# Ecological and Microbiological Features of the Soils of the City of Kogalym

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Abstract: The article presents the results of a biogeochemical study of the soils of the city of Kogalym. It is shown that the priority pollutants among heavy metals are mobile forms of copper and nickel, exceeding their MPC is observed in industrial areas of the city, especially in the Eastern zone. Changes in the state of soil microflora in industrial zones were recorded: the maximally reduced number of nitrogen cycle microorganisms: ammonifying, denitrifying and nitrogen-fixing. It is shown that the main phylum in soil microbocenoses is Bacillota, represented by bacteria of the genus *Bacillus* (10 species) and *Kurthia* (2 species). The phylum Actinomycetota is represented by bacteria of the genus *Aureobacterium* (2 species) and *Curtobacterium* (2 species), the phylum Pseudomonadota is represented by one species of bacteria of the genus *Pseudomonas*. According to the abundance index, the genus *Bacillus* dominated in the group of heterotrophic and hydrocarbon-oxidizing microorganisms, the genus *Kurthia* dominated in the group of denitrifying microorganisms, ammonifiers were represented in equal proportions by bacteria of the genera *Bacillus*, *Kurthia* and *Pseudomonas*. The taxonomic diversity of the soil microbocenoses of the city of Kogalym indicates a significant bioremediation potential for the functioning of ecosystems and its recovery in the event of man-made pollution.

## 1 INTRODUCTION

Khanty-Mansi Autonomous Okrug (KhMAO) is one of the main oil-producting areas in Russia. In this region, the long-term exploitation of oil and gas fields has affected the state of the natural environment, soils, and changed the natural landscapes of the district (Sokolov et al., 2017).

The city of Kogalym (KhMAO, Tyumen region) is a young and rapidly developing city with great economic potential. The main industrial sector of Kogalym is oil production, as well as processing and transportation of hydrocarbon products. There is the Kogalym oilfield, located 17 km northwest of Kogalym, the development of which began in 1985. The field belongs to the West Siberian Province – the largest oil and gas basin in the world. In case of soil contamination with petroleum products, a geochemical threat to ecosystems may arise, due to presence of heavy metals in crude oil and drilling fluids, such as lead, nickel, cadmium, chromium, vanadium and zinc (Mustafa et al., 2015). Heavy metals negatively affect the abundance and species diversity of the soil microbiota, exerting mutagenic, microbostatic and sterilizing effects, as well as disrupt the correct sequence of biochemical pathways

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in the biogeochemical cycles of the soil (Friedlová, 2010).

Solving the problem of soil protection and trying to increase it's fertility is impossible without knowledge of biological and geochemical soil characteristics and processes caused by anthropogenic impact. Soil is the main component of the terrestrial ecosystem, the functioning of which is largely provided by the soil microbiome, which determines the importance of studying microbial communities (Pospelova et al., 2015; Wolińska et al., 2016). Assessment of the plight and changes in soil microbiota is one of the most important issues of soil monitoring, since soil microorganisms are the most sensitive indicators of changes in soil and environmental conditions (Sumampouw, Risjani, 2014; Korneykova et al., 2021).

To assess the state of Russian and foreign urban soils, a wide range of microbiological indicators are determined: the number of pathogenic and opportunistic pathogenic microorganisms, taxonomic diversity and structure of microbial communities, functional diversity of soil microorganisms, microbial biomass content and ecophysiological profile, greenhouse gas formation, activity of soil enzymes (Tikhonovich et al., 2017; Ananyeva et al., 2021). The study of the microbiome of urban soils in Russia is realised mainly in large industrial cities and megapolises (Stoma et al., 2020). There is very little information on microbial communities in small towns.

The city of Kogalym holds a stable economic position and has great potential for further development. New oilfields are being developed, the population is increasing due to natural growth and the influx of prospective staff. Therefore, microbiological analysis of urban soils of Kogalym is of great importance both for characterizing the ecological and biological state of soils at the present stage, and for further forecasting the environmental consequences of human activity on this territory. In addition, the soils of the oil and gas region can be considered as the largest natural depository of hydrocarbon-oxidizing microorganisms.

