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ABSTRACT

of the dissertation for the degree of doctor of philosophy

MODELING OF MICROBIOLOGICAL PROCESSES IN SOILS UNDER ANTHROPOGENIC EFFECT (ON AN EXAMPLE OF SUMGAYIT CITY)

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GENERAL CHARACTERISTICS OF THE RESEARCH

Relevance and degree of completion of the topic. Nowadays, "*optimization of natural, including urbanized landscapes is one of the most important tasks of geoecological protection of territories*".[1] "*The ecological condition of the landscapes"* located in the territory of the Absheron economic district "*requires the bioecological assessment of the soil cover, as well as the formation of a paradigm of sustainable land use and the organization of long-term monitoring systems of technogenic impact zones*"[2,3]. In this regard, one of the most important problems of the efficient use and protection of urban soil is the development of methods for predicting changes in the physicochemical and biogenic, including, assimilation potential characteristics of Sumgayit city landscapes under the influence of technogenic processes. Thus, considering the specificity of the soil-climate characteristics, the system of soil protection measures developed for other areas of Azerbaijan (for example, the permissible limit (PL) of technogenic effects) is not suitable for the Sumgayit territory.

In the city of Sumgayit, "*under conditions of lack of natural resource potential, the anthropogenic impact load on the soil cover is increasing day by day, resulting in the change of water and air regimes, physicochemical parameters*"[4] and, accordingly, the direction of microbiological processes.

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[1] Заиканов, В.Г., Минакова, Т.Б., Булдакова, Е.В. Геоэкологическая безопасность урбанизированных территорий: подходы и пути реализации // Геоэкология. Инженерная геология, гидрогеология. – 2019. № 1, – с. 17-23.

[2] Исмаилов, Н.М. Глобалистика и экология Азербайджана. Баку: Изд-во Элм. 2006. -233с.

[3] Мамедов, М.Х. Эколого-географическая оценка состояния загрязненных земель на территории староосвоенных месторождений в Апшеронском экономическом районе Азербайджана // Географический вестник. Экология и природопользование. 2015. 1(32).-с. 61-73.

[4] Исмаилов, Н.М., Наджафова, С.И., Гасымова, А. Апшеронский промышленный регион – факторы экологической напряженности. Аридные экосистемы, Москва, т.21, № 3(64), 2015, -с.92 –100

According to the Decree of the President of the Republic of Azerbaijan, the goal of "evaluation of the ecological condition of contaminated lands and activization of work on the recultivation"[5] was set and using systematic analysis of the biological condition of Sumgayit city soils and modern biotechnologies, this determines the high relevance of the work in the direction of information-methodical provision of ecological projects on the optimization of their physicochemical and biological indicators.

The purpose and tasks of the research. The main purpose of the research was to study the essential regularities of the bioecological conditions of the Sumgayit soil cover, the development of different groups of microorganisms and effective modeling under conditions of anthropogenic and technogenic influence.

To achieve the set goal, the following tasks were performed:

- Using known methods of microbiology, the study of seasonal dynamics of the number of microorganisms in different zones of Sumgayit city;
- Studying the enzymatic activity and biological properties of soil bacteria in different zones of the city;
- Determination of the rate of microbiological destruction of plant residues and evaluation of the influence of various factors on these processes;
- Studying the possibilities of increasing the amount of humus and actual biogenicity in Sumgayit city soils through the use of microorganisms and regional bioresources;
- Development of modern biotechnologies, making recommendations for the solution of environmental problems of Sumgayit city soils - developing biogenicity and activating self-cleaning processes.

[5] “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

Research methods. The studies were conducted using generally accepted methods. The natural objects and processes that determine the bioecological characteristics of Sumgayit city soils were comprehensively analyzed. Field and laboratory research methods, modeling methods were used in the work. The research work is located at the intersection of microbiology, ecology, and soil science.

Main points presented to the defense of the dissertation:

- The negative effect of technogenic pollutants is characterized by the violation of the activity regularities of the soil microbiocenosis in the functional zones of Sumgayit city, which manifests itself in the quantitative dynamics and enzymatic activity of microorganisms.
- Microorganisms capable of assimilating paraffin and hydrocarbons of various structures are widespread in Sumgayit city soils.
- The use of an association of cellulose-degrading microorganisms to increase the microorganism pool and enzyme activity of urban soils has a stimulating effect on the soil remediation process.
- Application of the biopreparation obtained by the use of active groups of microbiocenosis, activated sludge microbiocenosis and plant residues opens prospects for increasing the biogenicity and assimilation potential of soils.

Scientific novelty of the research. The analysis of the modern bioecological situation carried out in Sumgayit showed that the city soil cover had been polluted as a result of the perennial and strong influence of technogenic factors, the ability to "assimilate" organic compounds of plant origin had been significantly weakened, the ability to restore the characteristics of resistance to decomposition and productivity had been lost. The analysis of the dynamics of most of the criteria selected for the assessment of land use, including microbiological and enzymatic criteria, showed that a significant violation of soil biogenicity had caused negative changes in the structure of microbiocenosis.

Considering the soil-climate characteristics of the region, integrated biotechnologies were developed for the technogenically disturbed soils of Sumgayit and their positive effect on soil biogenicity and assimilation potential was verified in a series of model experiments.

