəli Sərəncamı- Bakı, – 2014

selection of active strains from the soils and lakes of the Absheron Peninsula under study.

5. Selection of plant species characteristic of this soil-climatic zone that support the accumulation of hydrocarbon-degrading microbial populations in their rhizosphere.

6. Investigation of the self-purification capacity of the soil cover and lakes of the Absheron Peninsula from petroleum hydrocarbons and heavy metals, and the development of modern biotechnologies (microbiological preparations) using regional bioresources to enhance this capacity.

7. Study of the impact of the developed biotechnologies on the restoration dynamics of the bioecological properties of contaminated soils and water bodies.

Research methods. To address the objectives, a combination of established microbiological, chemical, and biotechnological methods and approaches was employed to study the soil and water environments. During the research, screening methods for microorganisms capable of actively degrading organic pollutants, phytotoxicity assays, phytoremediation techniques, and mathematical statistical analyses were utilized.

The main provisions of the defense.

1. In the gray-brown soils of the Absheron Peninsula, the quantitative dynamics of certain indicator microorganism groups and the enzymatic processes occurring in the soil exhibit specific characteristics depending on the level of technogenic impact. Furthermore, the quantitative and qualitative composition of the microbiocenosis differs from that of uncontaminated soils.

2. The use of hydrocarbon-oxidizing microorganisms (HOMs) isolated from technogenically polluted soils in the region for the development of modified biological preparations yields more effective results in restoring these soils.

3. In soils affected by technogenic pollution, the association between microorganisms and plants is accompanied by a sustained increase in the number and biomass of rhizosphere microorganisms, including those that degrade hydrocarbons, which underpins the effectiveness of the phytoremediation process.

4. Inoculating plant seeds with preparations containing hydrocarbon-degrading microorganisms has a positive influence on plant growth and development, as well as the biological remediation of contaminated soils.

5. Activated sludge and our modified multifunctional microbial preparation, 'Fermi-Start,' exhibit a high potential for heavy metal adsorption and can be used for the remediation of water bodies contaminated with heavy metals.

6. The extensive use of regional bioresources in the biological remediation of soils and water bodies contaminated by technogenic impacts contributes to the establishment of a closed-loop biotechnological system in the Absheron industrial region.

Scientific novelty of the research. For the first time, the bioecological condition of technogenically polluted soils and lakes of the Absheron Peninsula has been comprehensively studied as a unified, functioning ecosystem. It was established that the biological activity of both soil cover and water bodies varies according to the degree of technogenic pollution.

For the first time, modified biopreparations based on HOMs and regional biowaste were developed to enhance the biological activity of soils and their self-purification capacity from oil and petroleum products. Additionally, the optimal application rates for these preparations were established.

The potential of newly modified microbial–plant associations for remediating contaminated soils on the Absheron Peninsula has been demonstrated. Associations that include alfalfa *(Medicago sativa),* Bermuda grass *(Cynodon dactylon), Pseudomonas aeruginosa* strain No. 3, and the biological product *”Fermi-Start”* were found to enhance microbial populations in the rhizosphere, sustain their activity, reduce petroleum hydrocarbon concentrations in the soil, and bolster the resistance of gray-brown soils to hydrocarbon contamination.

It was determined that the concentrations of hydrocarbons and certain heavy metals in the lakes of the Absheron Peninsula, which are exposed to technogenic impacts, significantly exceed permissible limit values. These levels vary depending on the sampling location and the season.

The technogenically impacted lakes on the Absheron Peninsula were found to contain *Pseudomonas sp. strains No. 45, 21, 33, and 64; Rhodococcus sp. strains No. 7 and 12;* and *Micrococcus sp. strain No. 22*, all of which demonstrated high hydrocarbon-degrading potential. Based on these active microorganisms, the scientific foundations for the development of biological preparations were established.

For the first time, the scientific foundations for the modification of (multifunctional) biobonds based on active hydrocarbon-oxidizing bacteria have been developed for the effective remediation of lakes on the Absheron Peninsula.

Theoretical and practical significance of the research. The data obtained from this research will contribute to the expansion of the knowledge base on the microbiological characteristics and ecological conditions of technogenically polluted gray-brown soils and natural lakes on the Absheron Peninsula.

The developed technologies and approaches are effective for the bioremediation of gray-brown soils on the Absheron Peninsula, which have been impacted by technogenic activities, enabling their restoration and transformation into soils with enhanced productivity.

The principles and developed technologies for the remediation of water bodies on the Absheron Peninsula contaminated by oil, petroleum products, and heavy metals provide extensive opportunities for the efficient utilization of limited water resources in arid regions for recreational purposes.

For these reasons, the conducted research offers both environmental and economic advantages. The developed biotechnologies contribute to the conservation of natural resources by enabling the efficient utilization of renewable (secondary) raw materials, preventing the discharge of organic substances with high biological oxygen demand (BOD) into the environment, and reducing the need for substantial financial expenditures on environmental protection.

Publication, approbation and implementation of the dissertation. A total of 40 scientific works related to the dissertation topic have been published, including 1 book, 17 articles, and 22 conference proceedings.

Materials of the dissertation were presented at the İnternational Symposium: "Modern Biology: Current Issues" (Saint Petersburg, 2015) international conference; Eurasian Union of Scientists XV Int. Scientific and Practical Conf. "Modern Concepts of Scientific Research" (Moscow, 2015); "Natural and anthropogenic changes in arid ecosystems and the fight against desertification (Makhachkala, 2016) international conference; "Indication of the state of the environment: theory, practice, education" international conference (Moscow, 2017); 1st International Conference of the European Academy of Science: Federal Ministry of Education and Research. **(**Germany, 2018); the International Conference of the Russian Academy of Natural Sciences on “Regional Strategies and Projects: Environmental and Economic Aspects of Development and Implementation” (Russia, Moscow, 2020), the II International Scientific-Practical Conference on “Actual problems of land management, cadastre, and environmental management” (Russia, Voronezh, 2020), scientific-practical. conf. RANS "Strategic regional projects and ecological-economic and social development of territories" (Moscow, 2021); Int. scientific-practical. conference "Strategic projects and implementation of resource-saving and environmentally friendly technologies in the regions" RANS (Moscow, 2022); "Innovative biotechnologies for environmental protection environment: from theory to practice", (Minsk, 2024), "Actual problems of microbiology, biotechnology and biodiversity" International conference on the topic (Astana, 2024), National conferences: "Actual problems of the century and development prospects in the conditions of globalization" International scientific-practical conference (Ganja, 2014), "Actual problems of modern biology and chemistry" scientific-practical conference (Ganja, 2015), "Actual problems of modern chemistry and biology" International conference materials (Ganja, 2016), Conference dedicated to the anniversary of "Academician Hasan Aliyev -110" (Baku, 2017), Republican scientific-practical conference on the topic "New trends and innovations: prospects for the development of microbiology in Azerbaijan" (Baku, 2022); IV Republican scientific conference on the topic "Ecology: problems of nature and society" Baku-2023), Republican scientific conference "Actual problems of biology in the context of sustainable development" dedicated to the 100th anniversary of the National Leader Heydar Aliyev. (Baku, 2023), International Soil Science Congress on «Climate change and sustainable soil management» (ICEFSSS, Baku, 2023)

Name of the Institution Where the Dissertation Research Was Conducted. The dissertation research was conducted (during 2015-2025) at the Soil Microbiology Laboratory of the Institute of Microbiology, Ministry of Science and Education of the Republic of Azerbaijan.

Structure and Scope of the Dissertation: The dissertation consists of an introduction, a literature review (Chapter I), a description of the research objectives and methods (Chapter II), the experimental section (Chapters III–V), a final analysis of the research, conclusions, main results, practical recommendations, a list of used literature and abbreviations. All this consists of a total of 441866 characters.

CHAPTER I
FUNCTIONAL CHARACTERISTICS OF TECHNOGENIC LANDSCAPES

Sections 1.1 and 1.2 of the dissertations address the issues of bio- and ecodiagnostics of oil-contaminated soils, as well as the effects of oil pollution levels on the physicochemical properties of soils. Section 1.3 reviews various approaches to the systematic assessment of the functional-ecological characteristics, resilience, and self-purification capacity of soils. Section 1.4 (subsections 1.4.1–1.4.5) examines the biological diagnostics and indication methods for technogenically polluted soils, focusing on the impact of hydrocarbon contamination on the structure and functional activity of the soil microbiocenosis, algal communities, soil invertebrates, higher plants, and enzymatic activity. Section 1.5 discusses the microbial degradation of petroleum hydrocarbons and its influence on the intensity of natural self-purification processes. Section 1.6 is devoted to soil remediation methods, emphasizing the roles of higher plants and microorganisms in bioremediation. Section 1.7 (subsections 1.7.1–1.7.2) examines the problem of hydrocarbon and heavy metal contamination in water bodies, including the effects of petroleum hydrocarbons on aquatic ecosystems, the fundamental principles of natural water self-purification from organic pollutants, and the management of these processes. Finally, Section 1.8 provides a detailed analysis of the literature on the restoration of biological activity in water bodies contaminated with heavy metals, offering a comprehensive overview of the scientific research conducted in this area.

CHAPTER II
MATERIALS AND METHODS

2.1. Ecological Characteristics of the Study Area: Soil and Vegetation Features

This section presents information on the general characteristics of the study area, including climatic conditions, topography, hydrogeological features, and soil and vegetation cover. It also provides an analysis of the region's current ecological status. The research was carried out between 2015 and 2025, based on water samples collected from technogenically polluted gray-brown soils in various parts of the Absheron Peninsula (Bibi-Heybat, Binagadi, Balakhani, Sabunchu, Ramana, Bina, and Surakhani), as well as from natural lakes such as Boyuk-Shor and Zigh.

2.2. Soil Analysis Methods

Soil samples for the study were collected seasonally by established “standards”[14]. The isolation and enumeration of various

[14] ГОСТ «Охрана природы. Почвы. Методы отбора и подготовки проб для химического, бактериологического, гельминтологического анализа». – М., стандартинформ, – 2008, – с.7

physiological groups of microorganisms were carried out using the agar plate cultivation method.

Microorganisms were identified at the genus level based *on their cultural, morphological, and physiological characteristics"*[15]. "Soil "*enzymatic activity was assessed using generally accepted methods"*[16], while respiration intensity was determined using the *"Makarov method"*[17].

"*The degree of phytotoxicity was evaluated based on the germination rate of test plants"*[18].

The *"mineralization coefficient"*[19] was determined using Ismayilov's method. The phytoremediation of oil-contaminated soils was investigated using complex systems composed of plant–microorganism combinations. The experiments were conducted on soil samples artificially contaminated with crude oil at a concentration of 15 g/kg. Pollution-resistant plant species, including alfalfa *(Medicago sativa L.)* and Bermuda grass *(Cynodon dactylon (L.) Pers.),* were selected for the study. For seed bacterization, the hydrocarbon-oxidizing bacterial strain *Pseudomonas aeruginosa No. 3*, isolated during the experiments, was used in combination with the biological product "Fermi-start."

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[15] Лысак, Л.В. Методы оценки бактериального разнообразия почв и идентификации почвенных бактерий. / Л.В. Лысак, Т.Г. Добровольская, И.Н. Скворцова. – М.: МАКС Пресс, –2003. –121с.