The purpose of this study was to study the qualitative and quantitative composition of soil microbocenoses of the city of Kogalym in combination with an assessment of the ecological and geochemical properties of soils.

# 2 RESEARCH METHODOLOGY

The objects of the study were soil samples taken on the territory of the city of Kogalym (N 62°16', E 74°29'), which is equated to the regions of the Far North. Soils: podzols illuvial-humus; peat bog top and peat bog transitional with shallow thermokarst lakes; floodplain swampy. The soil–forming rocks are sandy (National Atlas, 2011).

In the course of this study, 53 soil samples were selected and studied at the intersection of the main highways of Kogalym (Figure 1). Samples from 1 to 26 were taken in the central residential part of the city, from 27 to 33 – in the Northern Industrial zone, and from 34 to 53 – on the territory of the eastern industrial zone and in the region of such villages as Pionernyj, Festivalnyj, Pribaltijskih Stroitelej. Background samples in the number of 10 were taken 10 km to the west from the territory of the city in identical urban geomorphological and geological conditions.

Samples were taken and prepared according to GOST 17.4.4.02-84 (2008). Sampling sites were chosen with the account the wind rose, microrelief, and the disposition of buildings and communications. According to the requirements of the GOST, we analyzed the top part of the "A" soil horizon (from 0- to 5-cm-thick), where most of the contaminants precipitating from the atmosphere are usually accumulated. The size of the test plots varied from 2-3 to 10 m$^2$. Samples were taken by the envelope method: one sample in the center, four at the corners of the site, and 2-3 samples around the tops of the envelope. The weight of the combined sample varied within 0.5-1.0 kg.


Figure 1: The layout of the testing areas on the territory of Kogalym: 1 – building districts, 2 – water sites, 3 – railway, 4 – highway, 5 – testing sites.

The number of cultivated microorganisms of the nitrogen cycle was taken into account on agarized selective media. The number of ammonifying microorganisms was determined on a selective medium of the following composition, g/L: peptone – 10.0; starvation agar – 20.0; denitrifying microorganisms on medium: GRM-agar – 38±2; $KNO_3$ – 1.0; nitrifying microorganisms – on Vinogradsky medium: $KNO_3$ – 10.0; $K_2HRO_4$ – 5.0; $MgSO_4$ – 2.5; NaCl – 2.5; $FeSO_4$ – 0.05; starvation agar – 20.0; nitrogen–fixing microorganisms – on Ashby medium: glucose – 20.0; $K_2HPO_4$ – 0.2; $MgSO_4$ – 0.2; NaCl – 0.2; $K_2SO_4$ – 0.2; $CaCO_3$ – 5.0; starvation agar – 20.0. The hydrocarbon-oxidizing microorganisms (HOMs) were detected on mineral medium agar M9 with 1 % of vaseline oil as the only source of carbon and energy (Mills et al., 1978).

The inoculation on nutrient agar (GRM-agar) and selective media for the analysis of the number of ammonifying (AMM), denitrifying (DNM), nitrifying (NM) and nitrogen-fixing (NFM) microorganisms was performed at dilutions of $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$ in several replications. The colonies grown were calculated within a 3- to 5-day period of cultivation in a thermostat at 28-30 °C (Foght, Aislabie, 2005).

Representatives of the main morphotypes were isolated on a beveled GRM-agar and identified based on results obtained from studying a complex of cultural, morphological, physiological, and biochemical traits according to generally accepted manuals (Bergey's Manual of Determinative Bacteriology).

Heavy metals in soil were determined by atomic absorption spectrometry with flame atomization on a Kvant-2AT spectrophotometer (KORTEK enterprise, Russia). Mobile acid-soluble forms of metals (Cu, Zn, Ni, and Pb) were determined in extracts of 1M $HNO_3$ (RD 52.18.191-89).

The experimental data obtained were subjected to statistical processing. To do this, the obtained digital values were checked for the normality of the distribution by the Kolmogorov-Smirnov test. The comparison of the average was carried out using the Fisher test and the indicator of the smallest significant difference ($P \leq 0.05$) in a one-way analysis of variance (ANOVA). Data was analyzed and processed using the Statistica 13.0 package (TIBCO Software Inc. 2017, StatSoft Russia).