The rehabilitation of technogenic urban soils is based on the use of modified biopreparations ("Fermi-start", activated sludge of cleaning facilities rich in cellulose-degrading bacteria, plant residues, and hydrocarbon-decomposing microorganisms). All biotechnologies tested in a large number have shown their effectiveness in increasing the biogenicity of soils contaminated with organic matter and in accelerating their recovery processes in laboratory model experiments.

Theoretical and practical significance of the research. The results of the research can be used in monitoring and diagnosing the state of urban soils and regional landscapes polluted with industrial organic substances, conducting ecological expertise, as well as assessing the risk of environmental impact, natural and technogenic disasters, and other nature protection measures.

Results allow ensuring the improvement of the ecological situation of Sumgayit city.

Publications, approbation, and application of the research. Fourteen scientific works related to the topic of the dissertation were published and the materials of the dissertation were presented at the conference on “Innovations in Biology and Agriculture to Solve Global Challenges” (Baku, 2018), the 1st international conference of the European Academy of Sciences (Germany, Bonn, 2018), the International scientific ecological conference on “Waste, causes of their formation and prospects for use” (Krasnodar, 2019), the II international scientific-practical conference on “Actual problems of land management, cadastre, and environmental management” (Russia, Voronezh, 2020), 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 III international scientific-practical conference on “Actual problems of land management, cadastre, and environmental management” (Russia, Voronezh, 2021).

The organization where the dissertation was performed. The dissertation work was performed at the Soil Microbiology Laboratory of the Institute of Microbiology of ANAS from 2018 to 2021.

The volume and structure of the dissertation. The total volume of the dissertation consists of 193 computer pages, which is 243380 characters in total.

CHAPTER I. FUNCTIONAL CHARACTERISTICS OF URBAN ECOSYSTEM SOILS

In this chapter, literature data on biological and ecodiagnostic properties, classification and diagnosis, functional zoning, as well as pollution characteristics of urban landscape soils have been analyzed. A comprehensive analysis of the results of scientific research conducted in these directions is also given in Chapter I.

CHAPTER II. MATERIALS AND METHODS OF THE RESEARCH

The Sumgayit city soil was used as a research object. Soil sampling was carried out in 4 seasons during 2018-2019 according to "standards"[6]. The biological activity and self-cleaning capacity of the soil were evaluated based on the enzymatic activity of the soil, the number of different physiological groups of microorganisms, the intensity of respiration, the amount of residual oil in the soil, and seed germination (phytotoxicity) in experimental and control samples.

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

The respiration intensity was determined by the "*Makarov method*"[7]. The separation and counting of different physiological groups of microorganisms were carried out by the "method of planting in agar nutrient mediums"[8] and was expressed in CFU/g soil. Hydrocarbon-degrading microorganisms were determined in a Raymond medium supplemented with n-hexadecane, the number of bacteria of Azotobacter genus in an Ashby medium and cellulose-degrading microorganisms (SDM) in a liquid Hutchinson medium with filter paper. Identification of the genera of microorganisms was based on their "*morphophysiological characteristics* "[8].

"*Enzymatic activity* of urban soils *was determined by generally accepted methods*"[9].

The effect of heavy metals on the enzymatic activity of soils and the amount of cellulose-degrading microorganisms (CDMs) was studied by adding their water-soluble salts to the soil at different concentrations.

The degree of phytotoxicity was determined based on the "*Trofimov method*"[10].

The effect of the use of biopreparations and various plant waste on the accumulation of total carbon and humus in the soil and on the amount of CDMs was verified by modeling under laboratory conditions.

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

[8] 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.

[9] 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.

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

Pollution by oil products is determined according to "*PNDF"*[11].

Statistical processing of the results was carried out using "*Lakin*"[12], "*Excel*" and "*Statistica V.4.5*" software packages. Average statistical data are shown in the tables.

CHAPTER III. SYSTEMATIC ANALYSIS OF THE ECOLOGICAL SITUATION OF SUMGAYIT CITY

3.1. Natural-climatic conditions of Sumgayit urban landscapes

Sumgayit is a large industrial city located in the northwestern part of the Absheron peninsula. Functional zoning has been established in the city. The relief of the region is soft, the climate is mild, warm semi-desert and dry desert - high solar radiation and low precipitation are characteristic.

When strong Khazri wind prevails, a large amount of dust rises into the air having a negative impact. A positive effect of Khazri is that it causes the spread of atmospheric pollutants (Qypisic calcisols soils).

The "*gray-brown"* [13] soil is characterized by low humus content (1.2–1.8%), alkaline reaction, low absorption capacity and semi-desert vegetation. Thus, the climate of the region favors the evaporation of organic pollutants falling on the soil, and especially the strong year-round wind protects the air from pollution by volatile traffic and industrial waste.

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[11] ПНДФ 16.1.21-98 «Методика выполнения измерений массовой доли нефтепродуктов в пробах почв на анализаторе жидкости «Флюорат-02-2М»». – М., 1998. -13с.

[12] Лакин Г.Ф. Биометрия. М.: Высшая школа, 1980. - 294 с.