[16] Казеев, К.Ш., Колесников, С.И., Вальков, В.Ф. Биологическая диагностика и индикация почв: методология и методы исследований. Ростов-на-Дону, Изд-во Рост, ун-та, – 2003. – 204 с.

[17] Макаров, Б.Н. Упрощенный метод определения дыхания почвы // Почвоведение, –1957. № 9, – с. 119-122.

[18] Trofimov, I., Pavliukh, L., Novakivska, T., Bondarenko, D. // Assessment of phytotoxic toxicity of mixed aviation fuels using plant testers / International independent scientific journal, –2020. №11. – p.9–17.

[19] Исмаилов, Н.М., Гаджиева В.И., Гасанова М.Г. Коэффициент минерализации углеводородов как показатель самоочищающей способности нефтезагрязнённых почв // Известия АН АзССР, серия биологическая, – 1984.№ 6. – с. 76-85.

2.3. Research Methods for Water Systems

Water samples for the study were collected seasonally using *"traditional methods"*[20]. *"Chemical analysis of the water was conducted using standard procedures"*[21]. The component composition of petroleum contamination in the samples was analyzed using *"chromatographic techniques"*[22]. The concentrations of heavy metals were determined by photometric analysis with a Palintest Photometer at a wavelength of 520 nm.

The identification of microbial species capable of degrading oil and petroleum products, as well as adsorbing heavy metals, was *"conducted using standard microbiological methods"*[23].

"Statistical analysis of the results was performed using the Lakin[24]*, Excel 2007", and "Statistica V6.0"*software programs. The Student's t-test was applied to assess the statistical significance of the mean values obtained. The results presented in the tables and graphs are expressed as means with standard deviations.

EXPERIMENTAL PART
CHAPTER III
ASSESSMENT OF THE BIOLOGICAL ACTIVITY OF TECHNOGENIC SOILS OF THE ABSHERON PENINSULA

3.1. Assessment of the Functional and Ecological Condition of Oil-Contaminated Soils in the Absheron Peninsula

This subchapter provides an analysis of the total area and degree of contamination of gray-brown soils on the Absheron Peninsula

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[20] Романенко В., Кузнецов С. Экология микроорганизмов пресных водоемов Ленинград: Наука, 1974. –192с.
[21] Определение массовой концентрации нефтепродуктов в воде. Методические указания. МУК 4.1.1013-01.-М.: 2001.
[22] Методика хроматогрвфического анализа: ГОСТ 2177-99. Нефтепродукты. Определение фракционного состава. М.: ИПК. -1999.
[23] Определитель бактерий Берджи, Том 1-422с; Том 2. Дж. Хоулт . 1997. – 325с
[24] Лакин Г.Ф. Биометрия. М.: Высшая школа, IV издание, 1990. - 352 с.

affected by technogenic pollution, along with a review of research studies addressing the issue of maximum allowable concentrations (MAC) for oil and petroleum hydrocarbons in these soils.

For the research, a total of 101 soil samples were collected from the territories of the Oil and Gas Production Department (named after A. Amirov, Bibi-Heybat, Balakhani, and Z. Taghiyev) to carry out a systematic analysis of the functional and ecological state of the soil cover. The total hydrocarbon content in the samples was determined. According to the analysis results on the degree of oil pollution in these areas, it was revealed that in some samples, the hydrocarbon content exceeded 10%, while in others, it was up to 10%.

Based on the average pollution indicators, an integrated ranking of the territories of the studied Oil and Gas Production Departments (OGPDs) was carried out according to the decreasing levels of soil contamination with hydrocarbons. According to the results, the OGPD territories are ranked as follows:

Bibi-Heybat > Z. Tagiyev > A. Amirov > Balakhani

3.2. Characteristics and Microbiological Activity of Gray-Brown Soils Affected by Technogenic Impact on the Absheron Peninsula

Gray-brown soils are predominant on the Absheron Peninsula, and arid climatic conditions, along with a xerophytic-ephemeral type of vegetation, shape their structure and properties. Under such environmental factors, the accumulation of organic matter in the soil is minimal, and its rapid mineralization is commonly observed. Consequently, the soils of Absheron, like other gray-brown soils of desert origin, are characterized by a very thin humus layer, low humus content, limited depth, and poor structural composition. The introduction of hydrocarbons into the environment affects not only the soil's physicochemical properties but also its biological characteristics. In the soil microbiota, both qualitative and quantitative changes are observed at the population and cellular levels. In the oil-contaminated soil samples collected from the studied

Table 1.

Amount of microorganisms isolated from soil samples taken from the Oil and Gas Production Areas

№	Sampling areas	Total number of microorganisms, CFU/g soil	Hydrocarbon-oxidizing microorganisms CFU/g soil	Denitrifying microorganisms, CFU/g soil	Nitrifying bacteria, CFU/g soil	Cellulose-decomposing microorganisms CFU/g soil
1	Bibi-Heybat	1,7±0,12 x 10^4	3,7±0,12 x10^4	4,9 ±0,2 x 10^4	1,1±0,12 x 10^2	1,2±0,11 x 10^2
2	A. Amirov	2,3±0,50 x 10^4	3,5±0,50 x10^4	3,4 ±0,4 x 10^4	1,4±0,23 ×10^2	1,3±0,3 x 10^3
3	Binagadi	3,7±0,61 x 10^4	2,1±0,61 x10^5	2,2 ±0,21 x 10^4	1,1±0,3 x10^2	1,7±0,14 x 10^3
4	Balakhani	3,2±0,2 x 10^4	4,5±0,2x10^4	2,1 ±0,11 x 10^4	2,3±0,5 ×10^2	2,1±0,22 x10^3
5	Sabunchu	2,4±0,11 x 10^4	2,4±0,11x10^4	2,3±0,12 x 10^4	3,2±0,2 x10^2	1,6±0,6 x 10^3
6	Ramana	3,6±0,43 x 10^4	2,6±0,43x10^4	2,6±0,2 x 10^4	1,5±0,42 x10^2	1,8±0,4 x10^3
7	Z. Tagiyev	2,2±0,11 x 10^4	2,5±0,11x10^5	2,2±0,2 x10^5	1,3±0,12 ×10^3	1,2±0,2 x10^3
8	Surakhani	3,7±0,11 x 10^4	2,7±0,11x10^5	4,2±0,23 x 10^4	1,2±0,24 x10^2	2,2±0,13 x10^3
9	Control	4,9±0,11 x 10^5	1,7±0,12x10^3	1,8±0,12 x10^3	4,0±0,12 x10^4	4,8±0,26 x10^4

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areas, the total number of heterotrophic microorganisms, as well as those belonging to various physiological groups, were isolated using specific nutrient media. It was determined that while the group composition of microorganisms in oil-contaminated soils is similar to that of uncontaminated soils, the proportional distribution of microbial groups differs significantly (Table 1).

In the soil samples collected from oil-contaminated areas, an increase was observed in the total number of microorganisms, particularly hydrocarbon-oxidizing and denitrifying bacteria, compared to the control (Mardakan- uncontaminated) soil samples.

Conversely, the abundance of cellulose-degrading and nitrifying bacteria showed a notable decline. This reduction in certain microbial groups may be attributed to the lower levels of nitrogen and plant residues in contaminated soils relative to clean soils. These conditions create an unfavourable environment for the growth and activity of specific microbial populations in oil-polluted soils. Following the analysis of 101 soil samples collected from the study areas, 215 strains of HOMs belonging to seven different genera were isolated in pure culture. The identified strains were found to belong to the genera *Pseudomonas sp., Arthrobacter sp., Micrococcus sp., Bacillus sp., Mycobacterium sp., Acinetobacter sp.* and *Rhodococcus sp*. (Table 2).

The HOMs were isolated using n-hexadecane as the primary carbon source. It is well known that crude oil is a complex, multi-component mixture in terms of its chemical composition. Therefore, if the isolated microorganisms are intended for use in the bioremediation of oil-contaminated soils, they must possess the metabolic capacity to utilize a broad range of hydrocarbons as nutrient substrates.

Under such conditions, the activity of the isolated microorganisms is considered to be more effective. To evaluate their metabolic versatility, various carbon substrates, including crude oil, a mixture of n-paraffins (C_{12}–C_{18}), kerosene, gasoline, and diesel fuel, were used as nutrient sources. The ability of the isolated HOMs to utilize individual hydrocarbons was examined (Table 3).

Table 2.

Hydrocarbon-Oxidizing Microorganisms Isolated from the Studied Soil Samples

№	Sampling sites	Microorganism genera	Number of isolated strains
1.	Bibi-Heybat	*Pseudomonas sp.* *Arthrobacter sp.* *Micrococcus sp.* *Bacillus sp.*	18 8 5 11
2.	A.Amirov	*Mycobactrium sp.* *Pseudomonas sp.* *Rhodococcus sp.*	7 3 12
3.	Binagadi	*Pseudomonas sp.* *Arthrobacter sp.* *Bacillus sp.*	11 9 14
4.	Balakhani	*Pseudomonas sp.* *Mycobactrium sp.* *Bacillus sp.*	15 12 8
5.	Sabunchu	*Mycobactrium sp.* *Acinetobacter sp.* *Rhodococcus sp.*	7 3 9
6.	Ramana	*Pseudomonas sp.* *Acinetobacter sp.*	10 6
7.	Z.Taghiyev	*Mycobactrium sp.* *Acinetobacter sp.* *Bacillus sp.*	7 9 15
8.	Surakhani	*Acinetobacter sp.* *Rhodococcus sp.* *Mycobactrium sp.*	5 8 3
	Total	*7*	215

Table 3.

Assimilation of Oil and Oil Products by Active Microorganism Strains Isolated from the Territories of Oil and Gas Production Departments (OGPDs)

№	**Strains**	**Oil**	**(C_{12}-C_{18})**	**Kerosine**	**Petro-leum**	**DF**
1.	*Acinetobacter sp.13*	-	+	+	-	+
2.	***Pseudomonas sp. 3***	+	+	+	+	+
3.	*Bacillus sp.7*	+	+	-	-	+
4.	*Arthrobacter sp.4*	-	+	-	+	-
5.	*Pseudomonas sp. 12*	+	+	-	-	+
6.	*Micrococcus sp. 8*	+	+	-	+	+
7.	***Bacillus sp.15***	+	+	+	+	+
8.	*Mycobactrium sp. 5*	+	+	-	-	-
9.	*Acinetobacter sp. 24*	-	+	-	-	+
10.	***Mycobactrium sp.11***	+	+	+	+	+
11.	*Rhodococcus sp. 19*	-	+	-	-	+
12.	***Bacillus sp.18***	+	+	+	+	+
13.	*Rhodococcus sp.26*	+	+	-	-	-
14.	*Micrococcus sp.19*	+	+	-	-	-
15.	***Pseudomonas sp.28***	+	+	+	+	+
16	*Micrococcus sp. 23*	+	+	-	-	+
17	***Pseudomonas sp.22***	+	+	+	+	+
18	*Mycobactrium sp. 1*	-	+	-	+	-
19	*Rhodococcus sp.9*	-	+	-	-	+
20	*Acinetobacter sp. 17*	-	+	-	-	+
21	***Bacillus sp.25***	+	+	+	+	+
22	*Micrococcus sp. 22*	+	-	+	+	+

DF- Diesel fuel

Based on the results obtained during the study, strains belonging to the genera *Pseudomonas sp. 3*, *Pseudomonas sp. 22*, *Pseudomonas sp. 28, Bacillus sp. 18, Bacillus sp. 15, and Mycobacterium sp. 11* were distinguished by their high activity in degrading oil and oil products. Among these, the strain *Pseudomonas sp.3* exhibited the highest degradation efficiency in terms of hydrocarbon breakdown time. Specifically, it utilized n-paraffins (C_{12}–C_{18}) and kerosene within 48 hours and gasoline and diesel fuel within 65 hours (Figure 1).