[13] Морфогенетические профили почв Азербайджана / Под ред. М.П. Бабаева, Ч.М.Джафаровой, В.Г. Гасанова. Баку: Элм, - 2004, -202с

3.2. Anthropogenic factors affecting the ecosystem of Sumgayit city

A systematic analysis of the ecological condition of Sumgayit urban landscapes was carried out. The main components of air pollution in Sumgayit are the same as in other large cities: dust, carbon monoxide, sulfur anhydride, nitrogen oxide, hydrocarbons, and hydrogen sulfide (Figure 3.1).

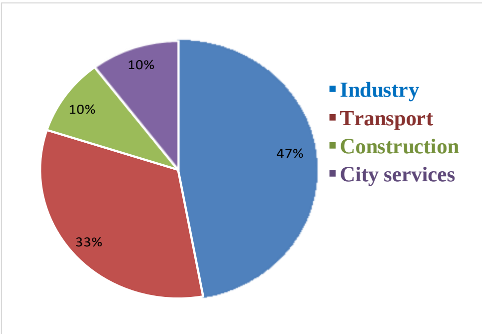


Figure 3.1. The amount of CO2 released into the atmosphere of Sumgayit city from various sources

The continuous growth of the population and the corresponding energy consumption should be noted especially. Because 50% of the population of Azerbaijan lives in the cities of Baku and Sumgayit.

Thus, "*in the 90s of the 20th century, the population of Sumgayit city was 240,000, while in 2021, it increased to 359,000 (increase - 51%)*"[14] (Figure 3.2).

[14] https://www.stat.gov.az/

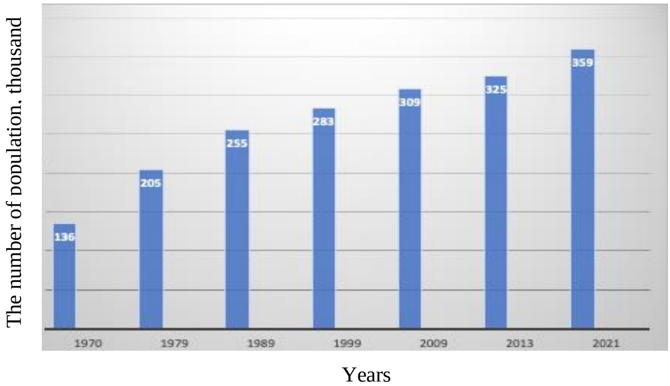


Figure 3.2. Population dynamics of Sumgayit city

In recent years, "*the number of vehicles is constantly increasing in Sumgayit, which causes numerous dust and toxic substances to enter the atmosphere*"[15] and pollution of air, soil and water facilities. The density of traffic flows is higher in the central part of the city.

Thus, population growth, aridity of the climate, low biodiversity, industrial and transport waste, high sensitivity to pollution, and low reproduction in relation to oxygen (compared to the norm of 27.4 million tons per year - only 0.8 million tons) affect the self-cleaning ability of the region and they are factors that cause the assimilation potential to be weak.

3.3 Pollution of Sumgayit city soils and its consequences

The dynamic growth of construction, industrial production, and transport load in Sumgayit causes deep changes in the soil cover, which leads to their alkalization, salinization, contamination with hydrocarbons and heavy metals, and, as a result, the degradation of the soil cover. Our research shows that the degree of soil organic

[15] Методика определения выбросов вредных веществ в атмосферный воздух от автотранспортных потоков, движущихся по автомагистралям Санкт-Петербурга //Утверждена Комит. по природопользованию, охране окружающей среды и обеспечению экологической безопасности от 8.12.2005, № 309. - с.205.

matter contamination depends on the soil-sampling zone: the industrial and transport zones have the highest degree of pollution, the least pollution is observed in the park zone.

The study of phytotoxicity revealed a direct relationship between the level of pollution and phytotoxicity.

According to the reduction of the degree of phytotoxicity, Sumgayit technogenic contaminated soils can be placed in the following order:

Industrial zone > Transport zone > Seliteb zone > Recreation zone

3.4. Self-cleaning capacity of the Baku-Sumgayit industrial region

According to our calculations, the number of factors that negatively affect the assimilation potential in this region is quite high. This enables the module of the anthropogenic impact of various organic pollutants on soil cover, surface, and groundwater, etc. to remain at a critical level in the natural landscape complex of the Absheron Peninsula. The self-cleaning capacity of the studied area is low and therefore, cannot effectively "neutralize" technogenic loads.

CHAPTER IV.
CHARACTERISTICS OF MICROBIOLOGICAL PROCESSES OF SUMGAYIT CITY SOILS

4.1. A brief history of the microbiological study of soils, including urban soils

In this sub-chapter, research on the microbiology of different soil types in Azerbaijan was analyzed.

4.2. The main regularities of bacterial microflora development in Sumgayit urban soils

As research objects, 25 soil samples were taken from 4 functional zones of Sumgayit. As a control, the soil of the Jeyranbatan reservoir area was used.

According to the results of the research, the highest number of heterotrophic microorganisms in the soil occurred in spring and autumn. Thus, in spring it changed from $1.2 \pm 0.27 x 10^5$ to $3 \pm 0.21 x 10^6$, and in autumn from $1.4 \pm 0.14 x 10^5$ to $4 \pm 0.33 x 10^6$ CFU/ 1g soil. This shows the effect of ecological factors on the number and activity of soil microorganisms.

The number of microorganisms also depends on the place of sampling. So, it was the most in the park zone soils: 2±0.23x106 - 4±0.33x106 CFU/ 1g soil.