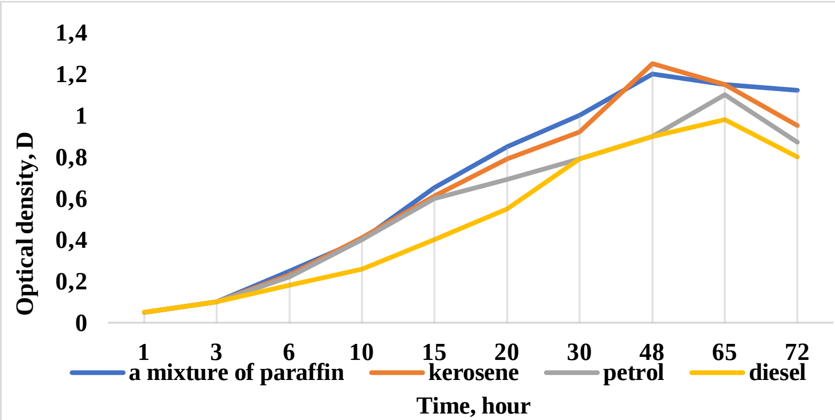

Figure 1. Assimilation of paraffin hydrocarbons (C_{12}–C_{18}) and petroleum products by *Pseudomonas sp*. strain No. 3.

The results obtained indicate that this strain of the genus can be used as a promising candidate for developing bioremediation methods for oil-contaminated soils.

3.3. Features of Rhizosphere Microbiota under Hydrocarbon Pollution Conditions

In the modern era, the impact of various pollutants on the environment and the mitigation of the resulting ecological problems are of significant importance. Since petroleum products are among the primary environmental pollutants, the impact of oil on the physicochemical properties of soils and the structure of soil microbiocenosis remains a consistent focus of scientific attention. Although the effects of oil pollutants on soil microbiota have been well studied to date, the ecological and functional responses of rhizosphere microorganisms to hydrocarbon contamination remain insufficiently explored. Therefore, to analyze the activity of rhizosphere microbiota in oil-contaminated soils of the Absheron Peninsula, a comparative study was conducted on the rhizosphere of Bermuda grass in relatively clean and crude oil-polluted soils. The effect of oil contamination on the population dynamics of the rhizosphere microbiota was investigated through model experiments. The effect of crude oil on soil microbial activity was observed over one month in both planted and unplanted soil samples. Ten days after contaminating non-vegetated soils with crude oil (15 g/kg), an increase in the total number of heterotrophic microorganisms was observed. However, as the experiment continued, later analyses of the soil samples indicated a decline in microbial numbers when compared to both the control sample and the initial measurement. This outcome results from the rapid proliferation of microorganisms during the initial stage of oil contamination, driven by the assimilation of readily degradable hydrocarbons. The experimental results are presented in Table 4.

Table 4.

Comparative analysis of rhizosphere microbiota activity in clean and oil-contaminated soils

Experimental variants	TMC, CFU/g soil		
	10 days	20 days	30days
Plant-free soil			
Control	$7{,}0 \pm 0{,}4\times 10^5$	$7{,}1 \pm 0{,}4\times 10^5$	$7{,}1 \pm 0{,}3\times 10^5$
Oil-contaminated soil	$7{,}7\pm 0{,}3\times 10^5$	$6{,}8 \pm 0{,}3\times10^4$	$6{,}5 \pm 0{,}2\times 10^3$
Rhizosphere of Bermudagrass			
Control	$7{,}5 \pm 0{,}4\times 10^5$	$7{,}3 \pm 0{,}4\times 10^5$	$7{,}9 \pm 0{,}4\times 10^5$
Oil-contaminated soil	$8{,}0 \pm 0{,}2\times 10^5$	$8{,}0 \pm 0{,}2\times 10^6$	$8{,}8 \pm 0{,}3\times10^6$

Control - uncontaminated soil, TMC- total microbial count

In the rhizosphere of the Bermuda grass plant, the number of microorganisms increased during the experiment and remained consistently high throughout the experiment. The investigation of structural and quantitative changes in the microbial communities of the root zone of Bermuda grass, as well as the impact of hydrocarbon contamination on their functional activity, revealed several general patterns. Analyses carried out to determine the structural composition of the microbiota in soils with and without vegetation revealed that microorganisms in both soil samples are represented by nearly the same physiological groups—those involved in nitrification, ammonification, denitrification, nitrogen fixation, and cellulose degradation. Furthermore, the analysis of the abundance of the main physiological groups of soil microorganisms revealed that all studied groups exhibit high abundance in the rhizosphere, and their mutual positive interactions may enhance the soil purification process.

3.4. Effect of Hydrocarbon Compounds on Soil Enzymes

"The significant impact of oil and petroleum product contamination on the enzymatic activity"[25,26] of soils has also been documented in the scientific works of several researchers.

In all the samples we studied, notable differences in enzymatic activity were observed between contaminated and uncontaminated soils. In particular, significant variations were observed in the catalase activity of control soils compared to those contaminated with oil. In the control samples of all studied soils, catalase activity ranged from 0.08 to 0.11 ml of 0.1 $KMnO_4$. In oil-contaminated soils (with an oil content of 2.3 and 7.4 g per 100 g of soil), this value increased to a range of 0.2 to 0.6 ml of 0.1 $KMnO_4$. This can be explained by the fact that under deteriorating air-gas conditions, the highly active oxygen produced with the involvement of the catalase enzyme supplies oxygen to microorganisms

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[25] Сергатенко С.Н, Федорова И.Л, Игнатова Т.Д. Влияние нефтяного загрязнения на активность почвенных ферментов классов оксидоредуктаз и гидролаз. Вестник Ульяновской государственной сельскохозяйственной академии, – 2022 (3 (59), – с.83-8.

[26] Булуктаев А.А. Фитотоксичность и ферментативная активность почв Калмыкии при нефтяном загрязнении. Юг России: экология, развитие, – 2017(4), – с 147-56.

participating in biodegradation processes, leading to an increase in the number of HOMs and an elevated level of reduction reactions associated with oil biodegradation.

Analysis of soil dehydrogenase activity revealed an inverse relationship with the concentration of oil in the soil. Laboratory studies showed that while the dehydrogenase activity in background soil was 3.8 mg TPP at oil concentrations of 2.3 and 7.4 g/100 g of soil, the dehydrogenase activity was 0.9 and 0.12 mg TPP per 10 g of soil, respectively. As is known, unlike hydrolytic enzymes, dehydrogenases are intracellular enzymes and are not secreted into the environment by microorganisms.

The activity of these enzymes is primarily attributed to the intracellular enzymatic systems of microorganisms, as well as the breakdown of dead cells and “the inhibitory effects of metabolites produced during hydrocarbon degradation”[27].

A decrease in invertase activity was also observed in the studied oil-contaminated soil samples. Specifically, when the oil content in the soil was 2.3 and 7.4 g per 100 g of soil, 19 and 14 mg of glucose were formed per 1 g of soil, respectively.

The decrease in invertase activity reflects a disruption in the interaction between the vital activity and functional state of the surface vegetation, which serves as the main source of this enzyme. The invertase enzyme, which is characteristic of all soil types, plays a crucial role in the carbon cycle within the biogeocenosis and is regarded as one of the key enzymes characterizing the biogenicity of soil. This enzyme participates in the hydrolytic decomposition of organic compounds, thereby playing a crucial role in enriching the soil with active nutrients that are available to plants and microorganisms. Furthermore, the activity of invertase is correlated with the amount of humus and the soil's fertility. It is known that oil, with its complex chemical composition, contains the main classes of hydrocarbons — paraffins, cycloparaffins, and aromatics. For this reason, we also studied the effects of petroleum hydrocarbons from different classes on the biological activity of gray-

[27] Хазиев Ф.Х., Фахтиев Ф.Ф. Изменение биохимических процессов в почвах при нефтяном загрязнении и активация разложения нефти // Агрохимия, – 1981. №10.-с.102-111

brown soils.

A clean gray-brown soil sample taken from the Balakhani area was contaminated in laboratory conditions with 1.0% crude oil, n-hexadecane, cyclohexane, and p-xylol, and the enzymatic activities of these contaminants were monitored. Three days later, the activity of the enzymes invertase, catalase, and dehydrogenase was examined in all samples. Based on the results obtained, it was determined that oil and various types of hydrocarbons had different effects on the activity of soil enzymes (Table 5).

Table 5.

The Effect of Oil and Hydrocarbons on Soil Enzymes

Experimental variants	Enzyme activity		
	Catalase ml 0,1 $KMnO_4$	Dehydrogenase mg, ÜFF/10 g soil	Invertase, 1g soil/mg glucose
Clean soil (control)	0,83	12,0	29
Clean soil + oil	0,79	11,8	14
p-xylol (C_8H_{10})	0,51	5,6	8
n-hexadecane ($C_{16}H_{34}$)	0,82	13,7	12
Cyclohexane (C_6H_{12})	0,78	11,2	17

It was found that aromatic hydrocarbons exert the most pronounced inhibitory effect on soil enzyme activity, whereas n-paraffins demonstrate the least inhibitory impact. This pattern closely mirrors the effects of petroleum contamination on plant physiological processes. Data presented in the table support the observation that n-paraffins are among the key petroleum components that enhance soil enzyme activity. Notably, these compounds also appear to stimulate the activity of soil dehydrogenases, further indicating their relatively favourable influence on microbial metabolic processes in contaminated soils.

The comparative analysis of enzyme sensitivity to petroleum hydrocarbons allows for their classification in the following order:

invertase > dehydrogenase > catalase

This ranking reflects the varying responsiveness of these enzymes to oil contamination, highlighting their distinct functional roles in soil biochemical processes.

The analysis of our experimental data shows that enzymatic activity and other biogenic indicators of soils are not stable under oil contamination. These parameters vary depending on the chemical nature and specificity of the pollutant, the degree of contamination, and the character and intensity of the biological processes occurring in oil-contaminated soils. Thus, the experiments revealed significant differences in enzymatic activity between oil-contaminated and uncontaminated soils. Catalase activity was higher in contaminated soils compared to the control. The activity of soil dehydrogenases showed an inverse relationship with the concentration of oil in the soil. In contrast, invertase activity in gray-brown soils was significantly reduced.

3.5. Phytotoxicity of soils exposed to technogenic pollution

To assess the toxicity of substances polluting the soil cover, the phytotest method, based on higher plants, is widely used. For this purpose, seed germination and morphometric parameters of the plants are analyzed. Scientific studies dedicated to investigating the phytotoxicity of soil cover contaminated with oil pollutants in various soil and climatic zones have shown that, compared to other bio-test objects, phytocoenosis exhibit higher sensitivity to soil oil contamination, *“allowing for more accurate results”*[28]. Under hydrocarbon contamination, the leading causes of soil cover phytotoxicity depend on several factors, including the direct toxic effects of hydrocarbons on phytocoenosis, the deterioration of the agrochemical and agrophysical properties of the soil, changes in the quantity and quality composition of soil microorganisms,and the impact on their

[28] Чудинова, О.А., Дзюба, Е.А. Схема биотестирования нефтезагрязненных почв на базе лаборатории экологии и охраны природы Пермского государственного университета // Экологическая безопасность в условиях антропогенной трансформации природной среды, Пермь: Пермский государственный национальный исследовательский университет, – 2022. – с. 425-431.

functional activity, among other characteristics. To study the phytotoxicity of technogenically polluted soils of the Absheron Peninsula, soil samples were collected from the Sabunchu, Surakhani, Ramana, and Bibi-Heybat areas. In total, thirty-two soil samples were taken: eleven from Sabunchu, nine from Surakhani, five from Ramana, and seven from Bibi-Heybat.

Samples from soils exposed to technogenic pollution with hydrocarbons were collected under generally accepted procedures, and the degree of phytotoxicity for higher plants was investigated. In all samples, the degree of soil phytotoxicity was studied using basil and alfalfa seeds as test plants.

The duration of the experiments was 17 days. To assess seed germination, the ratio of germinated seeds to the total number of seeds was calculated. The results of the phytotoxicity tests on soil samples collected from the study areas are presented in Table 6. The data in the table show that all 32 soil samples taken from four different oil-producing regions of the Absheron Peninsula had a high degree of phytotoxicity. The absolute germination ability of basil and alfalfa seeds used as test plants did not exceed 25–41%. According to the research results, the average phytotoxicity of all soil samples ranged from 75% to 61%, confirming that they possess a high level of phytotoxicity.

Thus, the experimental results demonstrated the negative impact of oil-contaminated soils on plant growth and development. This was evident not only in the number of germinated seeds but also in the length of the seedlings and roots (Figure 2). Depending on the degree of soil contamination and the plant species, differences in phytotoxicity levels were also observed. The obtained data are consistent with the research findings of other authors[29,30].

[29] Донец, Е.В, Скоридова Я.С. Влияние нефтяного загрязнения на прорастание семян сосны обыкновенной (Pinus sylvestris L.). Вестник Омского государственного аграрного университета. – 2017(3 (27)) –с.68-73.

[30] Пашутина, Е. Н. Исследование влияния загрязнения почвы дизельным топливом на прорастание семян подсолнечника // Перспективы внедрения инновационных технологий в медицине и фармации, –2019, Т 2. – Орехово-Зуево:– с. 209-211.

Table 6.

Phytotoxicity of soil samples taken from four oil field areas of the Absheron Peninsula

Number of samples	Seed germination %		Number of samples	Seed germination %	
	Clover *(Medicago sativa L)*	Cress *(Lepidium sativum)*		Clover *(Medicago sativa L)*	Cress *(Lepidium sativum)*
	Sabunchu			Bibi -Heybat	
1	25,7	23,5	1	29,3	35,1
2	25,8	22,1	2	28,4	29,3
3	35,5	29,4	3	27,5	28,2
4	36,1	24,3	4	38,7	41,9
5	31,6	31,7	5	41,1	43,4
6	37,8	29,2	6	35,2	34,7
7	38,5	27,9	7	34,6	35,3
8	28,1	29,6		Surakhani	
9	22,4	34,5	1	25,7	28,5
10	29,3	36,2	2	27,9	29,8
11	33,9	29,3	3	29,1	27,8
	Ramana		4	31,7	25,5
1	23,5	21,4	5	34,1	26,7
2	25,3	26,7	6	28,4	27,9
3	29,4	29,5	7	30,5	32,1
4	31,9	34,8	8	35,4	36,3
5	34,7	35,8	9	35,7	33,7

Figure 2. Phytotoxicity of soils exposed to varying degrees of oil contamination

CHAPTER IV
DEVELOPMENT OF BIOTECHNOLOGIES FOR THE REMEDIATION OF OIL-CONTAMINATED SOILS OF ABSHERON AND RESTORATION OF THEIR BIO-ECOLOGICAL FUNCTION

4.1. Phytoremediation of oil-contaminated gray-brown soils of the Absheron Peninsula

It is well known that biodegradation plays a crucial role in the intensity of self-purification processes of natural environments from various pollutants, including oil and petroleum products. Specifically, the high activity of the soil's native microbiota is one of the essential conditions for recovery processes. Current global practice also confirms the use of effective microorganisms related to soil biota and various plants as an efficient method in this direction. The ecological and economic advantages of these methods further increase their relevance and importance. For this purpose, several experiments were conducted to investigate the effectiveness of various plant and microorganism associations in the remediation process of oil-contaminated soils. The phytoremediation process of soil contaminated with oil at a concentration of 15 g/kg was studied using complex systems consisting of mixtures of plants and microorganisms.

During the study, seeds of dominant species resistant to hydrocarbon contamination were used: alfalfa *(Medicago sativa L.)* from the legume family and Bermuda grass *(Cynodon dactylon L. Pens.)* from the grass family. The seeds were bacterized with both the "Fermi-start" microbial preparation and its modified variant *("Fermi-start" + Ps aeruginosa strain No. 3)*. Before sowing, the seeds were soaked for 3–4 hours in a microbial suspension with a concentration of $1–3 \times 10^9$ cells/ml to carry out the bacterization process.

The individual application of *"Fermi-start," P. aeruginosa* culture, as well as the modified "Fermi-start,"resulted in a reduction of the toxic effects of crude oil on the plants.

The highest protective effect was observed with the combination of "Fermi-Start" and the *P. aeruginosa* modification (Figure 3). As a result, after 7 days, the length of the seedlings increased by 71% compared to the control (plant + oil).

Accelerated oil degradation was expected when applying *Ps. aeruginosa;* however, the residual oil concentration was higher compared to the *Ps. aeruginosa* + "Fermi start" treatment. These findings confirm that the oil + plant + modified microbiological product *"Fermi -start"* treatment variants demonstrated greater efficiency (Fig. 4).

Although the application of HOM resulted in an 18% degradation of oil (Figure 4), their protective effect on plants was limited. These results suggest that during the oil degradation process, microorganisms may accumulate intermediate compounds that are potentially toxic to plants. In contrast, the combined use of the "Fermi-start" biopreparation and the oil-oxidizing culture appears to have enabled the microorganisms present in the preparation—likely *Pseudomonas aeruginosa*—to utilize these intermediate compounds, thereby not only eliminating their toxic effects but also increasing the oil degradation rate to 24%. Higher efficiency was observed when Bermuda grass was used compared to alfalfa.

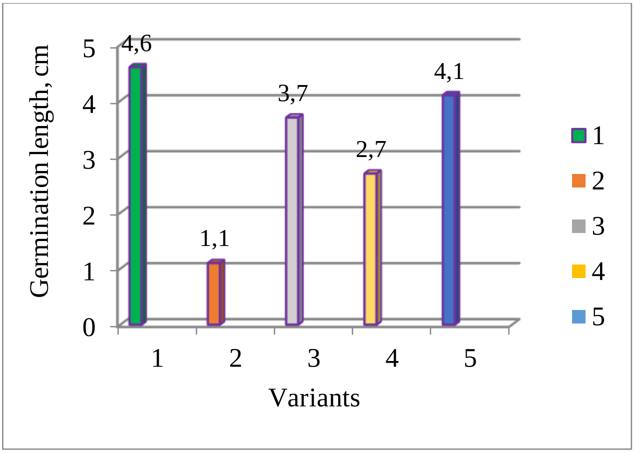
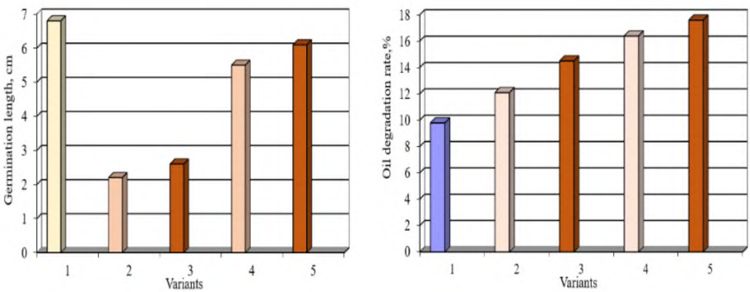


Figure 3. Length of alfalfa seedlings after 7 days of cultivation.
Note: 1) Control – plant + clean soil; 2) Plant + oil; 3) Plant + oil + "Fermi-start"; 4) Plant + oil + hydrocarbon-oxidizing microorganisms (HOMs); 5) Plant + oil + modified "Fermi-start" biopreparation

According to the results obtained during the experiment, the degradation of hydrocarbons in the soil beneath the plants reached 67%, which is 25–45% higher compared to the control without seeds.

The results indicate that treating plants with the modified biological product "Fermi-Strat" has a positive impact on the degree of residual hydrocarbon removal from gray-brown soil.



1) Control; 2) alfalfa +oil;3) bermuda grass + oil; 4) alfalfa + modified "Fermi-start" biopreparation 5) bermuda grass+ modified "Fermi-start" biopreparation

1) oil + modified biopreparation; 2) oil + alfalfa; 3) oil + bermuda grass; 4) oil + alfalfa + biopreparation; 5)oil + bermuda grass biopreparation

Figure 4.2. Length of plants (alfalfa and Bermuda grass) and degree of oil degradation after 12 days in the model experiment.

The research results enable the recommendation of a plant-microbe biosystem comprising alfalfa and Bermuda grass in combination with the modified "Fermi-start" biopreparation, for the phytoremediation of crude oil-contaminated gray-brown soils on the Absheron Peninsula.

4.2. Preparation of Bio Compost based on regional bioresources to remediate contaminated soils

This section presents a systematic analysis of biological resources (waste materials) that can be utilized to prepare modified bio compost for enhancing the fertility and biological activity of oil-contaminated soils in the Absheron industrial region following remediation. Based on data on the area of oil-contaminated soils on the Absheron Peninsula, the required volume of compost necessary for restoring and improving the productivity of these soils was calculated. The volume of regional biowaste needed to prepare modified composts of the required quality was examined.

Among the regional bioresources considered for the development of biocompost to improve the productivity of technogenic soils, the following were studied (Figure 5).