This difference in the number of microorganisms is explained by the less exposure of the zones to technogenic pollution. The obtained results show that as the degree of soil pollution increases, the number of microorganisms decreases and the intensity of respiration also declines. Based on the degree of reduction of the number of heterotrophic microorganisms, we can line up the soil cover of different zones of Sumgayit city as follows: Background > Recreation > Seliteb> Transport> Industry.

The number of hydrocarbon-oxidizing microorganisms (HOM) increased in the soil samples of transport and industrial zones.

4.3. Study of the ratio between heterotrophic and hydrocarbon-oxidizing microflora in different zones of urban soils

According to the obtained results, when the number ratio between hidrocarbon-oxidizing and saprotrophic microorganisms was 0.2-0.21 in the soils taken from the industrial zone, this ratio did not exceed 0.03-0.05 in the park zone soils.

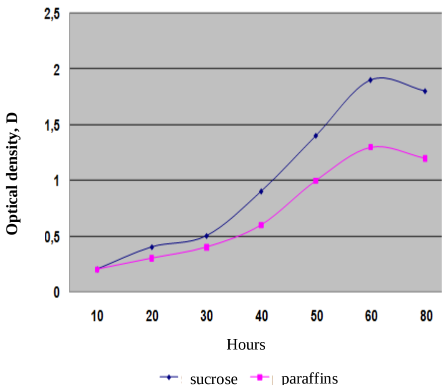
A similar pattern was found between the park zone and the roadside zone.

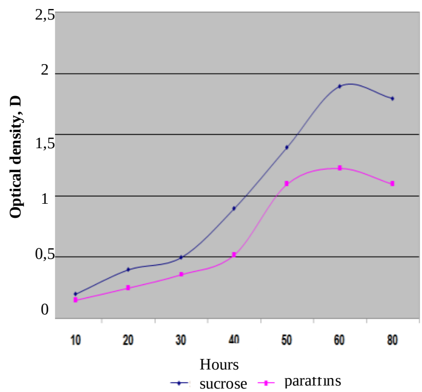
This shows that in urban soils with a high amount of hydrocarbons, about 10-15% of the structure of the microbiocenosis consists of microorganisms that break down petroleum hydrocarbons and participate in the self-cleaning processes of the soil.

Aerobic cellulose-degrading microorganisms constitute one of the indicator groups of the studied soils. Their number in technogenic soils ($6x10^2$ CFU/ 1g) was significantly lower compared to clean soils (1.4x10^4 CFU/ 1g). This is attributed to the low amount of nitrogen and plant residues in polluted soils.

The composition of the soil microbiocenosis, which ensures the physiological stability of the biosystem, changes in the soils of roadside and industrial zones, which can be observed on an example of microorganisms involved in the nitrogen cycle. Soil pollution caused a decrease in the activity of nitrifying microorganisms involved in the nitrogen cycle and a sharp increase in the number of nitrogen-fixing and ammonifying microorganisms. It is clear that the development of physiological groups of microorganisms in Sumgayit city soils varies according to functional zones, soil pollution leads to changes in soil microbiocenosis and a decrease in its diversity.

Considering that nitrogen-fixing bacteria use various organic compounds as well as carbohydrates as the only source of carbon and energy in soil biogeocenosis, we examined these properties of the isolated *Azotobacter sp.26* and *Azotobacter sp.11* strains in our laboratory model studies (Figure 4.3).





A

Figure 4.3. Growth of *Azotobacter sp.26* and *Azotobacter sp.11* (a) in sucrose and liquid n-paraffins

The results showed that the strains were actively grown in the n-paraffin C_{12}-C_{16} mixture, but the growth rate of the cultures was several times (21%-26%) lower in the medium with n-paraffins compared to the medium with sucrose.

Besides, Azotobacter strains grow better in the medium containing a mixture of n-paraffins and sucrose than in the medium containing only n-paraffin (Figure 4.4).

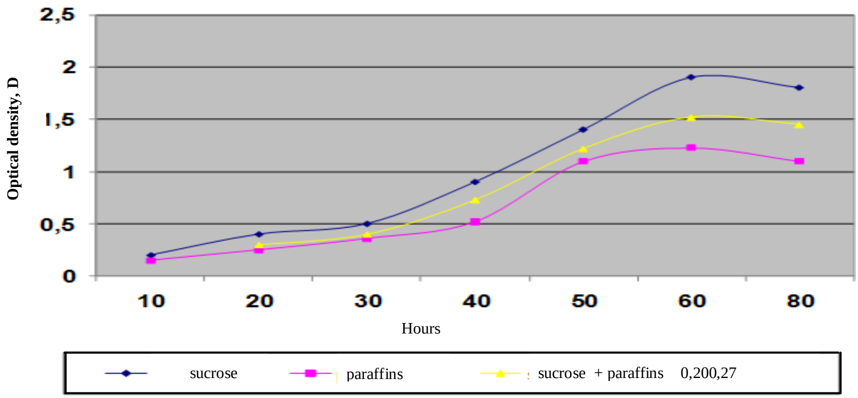


Figure 4.4. Growth of *Azotobacter sp.26* in sucrose and liquid n-paraffins, as well as in a mixture of these substrates.

This is explained y the effect of "co-oxidation" of hydrocarbons. Nitrogen fixation intensity was 23.3 mg/g on average.

This shows that Azotobacter strains isolated from polluted urban soils use hydrocarbons as the sole source of carbon and energy and are also capable of assimilating atmospheric nitrogen in these processes.

The introduction of Azotobacter strains to soils with technogenic pollutions had a positive effect on the growth and development of barley seedlings and reduced phytotoxicity (Figure 4.5).

In this regard, the introduction of Azotobacter cultures into contaminated urban soil is likely to stimulate the functional activity of HOM present in these soils. For this purpose, we studied the effect of *Azotobacter sp.26* and *Azotobacter sp.11* strains on the growth and development of microorganisms in activated sludge.

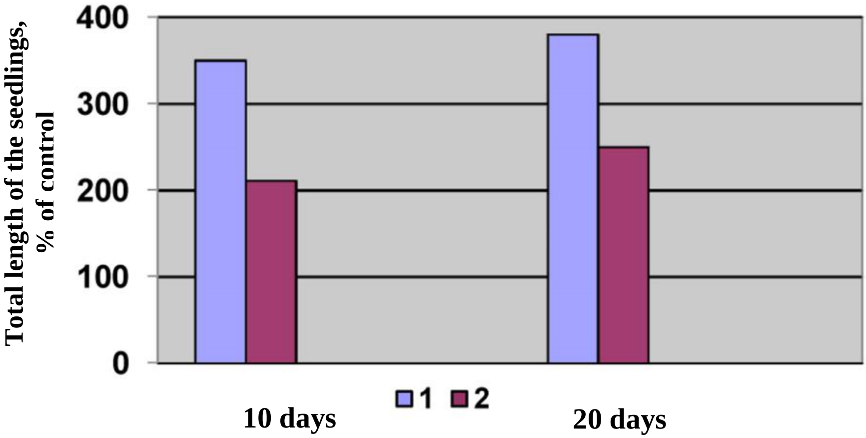


Figure 4.5. Effect of *Azotobacter sp.26* strain, introduced into the soil of roadside zones with technogenic contamination, on the total length of barley seedlings (% of control).

Note: 1- azotobacter cultures were introduced into the soil; 2 - control.

In the experiment, 100 ml of Raymond medium, 20 ml of activated sludge, 10 ml of studied nitrogen-fixing microorganisms, and 2 ml of liquid n-paraffin mixture C_{12}-C_{16} as the only carbon and energy source were placed in 250 ml flasks, and cultivation was performed in swings at a temperature of 28-29^{0}C. Samples were taken regularly to determine the amount of n-paraffins in the culture medium. The results are given in Figure 4.6.

This shows that the inclusion of azotobacter cultures in the activated sludge (AS) consortium increases the functional activity of the populations and leads to an increase in the degradation activity in relation to hydrocarbons.

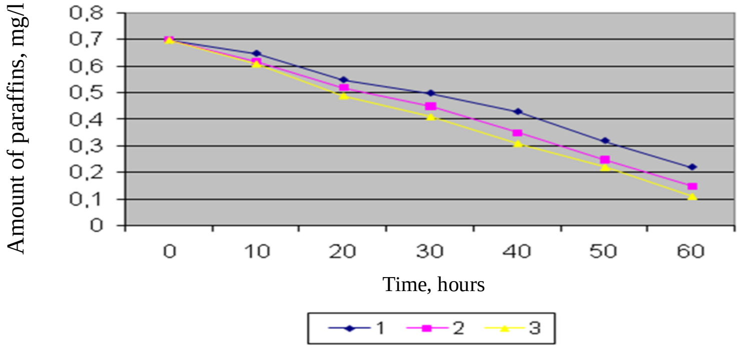


Figure 4.6. Development of different groups of microorganisms in n-paraffins

Note: 1- AS; 2- AS + *Azotobacter sp. 26*; 3- AS + *Azotobacter sp.11*.

These studies were continued in the form of soil model studies. 200 g of contaminated soil with a hydrocarbon content of 92.0 mg/kg was placed in three Petri dishes: the 1st variant was used as a control; the 2nd variant contained a consortium of AS microorganisms; A mixture of AS+azotobacter strains was added to the 3rd variant. Incubation was performed in a thermostat at 27-28°C and 50-60% humidity. In the 45-day model experiment, the amount of total hydrocarbons in technogenic contaminated soils was reduced by 2.2% in the control, 32.7% in the soil supplemented with AS, and 40.0% in the variant with the addition of the mixture of Azobacter cultures and AS. The obtained results showed that the application of AS accelerates the decomposition of hydrocarbons in the technogenically contaminated soil compared to the control, however, the introduction of sludge and the microbial consortium of azotobacter cultures into the soil increases the rate of decomposition of hydrocarbons in the soil.

After the model experiments, soil samples were examined for phytotoxicity (Table 4.3). It is clear that the combined use of AS and azotobacter cultures reduces the amount of hydrocarbons in the soil by 32.7-40%, thereby significantly reducing the phytotoxicity of the soil.

Table 4.3.