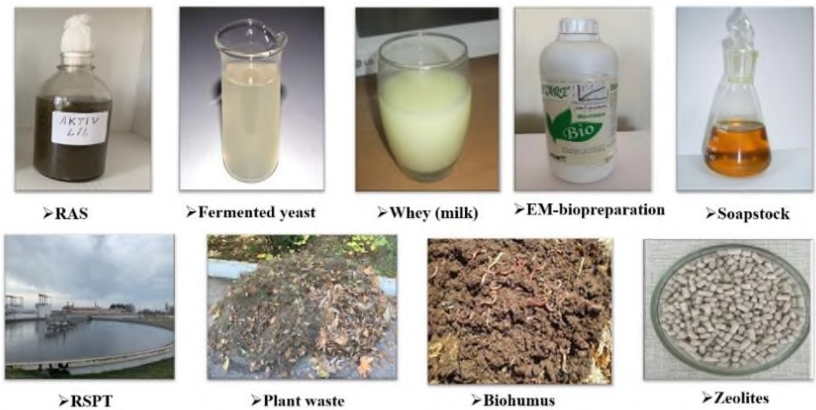


Figure 5. Regional Bioresources Used in the Preparation of Modified Biocompost to Improve Soil Fertility

- Residual activated sludge from biological treatment facilities (RAS) – 14,600 tons/year
- Raw sediment from primary tanks (RSPT), wet sludge – 3,000 tons/year
- Fermented yeast (waste from beer production) – 10,000 tons/year
- Plant waste (plant residues, fallen tree leaves, grass clippings from lawns, wood shavings, etc.) – 36,000 tons/year
- Whey – 15,000 tons/year
- Effective microorganisms
- Biohumus
- Zeolites (from the Ay-Dag deposit)

These bioresources were studied both individually and in various combinations in the study.

Composting is a purification process that enables the production of a valuable product through the biological decomposition of organic waste. On the other hand, this process also helps prevent the environmental issues caused by waste.

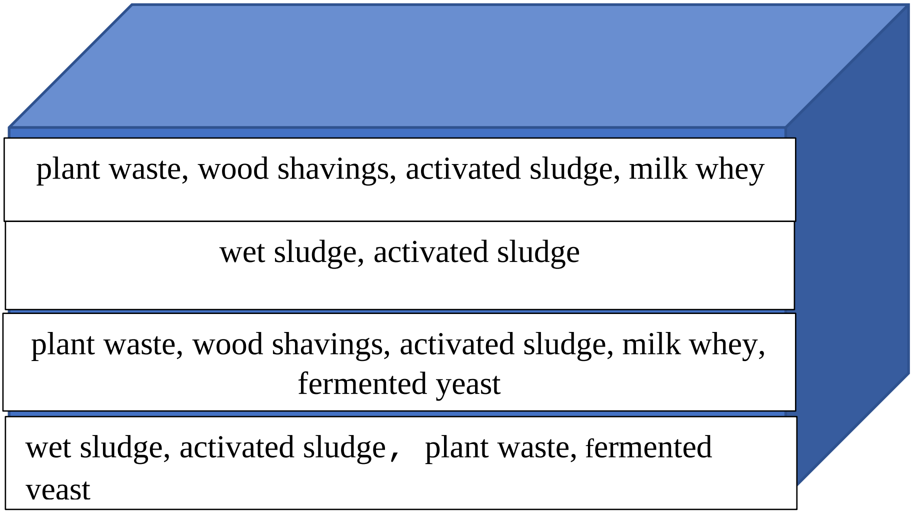


Figure 6. Schematic diagram of regional organic waste composting

The product obtained from the composting process is a valuable organic fertilizer used to improve soil structure, enhance soil biogenicity, and contribute to solving ecological problems such as waste utilization.

A schematic plan for composting regional bio-waste has been developed (Figure 6). Organic components used for composting included plant waste such as fallen leaves, wood chips, and mowed lawn grass. To accelerate the fermentation rate of the composting material, regional bio-wastes such as active clay, spent yeast, whey, and moist clay were selected and added at a 5% volume of the total material. The composting process was carried out under conditions of 28–35°C temperature, an optimal moisture content of 45–55%, a C:

N ratio of 24–26, a pH of 6.8–7.4, and aeration through periodic mixing, among other parameters.

4.3. Development of optimal modifications for composting regional bioresources

In laboratory model studies, plastic containers with a volume of 1 kg were used for composting regional bioresources. Composting studies were conducted in 4 variants:

1. Plant waste + "Fermi-start" microbial preparation
2. Plant waste + "Fermi-start" + HOM
3. Plant waste + "Fermi-start" + HOM+ RSPT (Raw sediment from primary tanks) + whey + fermented yeast
4. Plant waste + "Fermi-start" + HOM + fermented yeast + whey + RAS (residual active sludge from biological treatment plants)

During the study, active strains of HOM isolated from the region's oil-contaminated soils were included in the composted materials. These strains consisted of *Pseudomonas sp. Nos. 3, 22, 28; Mycobacterium sp. 11; and Bacillus sp. Nos. 18 and 15.* The purpose of applying KOM suspensions was to adapt them to the organic substances during the composting process. The cell concentration in the HOM suspension was 0.7×10^4 cells/l. Composting was conducted under laboratory conditions at a temperature of 22–26°C, a humidity of 45–55%, and a pH of 6.8–7.4. The experiment lasted 60 days. The resulting compost exhibited the following characteristics: a particle size mostly not exceeding 4–6 cm, a soft, lump-like structure, a moisture content of 50–70%, a near-neutral pH of 6.9–7.1, an organic matter content of approximately 50%, and a C: N ratio of 25:30.

Research was also conducted on the biological properties of the compost during the composting process. The total number of microorganisms, as well as the count and activity of HOM, in the resulting compost were studied (Table 7).

Table 7.

Microbiological indicators of the composted material

Composting variants	Biological indicators	
	TMC CFU/g compost	HOM, CFU/g compost
1	1,2-1,4 x 10^4	0,7-1,0 x 10^2
2	1,8-2,0 x 10^5	0,9-1,1 x 10^3
3	1,9-2,3 x 10^5	0,8-1,0 x 10^3
4	1,8-2,2 x 10^7	0,9-1,3 x 10^4

**** TMC -Total Microbial Count, HOM- hydrocarbon-oxidizing microorganisms***

The obtained results showed that the TMC (Total Microbial Count) reached 1.8–2.2 × 10^7 CFU/g after 30 days in the fourth variant, and the number of HOMs remained between 0.9–1.3 × 10^4 CFU/g throughout the period (Table 7).

Both the total microbial count and HODB levels were highest in this variant compared to all variants, which is attributed to the use of activated sludge suspension in that composition.

The final product of regional waste composting is a humified compost containing biogenic nutrients (N, P, K). In its dry mass, it contains approximately 1.1% nitrogen, 0.9% phosphorus, and 15–18% humic substances.

To enhance the productivity of technogenically polluted soils of the Absheron Peninsula following preliminary remediation, the potential use of zeolite and biohumus was investigated. The study focused on identifying their optimal ratios and evaluating their effectiveness. After a two-month composting process, the resulting compost was modified by mixing with zeolite and biohumus in an 80:15:5 ratio.

Table 8.

Biological and Physicochemical Properties of Modified Composts Prepared from Regional Bioresources

Modification Scheme and Component Ratios	Indicators				
	TMC (CFU/g)	Quantity of Hydrocarbon-Oxidizing Microorganisms (CFU/g)	pH	Amount of Humic Substances, %	Nitrogen content %
Biocompost + Zeolite + Biohumus	1,4-1,7×.10^7	1,2-1,3 ×10^4	7,1	9,9	3,4

TMC- total microbial count

The biological and physicochemical properties of this modified compost were then analyzed. The results are presented in Table 8.

Since the environmental efficiency of composts and their long-term effectiveness as complex fertilizers depend on their chemical composition, the presence of a large number of mesophilic (TMM) and HOM microorganisms indicates the high biological activity of the resulting composts and their potential to degrade hydrocarbons.

4.4. Development of biopreparations for the remediation of oil-contaminated gray-brown soils

This section presents research focused on the development of biotechnological solutions for the remediation of gray-brown soils in the Absheron Peninsula affected by crude oil contamination. The studies involved the selection of appropriate compounds to enhance the sorption capacity of the adsorbents used, specifically, biohumus and zeolite. In parallel, screening was conducted to identify effective strains of microorganisms capable of improving the performance of biological preparations. These investigations aim to optimize the composition and efficacy of biopreparations for improved soil recovery and environmental sustainability.

To regulate the hydrophilicity of the sorbents, they were treated with 4% soapstock and 3% primary tank raw sludge (RSPT).

Table 9.
The effect of zeolite- and biohumus-based biopreparations on oil degradation in soil

Variants	Oil concentration, g/kg soil		Degradation %
	Before	3 years later	
Soil (control)	30	28	7
soil +zeolite	30	25	17
Soil + zeolite + soapstock	30	20	34
soil +zeolite +RSPT	30	18	40
Soil + zeolite + soapstock + AS	30	9	70
soil +zeolite +RSPT+AS	30	6	80
soil + biohumus	30	22	29,7
soil + biohumus + soapstock	30	11	65,1
soil + biohumus +RSPT	30	10	66,7
soil + biohumus + soapstock + AS	30	3	90,0
soil + biohumus + RSPT + AS	30	2	93,7

The biopreparation was formulated using adsorbents and polyfunctional microorganisms and tested through experimental trials. As sources of such organisms, activated sludge (AS) and previously isolated active HOMs from oil-contaminated soils in the study area were used. These research findings were evaluated across various experimental setups to assess their impact on the degradation rate of oil in soils contaminated with hydrocarbons (3%). The results are presented in Table 9.

The experiments were conducted over 60 days at a temperature of 25°C, maintaining a moisture content of 40-50%. After 60 days, the oil concentration in the soil was determined using the gravimetric method. The most successful results were observed in the variants where the sorbents were treated with soapstock, RSPT, and AS. These combinations increase the oil retention capacity of the sorbent, and multifunctional microorganisms actively assimilate hydrocarbons.

As shown by the data in the table, the treatment of contaminated soil with biohumus and zeolite accelerated the degradation processes of hydrocarbon pollutants. The use of biosurfactants facilitates the active desorption of contaminants from the soil surface. It *"induces changes in the structure of the bacterial cell surface"*[31], which creates favourable conditions for accelerating bioremediation processes in polluted areas.

These findings confirm that the addition of structural materials to the soil cover—such as peat, sawdust, etc.—has a positive effect on the bioremediation process. These materials possess the ability to absorb hydrocarbon pollutants while simultaneously improving soil aeration, which aligns with the results reported by other researchers"[32].

It is also known that microorganisms proposed for bioremediation technologies of oil-contaminated landscapes must not possess toxic properties toward living organisms.

In this regard, to exclude any negative impact of the RAS on the biological activity of the soil biota, the phytotoxicity of the microorganisms contained in it was initially studied, and it was determined that they did not inhibit the germination of the tested plant seeds.

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[31] Kaczorek E, Pacholak A, Zdarta A, Smułek W. The impact of biosurfactants on microbial cell properties leads to an increase in hydrocarbon bioavailability. Colloids and Interfaces, – 2018, 26;2(3):35.; https://doi.org/10.3390/ colloids 2030035

[32] Морозов Н. В. Управляемая биоремедиация нефтезагрязнений в природных водах органическими сорбентами разнообразного происхождения // Вестник Казанского технологического университета. 2017. Т. 10. - №11. - с. 137-142.