Phytotoxicity of technogenically polluted soils after the use of microbial preparations

Variants	Amount of hydrocarbons, mg/kg soil		Phytotoxicity (germination of barley seeds, %)	
	before	after	before	after
Control (without additions)	92.0	90.0	72.0	68.0
AS	92.0	62.4	72.0	89.0
AS+ azotobacter cultures	92.0	55.2	72.0	92.0

Undoubtedly, the creation of new adaptation mechanisms in plants and microorganisms and their strengthening in subsequent generations allows them to constantly adapt to extreme forms of anthropogenic (technogenic) effects and continue their life activities. Gradually, a new natural structure - the biocenosis of technologically polluted landscapes of Sumgayit city is developing. At the same time, the issue of the possibility of managing these processes to increase the actual biogenicity of urban soils is relevant.

4.4. Study of hydrocarbon-oxidizing microorganisms in different soil areas of Sumgayit city

A total of 26 strains were isolated from two soil samples. HOM cultures were isolated from the clean zone (6 strains) and the industrial zone (20 strains). All selected strains were studied for their absorption of hydrocarbons of different structures (n- C_{12}-C_{18}

paraffin mixture, toluene, and p-xylene), crude oil, as well as petroleum products (kerosene, gasoline, and diesel fuel).

Studies have also shown the presence of cultures capable of assimilating petroleum hydrocarbons among the microorganisms of different physiological groups in microbiocenosis of urban soils. All examined hydrocarbons, as well as oil products, were isolated from oil-contaminated soils of the "Chemical Industry" area and 4 active strains - *Pseudomonas sp. 2,* and *sp.9, Bacillus sp.19, Rhodococcus sp.19* had the ability to assimilate. The existence of such groups of microorganisms capable of breaking down petroleum hydrocarbons is attributed to the continuous pollution of the soil by industrial and transport waste under Sumgayit conditions.

CHAPTER V.
ENZYME ACTIVITY IN URBAN SOILS

5.1. The main regularities of the activity of soil enzymes in different zones of urban soils

Enzyme activity was studied in Sumgayit urban soils. Soils in various zones were found to differ in enzymatic activity. Catalase activity varied in the range of 0.41-0.89±0.03 ml O_2/min/g soil. The activity of catalase was found to be higher in the soils of industrial and roadside zones most exposed to technogenic impacts (0.70-0.89±0.03 ml O_2/min/g soil), which indicated that the process of self-cleansing from organic pollutants occurred in the soil.

Besides, the soils were characterized by high levels of the polyphenol oxidase enzyme. The polyphenol oxidase activity varied between 8.2 ± 0.88 and 16.5 ± 0.88 mg purpurogallin 100g soil/30min. It is believed that this is related to the high amount of organic pollutants, including phenols, entering the soil as a component of industrial and transport waste.

The invertase activity varied between 5.0 ± 0.02 and 8.8 ± 0.02 mg glucose/g soil within 24 hours. The results showed that the invertase activity was significantly reduced in the polluted soils, and this reduction reflected the disruption of the relationship between the vital activity of the vegetation and the soil and the functional status of the plants.

Urease activity was between 2.6 ± 0.09 and 5.8 ± 0.09 mg NH_3/ 24 hours in 10 g of soil. An inverse correlation was detected between the soil dehydrogenase activity and oil concentration in the soil.

The activity of dehydrogenases in contaminated soils was on average 8.9-9.6 mg TF per 10 g of soil. In the soils of the park zone, this indicator was low and varied between 8.4-8.9 mg of TF on average in 10 g of soil.

The effect of various petroleum hydrocarbons on the biological activity of soils was studied under laboratory conditions. Clean soil taken from the recreation area was contaminated with crude oil and petroleum products (PP) (pollution degree - 1%).

After a month, the activity of soil enzymes - catalase, dehydrogenase and invertase was determined in all samples (Table 5.1).

Table 5.1.

Effects of oil and hydrocarbons on soil enzymes

Variants	Enzyme activity		
	Catalase, ml 0.1N $KMnO_4$	Invertase, mg glucose /1g soil	Dehydrogenase, mg TF/10g soil
Soil (control)	0.82	27	11.0
Soil+ petroleum	0.77	16	11. 6
Soil + n- hexadecane	0.89	11	12.7
Soil+ cyclohexane	0.79	12	10.2
Soil+p-xylene	0.46	9	4.8

Oil and PP had different effects on the activity of soil enzymes, which is due to the structure of hydrocarbons. Thus, aromatic hydrocarbons had the most inhibitory effect on the activity of soil enzymes, and paraffins had the least inhibitory effect, even n-paraffin hydrocarbons stimulated the activity of catalase and dehydrogenase enzymes.

In general, enzymes can be ranked according to the degree of sensitivity to oil and PP contamination of the soil as follows:

catalase > dehydrogenase > invertase

According to the results of our experiments, the microbiological and enzymatic indicators of the soils of different zones of Sumgayit city are unstable and reflect the specificity of the pollutant, the degree of pollution, the nature and intensity of the biological processes occurring in the soils affected by industrial and transport waste.

5.2. Effects of heavy metals on cellulose-degrading microorganisms and soil enzyme activities

Considering the negative impact of heavy metals on microbiological and biochemical processes in soils, we studied their effect on catalase activity and CDM content of soils at different pollution levels. For this, water-soluble salts of Cu, Zn, PB, and Co (1%) were included in the soil samples selected from the territory of the Jeyranbatan reservoir according to the following scheme: 1) control; 2) 1 PL; 3) 2 PL; 4) 5 PL; 5) 10 PL.