4.5. Research of modified biocompost for the remediation of biological properties of technogenic soils

To restore the biological properties of the studied soils, biocompost modified with zeolite was tested under laboratory conditions. Soil samples were collected from the Surakhani, Sabunchu, Ramana, and Bibi-Heybat areas. Different amounts of soil were mixed with 100 g of modified compost and allowed to stand for 24 hours, after which their phytotoxicity was assessed. For this purpose, garden cress (*Lepidium sativum*) and barley (*Hordeum*) seeds were used as test plants. Among all studied samples, the most effective result was obtained in the 400 g soil + 100 g compost treatment. The phytotoxicity of untreated (control) soils ranged between 89% 93%, while the addition of modified compost significantly reduced phytotoxicity. After treatment, the germination rate of test seeds in the technogenically degraded soils increased to 65.3–81.6%.

The 2–3fold reduction in phytotoxicity observed in compost-treated soils confirms a decrease in toxicity to plants and improvement in the soils' physicochemical properties. During the 30-day experiment, the microbiological indicators, enzymatic activity, hydrocarbon mineralization coefficient (used as an indicator of overall microbial activity), and residual hydrocarbon content in compost-treated soils were analyzed (Table 10). For 1 kg of technogenically degraded soil, 150–200 g of modified biocompost was determined to be the optimal dosage.

The observed increase in catalase activity in the treated gray-brown soils compared to control samples can be explained by a rise in the total microbial population—including HOMs — and an intensification of biodegradation processes involving oxidation-reduction reactions of petroleum hydrocarbons.

Table 10.

Biological properties of technologically polluted soils after the introduction of modified biopreparations

	Variants	Indicators					
		Quantity of saprotrophs, CFU/g	Quantity of hydrocarbon-oxidizing microorganisms (HOM), CFU/g	Enzyme activity		Mineralization coefficient, CO_2, mg/100g soil per 24 hours	Total hydrocarbon content, %
				Catalase activity, ml 0.1 MnO_4	Invertase activity, mg glucose per g soil		
1	C- Surakhani	$2,2\times10^5$	3×10^3	0,054± 0,03	28± 0,03	57± 0,03	1,3± 0,03
	400gS+100g MC	$4,4\times10^7$	$5,1\times10^4$	0,85± 0,03	122± 0,03	87 ± 0,03	0,5± 0,03
	900gS +100g MC	$3,5\times10^7$	$4,3\times10^4$	0,76± 0,03	98± 0,03	81 ± 0,03	0,6 ± ,03
	1900g S+100g MC	$2,5\times10^7$	4.0×10^4	0,64± 0,03	78± 0,03	75 ± 0,03	0,8 ± ,03
2	C - Bibi-Heybat	$1,5\times10^5$	$2,9\times10^3$	0,054± 0,03	28± 0,03	55± 0,03	1,5± 0,03
	400g +100g MC	$4,2\times10^7$	$3,0\times10^5$	0,85± 0,03	122± 0,03	85 ± 0,03	0,6± 0,03
	900g +100g MC	$3,9\times10^7$	$2,5\times10^5$	0,76± 0,03	98± 0,03	79 ± 0,03	0,7± 0,03
	1900g S+100g MC	$3,2\times10^7$	$1,9\times10^5$	0,64± 0,03	78± 0,03	73 ± 0,03	0,8 ± 0,03

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Table 10 (continues)

	Variants	Quantity of saprotrophs, CFU/g	Quantity of hydrocarbon-oxidizing microorganisms (HOM), CFU/g	Catalase activity, ml 0.1 MnO_4	Invertase activity, mg glucose per g soil	Mineralization coefficient, CO_2, mg/100g soil per 24 hours	Total hydrocarbon content, %
3	C - Sabunchu	$7×10^5$	$4,1×10^3$	0,054± 0,03	28± 0,03	53± 0,03	1,1± 0,03
	400g S+100g MC	$6,1×10^7$	$4,2×10^5$	0,85± 0,03	122± 0,03	83 ± 0,03	0,4± 0,03
	900g +100g MC	$5,3×10^7$	$5,1×10^4$	0,76± 0,03	98± 0,03	75 ± 0,03	0,6 ± ,03
	1900g S+100g MC	$4,2×10^7$	$7,2×10^4$	0,64± 0,03	78± 0,03	70 ± 0,03	0,8 ± ,03
4	C - Ramana	$2,0×10^5$	$3,0×10^3$	0,054± 0,03	28± 0,03	59± 0,03	1,6± 0,03
	400g S +100g MC	$3,1×10^7$	$4,2×10^5$	0,85± 0,03	122± 0,03	88 ± 0,03	0,8± 0,03
	900g S +100g MC	$3,0×10^7$	$3,9×10^5$	0,76± 0,03	98± 0,03	77 ± 0,03	0,9± 0,03
	1900g S +100g MC	$2,5×10^7$	$2,5×10^4$	0,64± 0,03	78± 0,03	73 ± 0,03	1,2 ± 0,03

Note* C- control, S – oil-contaminated soil, MC – modified compost

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Invertase activity is one of the key enzymes characterizing the biogenicity of the soil. Invertase activity is correlated with humus content and soil fertility. As the obtained data show, the increase in the proportion of modified compost in the composition of technologically disturbed soils leads to a rise in invertase activity, which confirms the improvement of soil fertility in technogenically degraded areas.

4.6. Research on the Effect of Modified Compost on the Growth and Development of Higher Plants in Technogenic Soil

To assess the growth and development of higher plants and the productivity of technogenically disturbed soils after the application of modified compost, wheat, barley, tomato, and cucumber seeds were used as test plants (Figure 7).

Figure 7. Laboratory Modelling of the Effect of Modified Composts on Plant Growth and Development

Note: No. 1 – Soil without compost application, No. 2 – Soil with modified compost application

After the application of modified composts to the soil, the germination of seeds of all the tested cultivated plants increased. In contrast, seed germination was very poor in technogenically contaminated soils containing 1.2% residual oil. In all experimental variants, the germination rate was more than 2.5 to 3 times higher compared to the control. These results provide a strong basis for proposing the most effective technology for increasing the fertility and restoring the biological activity of the technogenically degraded soils of the Absheron Peninsula.

CHAPTER V
DEVELOPMENT OF BIOTECHNOLOGIES FOR THE PURIFICATION OF ORGANIC AND INORGANIC POLLUTANTS FROM THE WATER BODIES OF THE ABSHERON PENINSULA

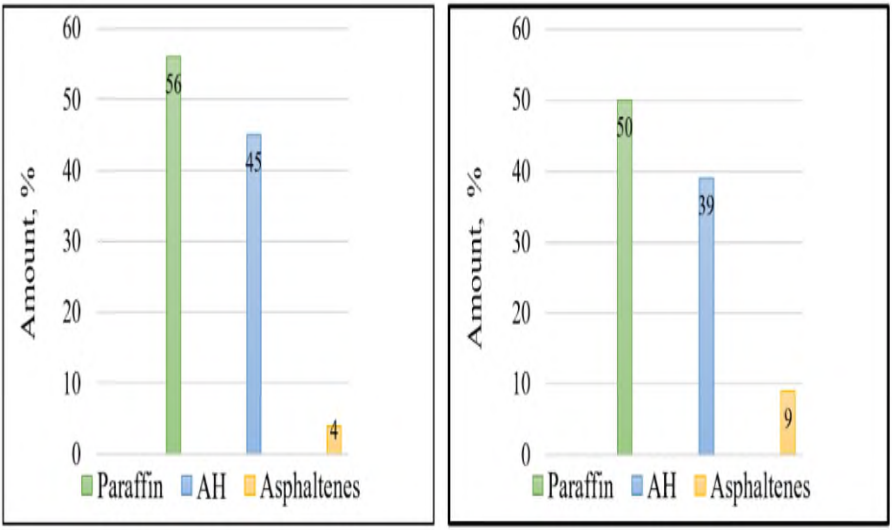
5.1. Pollution Problems of Natural Lakes in the Absheron Peninsula

This section analyzes the bioecological condition of the peninsula’s natural lakes, which are exposed to anthropogenic impacts, using Boyuk-Shor and Lake Zigh as examples. It provides information on the dominant pollutants present in these water bodies and their concentrations.

5.2. Determination of Pollutants and Microbiological Analyses of Boyuk-Shor and Zigh lakes water

For this research, water samples were collected from Lake Boyuk Shor and Lake Zig. In all water samples taken from these water bodies, total hydrocarbons were detected. It was found that the concentration of hydrocarbons varied depending on the location from which each sample was taken.

For the waters of Lake Zig, the concentration of total hydrocarbons ranged from 0.01–0.03 mg/L to 12–34 mg/L, while for Lake Boyuk Shor, the maximum concentration was found to be 25–34 mg/L.



a **b**

Figure 8. Component composition of residual hydrocarbons in Boyuk-Shor Lake (a), Zigh lake (b)

The analysis of the component composition of oil pollution in the water samples showed that the hydrocarbon composition was similar in samples taken from both lakes. However, in the samples from Lake Boyuk Shor, which is under greater technogenic pressure, the proportion of aromatic hydrocarbons (AHs) and asphaltenes within the total hydrocarbon content was higher compared to the waters of Lake Zig (Figure 8).

This indicates an increase in the proportion of hydrocarbons that are potentially prone to settling at the bottom, are difficult to degrade, and may remain outside the activity range of aerobic microorganisms for extended periods. Such conditions can have a negative impact on the aquatic biota.

Table 11.

Average annual abundance and biomass of saprotrophic and hydrocarbon-oxidizing microorganisms in the analyzed water samples

№	Sampling location	Microorganism Count (CFU/ml) and Biomass (mg/l)	
		Saprotrophs (CFU/ml)	Quantity of Hydrocarbon-Oxidizing Microorganisms (CFU/g)
Boyuk- Shor Lake			
1	Sample 1 (South part)	$3{,}0\pm2{,}1\times10^{5}$/66	$3{,}0\pm1{,}4\times10^{4}$/36
2	Sample 2 (South part)	$3{,}0\pm2{,}4\times10^{6}$ /72	$3{,}0\pm1{,}5\times10^{5}$/41
3	Sample 3 (North part)	$3{,}0\pm2{,}1\times10^{4}$ /54	$3{,}0\pm1{,}8\times10^{3}$ /32
4	Sample 4 (North part)	$3{,}0\pm2{,}3\times10^{4}$/ 55	$3{,}0\pm1{,}7\times10^{3}$/31
Zigh lake			
5	Sample 1 (North part)	$3{,}0\pm1{,}9\times10^{4}$ /42	$3{,}0\pm2{,}3\times10^{3}$ /26
6	Sample 2 (South part)	$3{,}0\pm2{,}1\times10^{5}$/55	$3{,}0\pm2{,}4\times10^{4}$/34
7	Sample 3 (North part)	$3{,}0\pm1{,}8\times10^{4}$/44	$3{,}0\pm2{,}6\times10^{3}$ /30
8	Sample 4 (East part)	$3{,}0\pm1{,}9\times10^{6}$ /74	$3{,}0\pm2{,}2\times10^{5}$/54
9	Sample 5 (West part)	$3{,}0\pm1{,}7\times10^{4}$/ 41	$3{,}0\pm2{,}5\times10^{3}$/24

According to our data, the number of HOMs in the studied reservoirs ranged between 10^3–10^5 cells/mL, which justifies classifying these water bodies as polluted, since reservoirs with 10^3–10^7 cells/mL of HOMs are considered contaminated. During the vegetation period, the number and biomass of saprotrophic microorganisms in the water were 10–20 times higher than in winter, most likely due to temperature influences—this observation aligns with existing literature (Table 11).