The results of the studies showed that CDM activity was significantly reduced by 1 PL and catalase activity by 5 and 10 PL as a result of soil contamination by heavy metals after 25 and 50 days of incubation. Thus, under the effect of high doses of Cu, cellulose-degrading activity was reduced by 51%, catalase activity – by 45% compared to the control; Cellulose-degrading activity with Zn application decreased by 45%, the catalase enzyme activity decreased by 67%. Under the influence of Pb, the decrease in cellulose-degrading activity was 54%, and in catalase activity was

51%. Under the effect of Co, cellulose-degrading activity decreased by 32% and catalase activity by 28%.

To evaluate the inhibitory effect of metals on the number of CDMs, according to the results of our experiments, they can be shown in the following order: Pb < Co < Cu < Zn.

The negative effect of heavy metals (HM) on the activity of cellulose-degrading bacteria is caused by their ability to connect with sulfhydryl groups of proteins, which disrupts the synthesis and activity of enzymes. The results of the research showed that among the studied enzymes, the catalase enzyme had the greatest response to soil lead contamination. This allows the use of soil catalase activity as an indicator of pollution of Sumgayit city soils with HM, including lead.

CHAPTER VI.
DEVELOPMENT OF INTEGRATIVE BIOTECHNOLOGIES FOR INCREASING THE BIOGENICITY, ASSIMILATION POTENTIAL AND SELF-CLEANING CAPACITY OF SUMGAYIT URBAN SOILS WITH THE PURPOSE OF SUSTAINABLE NATURE MANAGEMENT

6.1. Ways to increase soil cover biogenicity, assimilation potential and productivity of Sumgayit urban landscapes

According to the results of our research, the biogenicity of Sungayit urban soils has decreased because of intensive mineralization of organic substances, low humus content, high degree of pollution with organic and inorganic substances.

Recultivation methods should be developed for Sumgayit urban soils to activate microbiocenosis, assimilation potential and self-cleaning processes. In the research process, we aimed to determine whether it is possible to increase the amount of humus in the soil cover of Sumgayit city in a controlled regime by using biopreparations, including those containing CDMs. For this purpose, we conducted laboratory and field studies. For laboratory experiments, we included various modifications of plant-derived organic matter simultaneously with biopreparations in the soil samples. A microbial community was created based on three cultures of cellulose-degrading microorganisms (*Pseudomonas sp.2, and 9* and *Bacillus sp.19)*. Cultures were grown in Raymond's medium in the presence of n-C_{16} and biomass of mature cultures was collected.

During the experiments, 1 kg of a soil sample taken from clean areas (Jeyranbatan reservoir area) was placed in 2 kg containers, on which a mixture of chopped straw, tree bran, and leaves of large-leaved plants and coniferous trees was added in a ratio of 1:1 as a cellulose-containing material, and was kept at 55-60% of full field humidity. After that, biopreparations in the volume of 20 ml were added to the soil: association of cellulose-degrading microorganisms, activated sludge and “Fermi-start” biopreparation alone and in combination.

It was found that the use of biopreparations such as CDM association, activated sludge, "Fermi-Start" led to an increase in the number of CDMs (3-10 times), stimulated the enhancement of the accumulation of total carbon and humus in the soil by 20-55%. Microorganisms included in AS and CDM associations, for example, bacteria of the genus *Pseudomonas* and *Bacillus*, can directly participate in the degrading processes of cellulose compounds. In addition, some of the activated sludge microorganisms participate in the soil as a source of nitrogen, phosphorus, and other biogenic elements, which ultimately ensures the high activity of CDMs.

An increase in the number of total microorganisms was observed in all variants of the application of cellulose-containing materials and biological preparations in the soil, which indicates that the activity of microorganisms in the soil has increased and favorable conditions for their activity have been formed. In these processes, the role of “Fermi-Start” biopreparation, which contains various functional groups of microorganisms involved in the formation of soil fertility, is also positive. Thus, the use of biopreparations and the introduction of cellulose-containing materials into the soil allows for a significant increase in the amount of humus in gray-brown soils within 6 months, under the condition of maintaining optimal moisture.

The results of the studies showed the positive role of CDMs in the accumulation of total carbon and humus in the soil. This means that biological preparations based on CDMs can be promising for the acceleration of the composting process of organic waste. Laboratory modeling experiments were conducted on the effect of these biopreparations on the assimilation potential of Sumgayit urban soils. 300g of clean soil (Jeyranbatan reservoir area) was placed in vegetation containers and was contaminated (10%) with crude oil from the Surakhani field. This degree belongs to the category of high pollution.

A complex of biopreparations - a mixture of CDM associations, AS, and “Fermi-start” biological preparations (10 ml of each in 100 g of soil), as well as a mixture of plant substrates in the amount of 10% of the soil volume (wood bran, chopped straw, leaf litter) were added to the contaminated soil. For the cultivation, the pots were kept at 25^0C for 6 months with 50-60% of full field humidity.

After six months, the amount of residual hydrocarbons and the number of total and oil-degrading microorganisms were determined in all variants. The results are given in Table 6.1.