The prominent representatives of HOMs isolated from the studied water bodies belong to the genera *Acinetobacter*, *Pseudomonas*, *Arthrobacter*, *Bacillus*, *Micrococcus*, and *Rhodococcus*. The majority of the cultured isolates were identified as belonging to the *Pseudomonas* genus. Based on their hydrocarbon-oxidizing capabilities, 16 bacterial strains were selected. Among them, *Pseudomonas* sp. strains No. 45, 21, 33, and 64; *Rhodococcus* sp. strains No. 7 and 12; and *Micrococcus* sp. strain No. 22 demonstrated active utilization of crude oil and petroleum hydrocarbons (Figure 9).

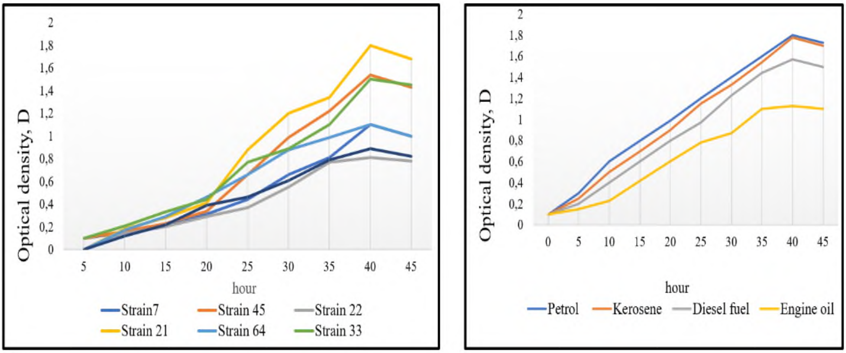


Figure 9. Utilization of crude oil and petroleum hydrocarbons by active strains
(Example: Pseudomonas sp. 21)

It was determined that *Pseudomonas sp*. strains No. 45, 21, and 33 demonstrated a high capacity to degrade crude oil, with degradation rates ranging from 72–76%. In contrast, the activity of *Pseudomonas sp. No. 64, Rhodococcus sp. No. 7* and 12, and *Micrococcus sp. No. 22* was comparatively lower, approximately 55–58% (Figure 9).

Under laboratory conditions, the consumption intensity of petroleum products (gasoline, kerosene, diesel fuel, and motor oil) in a liquid mineral medium supplemented with crude oil was investigated for the three most active strains. Based on the results, the petroleum products can be ranked by degradation efficiency as follows:

Petroleum > Kerosene > Diesel fuel > Motor oil.

5.3. Possibilities of using biological preparations to clean the lakes of the Absheron Peninsula

This biopreparation was developed using strains of oil- and hydrocarbon-oxidizing bacteria belonging to the genus *Pseudomonas* (strains No. 45, 21, and 33). The biological product was formulated by combining the biomass of individual cultures or their associations with an adsorbent (0.1–0.2 mm wood bran) (Table 12).

Table 12.

Crude oil degradation induced by the application of the bioremediation agent

№	Variants	Biodegradation, g						
		Initial	10 days	20 days	30 days	40 days	50 days	60 days
1	Water + oil (control)	50g	50	49	48	47	45	43
2	Water +oil + adsorbent	50g	48	41	38	34	31	29
3	Water+oil+biological preparation 1	50g	44	40	35	30	26	23
4	Water+oil+biological preparation 2	50g	43	40	34	29	24	21
5	Water+oil+biological preparation 3	50g	42	39	31	26	21	19

Microorganism consortia were selected based on their response to crude oil and individual hydrocarbons. When biopreparations were developed from particular strains of HOM, the crude oil degradation rate reached 54–62% over 60 days. In contrast, the use of a biopreparation based on an HOM consortium resulted in a 78% degradation rate.

These results suggest that the selected microbial strains can be effectively used in the development of biobooms for the collection and degradation of crude oil and petroleum products in aquatic environments (Table 3).

Table 13.

Degradation of Crude Oil in Water Using Biobooms

№	Variants of Microecosystems	Crude Oil Degradation Rate, %			
		after 3 days	after 10 days	after 20 days	after 30 days
1	Water + oil	0,2	1,3	4,3	6,1
2	Water + oil + boom filled with adsorbent	8,7	14,5	21,4	30,2
3	Water + oil + bioboom	12,3	17,9	34,7	67,8

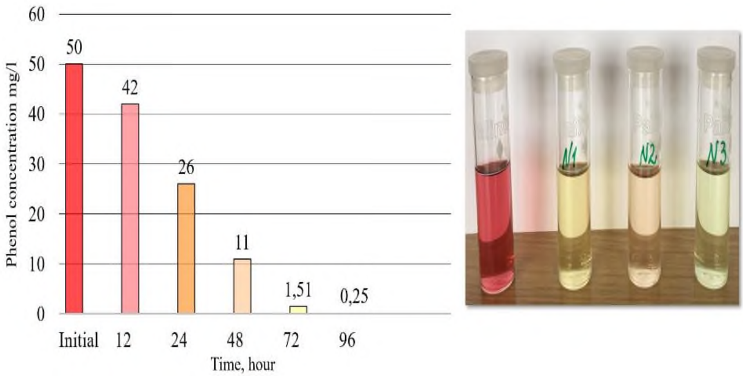


Figure 10. Phenol degradation by activated sludge microorganisms

In the subsequent phase of the study, work was also conducted to investigate the potential of using a microbial biosystem derived from activated sludge (RAS) in combination with adsorbents for removing phenols from contaminated water bodies (Figure 10).

The results demonstrated that after a 5-day dynamic cultivation process (28°C), the concentration of phenol in the medium (initially 50 mg/L) significantly decreased to 1.51 mg/L. This indicates that the consortium of HOMs, immobilized on the adsorbent, can be effectively utilized as a biotechnological tool for the purification of water bodies from organic pollutants.

5.4. Laboratory Modelling of the Biological Removal of Heavy Metals from the Waters of Boyuk-Shor and Zigh Lakes

The technogenic lakes located in the Absheron Peninsula are exposed not only to organic pollutants but also to contamination with heavy metals. In our studies, the bioremediation of water from heavy metals was investigated using copper salt as a model contaminant. The experiments utilized residual activated sludge (RAS) microorganisms, the biological preparation "Fermi-Start," and wood chips as an adsorbent for the removal of heavy metals from the water.

The bioaccumulation capacity of microorganisms for Cu^{2+} ions was evaluated by measuring the concentration of copper ions in the culture medium. The copper concentration was determined using a Palintest photometer at a wavelength of 520 nm (Figure 11).

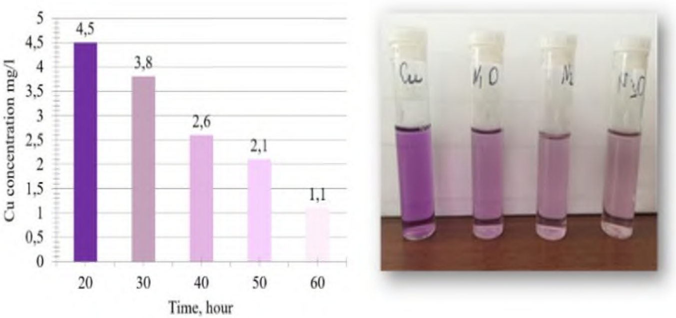


Figure 11. Laboratory Modelling of the Biological Removal of Heavy Metals from Water Samples

In the model experiment, the initial copper concentration in the medium decreased from 4.5 mg/L to 1.1 mg/L.

Based on the obtained data, the purification process achieved a removal efficiency of 76%, which represents a significant result.

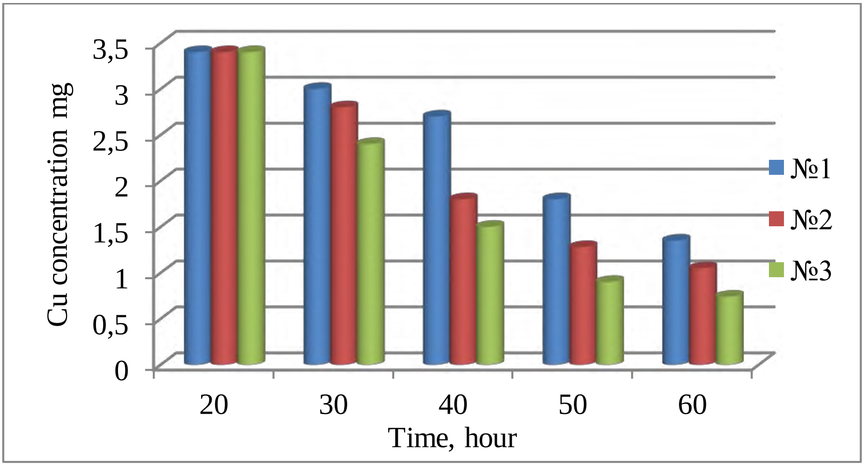


Figure 12. Copper Adsorption by RAS Microorganisms

Note: №1 – 0.1 g RAS biomass, №2 – 0.5 g RAS biomass, №3 – 0.75 g RAS biomass.

Based on the research results, the saturation of the RAS microbial consortium with copper occurs after 40 minutes (Figure 12). The amount of absorbed copper primarily depends on the initial concentration of the metal in the solution. As the metal concentration increases, the degree of its uptake by microbial cells also increases within the same time frame.

In another experimental variant, the sorption of heavy metals was comparatively studied using the “Fermi-Start” biological preparation, RAS microorganisms, and wood shavings. For this purpose, the tested water sample was passed through the biofilter three times, and the residual copper concentration in the water was determined photometrically (Table 14).

Table 14.

Degradation of crude oil during use of biopreparation

№	Variants	Removal Efficiency, %			Total Removal, %
		1st Filtration	2nd Filtration	3rd Filtration	
1	Dry Biomass of "Fermi-Start"	57	14	9	80
2	Dry Biomass of "Fermi-Start" + Wood Shavings	58	18	13	89
3	Dry Biomass of Activated Sludge	35	11	5	51
4	Dry Biomass of Activated sludge + Wood Shavings	38	18	6	62

The data presented in Table 14 indicate that the adsorption capacity of activated clay (RAS) was significantly lower compared to that of probiotic microorganisms. This finding is consistent with previous studies demonstrating metal accumulation by probiotic microorganisms. The likely reason for this is that the activated clay used in our laboratory experiments had already become saturated with heavy metals present in the wastewater treated at the aeration station in Baku, which led to a reduction in the active adsorption surface of the microbial community.

Passing the studied water sample through a biofilter composed of activated sludge three times resulted in an average copper adsorption efficiency of 51–62%. In all cases, the addition of sawdust as an auxiliary adsorbent material increased the degree of copper removal from the water. Thus, the findings of our study demonstrate that the association of RAS and the probiotic microorganisms in "Fermi-Start" exhibits a high adsorption potential for heavy metals.

These results suggest that this combined biosystem may be effectively applied in the development of biotechnologies for the remediation of heavy metals and organic pollutants in the water bodies of the Absheron industrial region.

FINAL ANALYSIS OF THE STUDY

As a region that concentrates 70% of the country's industrial potential, the Absheron Peninsula has also been subjected to intense ecological pressure.

One of the region's major environmental issues is the contamination of soils with crude oil and petroleum products. Out of the total 222,000 hectares of land on the Absheron Peninsula, approximately 33,000 hectares are classified as degraded, including more than 20,000 hectares contaminated with petroleum.

The primary causes of these ecological problems include the pollution of soil with oil and formation water during oil and gas extraction and drilling activities, the uncontrolled discharge of formation water over many years, leading to the creation of oil-contaminated artificial lakes and ponds, and the accumulation of waste from oil refining processes in open areas.

The long-term continuation and expansion of oil production in the region have further exacerbated the severity and urgency of these environmental challenges.

The complex chemical composition of crude oil—including aromatic hydrocarbons, aliphatic hydrocarbons, and asphaltenes—leads to multifaceted environmental impacts, contributing to the contamination of soil, water, and air, and disrupting ecological balance.

The natural self-purification capacity of ecosystems weakens in proportion to the concentration and duration of exposure to these pollutants, ultimately resulting in the disruption of natural ecological processes.

Although various multidisciplinary studies have been conducted to address the environmental problems of the Absheron Peninsula, the lack of comprehensive solutions highlights the need for novel methods and approaches.

Considering this critical situation, the scientific foundations for a biotechnological strategy aimed at restoring the fertility of oil-contaminated technogenic soils in the Absheron Peninsula have been developed. Investigations were carried out to accelerate the processes that enhance the biological properties and ecological recovery of these degraded soils.

The importance of this research lies in its application of modern microbial technologies—particularly the "effective microorganisms" (EM) approach—alongside the use of regional natural minerals (zeolites) and renewable bioresources (such as biological waste) to address pressing environmental challenges. Technologies based on microorganisms capable of ensuring the efficient remediation of ecologically hazardous water bodies have been developed.

For the first time, the feasibility of combining "effective microorganisms" with regional bioresources—including plant waste, biohumus, whey, activated sludge, and zeolites—was investigated. The effectiveness of such a modified biosystem was comprehensively evaluated for the restoration of degraded, low-fertility technogenic soils and for improving their productivity.

The results demonstrated that the developed biologically active preparation possesses multifunctional properties. Its application leads to an increase in the biological potential of the soil (e.g., respiration intensity, number of multifunctional microorganisms, and biochemical activity), as well as improvements in physical characteristics and the soil's water and thermal regimes. Notably, the water retention capacity of the soil increased by 12–15%. These enhancements facilitate the mineralization of residual hydrocarbons in the soil down to background levels. Additionally, the preparation exhibits water-retention capacity, which reduces the leaching of essential mineral nutrients and prolongs their availability to plants in an absorbable form.

The study's findings confirm that the restoration (reclamation) of anthropogenically degraded soils using plant-microbe interactions and associated heterotrophic biota is a promising bioremediation strategy.

Furthermore, based on the microbial biopreparation, biotechnologies (such as biobons) have been developed to ensure the effective treatment of ecologically hazardous lakes on the Absheron Peninsula.

Considering the development of the field of "digital ecology", a new scientific and practical direction of "digital technologies" in the modern era, the obtained results can be effectively used to create a broad "information base" in the development of innovative digital technologies for the optimization of natural landscapes currently characterized by extremely unfavorable ecological indicators and their sustainable development. Thus, the obtained research results are of a resource-innovative nature and have ecological, economic, technological, social and scientific-practical significance.

RESULTS

1.The bioecological status of technologically polluted soils and water bodies on the Absheron Peninsula has been thoroughly investigated as a result of this research.

As a result of the research conducted, the bioecological state of the soil and water basins of the Absheron Peninsula, which have been exposed to technogenic pollution, has been comprehensively studied for the first time.

Based on the degree of oil contamination, the affected areas can be ranked as follows: *Bibi-Heybat* > *Z. Tagiyev* > *A. Amirov* > *Balakhani* [1,5,16,18,19,37].

2. It was found that with increasing technogenic pressure, the total number of aerobic heterotrophic bacteria, as well as nitrifying and cellulose-degrading bacteria, decreased in grey-brown soils. In contrast, the number of hydrocarbon-oxidizing bacteria, nitrogen-fixing bacteria, ammonifying and denitrifying bacteria increased compared to the control soils [19,29,36].

3. There was a significant difference in enzymatic activity between polluted and control soils. Catalase activity was higher in polluted soils than in control (0.09 ml vs. 0.4 ml of 0.1 $KMnO_4$ solution). Dehydrogenase activity showed an inverse correlation with oil concentration in the soil (3.9 mg TPF in control vs. 0.11 mg in

polluted soil). Invertase activity was substantially reduced (122 mg glucose/g in control vs. 9 mg in polluted soil). Based on sensitivity to oil and petroleum product contamination, the studied soil enzymes can be ranked as follows: *Invertase* > *Dehydrogenase* > *Catalase* [20, 21, 29].

4. In all soil samples tested, the germination capacity of test plants (garden cress and alfalfa) did not exceed 25–41%, indicating a high level of phytotoxicity (61–75%) in these soils [9,18].

5. A collection of rhizosphere microorganisms—specifically, hydrocarbon-degrading strains—was established. Among them, promising species were identified with the ability to degrade crude oil and various petroleum hydrocarbons (at concentrations of 10–15 g/L, degrading 71–77% of crude oil within 10 days). Notably, Pseudomonas aeruginosa strain No. 3 demonstrated high degradation potential and may be utilized in the development of bio- and phytoremediation technologies [2,3,13,17,26,34].

6. The effectiveness of plant–microorganism associations in the phytoremediation of oil-contaminated soils was investigated, and the scientific basis of a microbial-phytoremediation approach was developed. In model experiments, seed inoculation of selected plant species (alfalfa [Medicago sativa] and smooth brome [Bromus inermis]) with a microbial preparation (including Pseudomonas aeruginosa strain No. 3 and the biological product "Fermi-Start") led to a 78% reduction in crude oil concentration in the soil over 3 months, along with a 29% increase in plant biomass [26,33,34,38].

7. As a result of the conducted research, a modified biocompost was developed using regional resources and a microbial biopreparation to enable the effective removal of organic pollutants and restoration of bioecological functions in technologically contaminated soils of the Absheron Peninsula. Application of this biocompost in model experiments significantly accelerated the degradation of crude oil in soil, achieving 80–93.7% breakdown within three months [4,10,11,12,15,24,25,28,31,32,35,36].

8. It was determined that in the technogenically impacted lakes of the Absheron Peninsula, the concentrations of hydrocarbons and certain heavy metals significantly exceed the Maximum Allowed

Concentration (MAC). For hydrocarbons, this concentration was found to be 25–34 mg/L in both Lake Boyuk Shor and Lake Zigh, whereas the MAC is 0.02–2.00 mg/L. For heavy metals, specifically copper (Cu), while the MAC is 0.3–46 µg/L, the concentration detected in the lakes reached 4.2–5 mg/L [7,23].

9. It was revealed that in the technogenically impacted lakes of the Absheron Peninsula, the concentration of hydrocarbons varies depending on the sampling location and the season of the year. It was determined that the average annual number of saprophytic microorganisms in the study's water bodies is 3×10^4 to 3.2×10^6 CFU/L, and hydrocarbon-oxidizing microorganisms are 10^3–10^5 CFU/L, which allows classifying these water bodies as polluted [6,23].

10.The ability of *Pseudomonas sp.* strains № 45, 21, 33, and 64; *Rhodococcus sp*. strains № 7 and 12; and *Micrococcus sp*. strain № 22 isolated from lakes exposed to the technogenic influence of the Absheron Peninsula's capacity to degrade crude oil and oil products was revealed. It was determined that the degradation efficiency of strains of the genus *Pseudomonas (№ 45, 21, 33) is* 72–76% while other strains demonstrated a degradation capacity of 55–58% *Petroleum* > *Kerosene* > *Diesel fuel* > *Engine oil* [22,23,27].

11. Scientific foundations have been developed for creating biological preparations based on screened oil-oxidizing microorganisms to clean organic pollution in the water bodies of the Absheron Peninsula. This biopreparation was formulated using Pseudomonas sp. strains № 45, 21, and 33. Key findings include: Oil degradation rate with individual strains: 54–62%. Oil degradation rate using the strain association (consortium): 78%. These results confirm that a resource potential of oil-oxidizing microorganisms has been established, forming a scientific basis for the development of effective biopreparations aimed at remediating petroleum-contaminated aquatic ecosystems [22, 30, 39].

12. For the first time, the scientific foundations for the development of multifunctional biobons (dynamic, stationary, and cascade types) have been established to ensure the effective remediation of the heavily polluted lakes of the Absheron Peninsula.

These biobons possess the capacity to Degrade petroleum hydrocarbons and adsorb heavy metals. Thus, it enables the simultaneous removal of both organic and inorganic pollutants from aquatic environments. Modelling and laboratory experiments demonstrated that the proposed biotechnological approach allows for: Up to 96.7% degradation of petroleum hydrocarbons, and 78–86% removal of heavy metals. These findings underscore the potential of the developed biobons as an efficient and promising tool for addressing complex environmental pollution in industrial water bodies [7, 8, 22, 27,40].

Practical Recommendations

1. The developed efficient biotechnologies offer a viable solution to one of the most critical environmental problems of the Absheron Peninsula. Specifically, they enable the complete restoration of the bioecological functions of more than 25,000 hectares of technogenically degraded soils and 50 km^2 of polluted lake waters — ecosystems that have been excluded from biospheric processes for decades.

2. The proposed biotechnological approaches can be effectively applied to the remediation of multi-factorially contaminated soils of the Absheron region. This is due to the broad functional diversity, high degradative activity, and bioaccumulation capacity of the microorganism consortia used in these formulations.

3.The use of plant-microorganism associations presents a promising strategy for the phytoremediation of petroleum-contaminated grey-brown soils of the Absheron Peninsula. These associations enhance both hydrocarbon degradation and plant biomass productivity, supporting sustainable soil recovery.

4.Through various biochemical processes, it is possible not only to remove residual hydrocarbons from contaminated soils but also to enhance soil fertility and restore essential biological functions. The developed modified biocomposts can be applied effectively in the remediation of polluted soils and in improving their eco-biological condition. The preparation protocol and optimal application dosage for these biocomposts have been scientifically established.

5.The multifunctional biobons, developed for the effective purification of aquatic ecosystems, can be used in different configurations—stationary, dynamic, or cascade systems—depending on the hydrological characteristics, pollution levels, and types of contaminants present. These biobons are capable of adsorbing and degrading oil residues, including microspills, and play a vital role in protecting lakes from organic contamination. Their use contributes to the improvement of sanitary and hygienic conditions, expansion of recreational use, and supports the development of aquaculture and fisheries in the treated water bodies.

6. The integration of untapped biowaste materials as secondary resources into biotechnological processes not only enhances economic and environmental efficiency but also holds substantial value for environmental protection. This approach promotes sustainable resource management while contributing to the solution of pressing ecological challenges.

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