The results of laboratory modeling show that the addition of ameliorants to contaminated soils helps to reduce the amount of oil in the soil by 65-94% compared to the control. The obtained results show that the main advantages of using plant litter and other cellulose-containing waste (wood bran, mowed lawn, etc.) are the increase in the amount of organic substances necessary for plants in the soil, biogenicity and assimilation potential, durability and self-cleaning ability against pollution of Sumgayit urban soils with organic substances. Applying biopreparations to contaminated soil accelerates the biological decomposition of pollutants, restores the number of microorganisms, enzyme activity, assimilation potential and reduces phytotoxicity.

Table 6.1

Self-cleaning ability of soils when applying biopreparations and plant residues

Variants	Indicators				
	TNM CFU/g soil	RI, CO_2 mg/100 g soil	HOM, CFU/g soil	Amount of hydrocarbons, %	
				Initial	After 6 months
Contaminated soil (control)	$1.5.10^3$	52	$1.5.10^2$	10	9.1
Soil + biopreparations	$1.8.10^5$	68	$1.9.10^3$	10	3.2
Soil+ biopreparations + plant waste	$2.3.10^5$	70	$2.5.10^3$	10	1.5

Note: control - Soil taken from the Jeyranbatan reservoir area,
TNM-total number of microorganisms,
RI – respiration intensity,
HOM- hydrocarbon-oxidizing microorganisms.

The use of biopreparation enriches the soil with a complex of active microorganisms. The biopreparation contributes to the decomposition of hydrocarbons both due to its own oil destructor-microorganisms and by activating the aboriginal microbiota of the soil, which can decompose hydrocarbons. As a result, soil phytotoxicity decreases and normal growth and development of vegetation are ensured (Figure 6.1).

a park Seliteb industrial roadside

b park Seliteb industrial roadside

Figure 6.1. Degree of phytotoxicity of soils in different zones (park, seliteb, industrial, roadside): before (a) and after (b) application of biopreparations under laboratory conditions

FINAL ANALYSIS OF THE STUDY RESULTS

Because of the expansion of urban ecosystems in modern times, urban lands are exposed to various technogenic effects, which cause important changes in the structure of the soils. In the dissertation work, during the complex research conducted on the soils of Sumgayit city, the main regularities of microbiological and biochemical processes were studied, as well as the factors and mechanisms that determine the optimal course of these processes were studied, and the scientific basis of improving the biological functions of urban soils and bioremediation technology was developed.

Soil parameters were studied using biological, chemical, and technological methods. Studies were conducted at the interdisciplinary level that allowed the acceleration of the process of activating the biological properties of the soils with technogenic damage in Sumgayit, which is considered a large industrial city. For the first time in the research, the possibility of combined use of regional bioresources with the association of activated sludge and CDMs with modern microbial technologies - "effective microorganisms" (Fermi-start) to solve the problem was considered and the analysis of the effectiveness of such a modified biosystem in order to restore and increase the fertility of soils with technogenic pollutions was conducted.

This biopreparation obtained during research was submitted to the Greening Department of Sumgayit city and was accepted for field testing. The biopreparation is promising for use in environmental projects in the future.

CONCLUSIONS

1. For the first time, a complex study of the activity of microorganisms and some regularities of the activity of biochemical processes was conducted in Sumgayit urban soils. It was found that as a result of pollution, the number of microorganisms and the ratio of their functional groups varied in different zones compared to background soils. According to the degree of decrease in the number of heterotrophic microorganisms, the soils of different zones can be lined up as: Background > Recreation > Seliteb > Roadside > Industrial [1, 7, 8, 9, 11].
2. The number of hydrocarbon-oxidizing microorganisms increased in the soil samples of transport and industrial zones. 26 strains of bacteria spread in these soils were extracted into pure cultures, active strains (*Pseudomonas sp. 2,* və *sp.9, Bacillus sp.19, Rhodococcus sp.19*) capable of breaking down hydrocarbons of various structures (n-C_{12}-C_{18} paraffin mixture, toluene, and p-xylene), crude oil, as well as oil products ((kerosene, benzine, and diesel fuel) were identified (42.7-53.9%) [4, 10].
3. The activity of catalase in polluted soils was found to be higher compared to the control, and the activity of invertase was

significantly reduced. The activity of dehydrogenase was between the activities of catalase and invertase. According to the degree of sensitivity of soils to pollution with organic substances, the order of soil enzymes of Sumgayit city can be shown as follows: catalase > dehydrogenase > invertase [3, 5, 14].

4. For the first time, methods for increasing biogenicity based on the application of cellulose-degrading microorganism associations, “Fermi-start” preparation, as well as activated sludge (containing associations of natural hydrocarbon-oxidizing microorganisms) with plant waste were developed for the restoration of Sumgayit urban soils. The use of these biopreparations has a stimulating effect on the increase in the number of cellulose-degrading bacteria (CDB) (3-10 times) and the decomposition of cellulose compounds [12].

5. Cellulose-degrading bacteria in the soil have a positive effect on total carbon and humus accumulation. Thus, the introduction of cellulose-containing materials into the soil together with biopreparation (under the condition of optimal moisture protection) causes a 20-55% increase in the amount of humus in gray-brown soils within 6 months [2, 12].

6. All tested integrated modern biotechnologies have shown high efficiency in reducing phytotoxicity, increasing biogenicity and accelerating recovery processes of Sumgayit urban soils contaminated with organic substances. All the mentioned results allow us to ensure the improvement of the ecological situation in Sumgayit city [1, 6, 12].

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