# 3 RESEARCH RESULTS

According to the data of our ecological and geochemical analysis (Table 1), the content of mobile forms of Pb in the soil of the city of Kogalym was in the range from 0.08 to 2.93 mg/kg of soil, not exceeding the MPC (6.0 mg/kg of soil) (GN 2.1.7.2041-06). The content of mobile forms of Zn in most samples was within the normal range, varying from 1.76 to 21.56 mg/kg of soil. Only in three samples the concentration of mobile forms of Zn slightly exceeded the MPC (23.0 mg/kg of soil) (SanPiN 42-128-4433-87).

In the studied area of Kogalym, the concentrations of mobile forms of Cu varied from 0.16 to 1.55 mg/kg of soil, in 12 samples it exceeded the MPC (3.0 mg/kg) (Kuznetsov et al., 1992). Concentrations of mobile forms of Ni varied from 0.24 to 8.73 mg/kg of soil, exceeding the MPC (4.0 mg/kg) (GN 2.1.7.2041-06) in 14 samples. The Eastern industrial district of the city was singled out, in which the excess of mobile forms of Cu and Ni over the MPC in most samples was noted. The maximum excess of the content of mobile forms of Cu (3.85 MPC) was found in sample No. 38 (Figure 1), the maximum excess of the content of mobile forms of Ni (2.2 MPC) was detected in sample No. 47.

Table 1: Results of ecological-geochemical and microbiological analysis of Kogalym soil samples.

| Parameters | Minimum value/ Maximum value | Average value | Standard deviation (S) |
|---|---|---|---|
| | Metal concentration, mg/kg of soil | | |
| $C_{Ni}$ | 0.24/8.73 | 2.96 | 0.50 |
| $C_{Cu}$ | 0.16/11.55 | 2.60 | 0.55 |
| $C_{Pb}$ | 0.08/2.93 | 0.99 | 0.13 |
| $C_{Zn}$ | 1.76/26.01 | 12.15 | 2.60 |
| The amount of microorganisms, CFU/g of soil | | | |
| TNHM | $5.0 \times 10^3/1.0 \times 10^{10}$ | $1.5 \times 10^5$ | 0.70 |
| Number NFM | $1.0 \times 10^4/1.0 \times 10^7$ | $4.5 \times 10^5$ | 1.30 |
| AMM | $2.0 \times 10^4/5.0 \times 10^{10}$ | $1.9 \times 10^5$ | 0.95 |
| DNM | $5.0 \times 10^3/5.0 \times 10^6$ | $5.4 \times 10^4$ | 1.45 |

The average concentration of mobile forms of heavy metals in background samples was significantly lower than the MPC (tens and hundreds of times), amounting to: Cu and Ni – 0.18 mg/kg, Zn – 0.04 mg/kg; Pb – 0.61 mg/kg of soil.

As our studies have shown (Table 1), the soils of Kogalym didn't differ in the abundance of heterotrophic microorganisms on the scale of D.G. Zvyagintsev (1978), very depleted ($<1 \times 10^6$ CFU/g of soil) and depleted ($1\text{-}2 \times 10^6$ CFU/g of soil) by ammonifying bacteria; very depleted ($<1 \times 10^6$ CFU/g of soil) by denitrifying microorganisms, very depleted ($<2 \times 10^6$ CFU/g of soil) and depleted ($2\text{-}4 \times 10^6$ CFU/g of soil) by nitrogen-fixing microorganisms. The number of nitrifying microorganisms was low in both urban and background soil microbiocenoses, namely less than 1000 CFU/g of soil. Nitrifying microorganisms in the amount of $4.0 \times 10^4$ and $2.0 \times 10^5$ CFU/g of soil, respectively, were detected only in two samples of urban soils of the central residential zone of Kogalym (No. 4 and 15).

The average number of cultivated aerobic heterotrophic microorganisms in urban soils of the studied territory was $1.5 \times 10^5$ CFU/g of soil, ammonifying microorganisms – $1.9 \times 10^5$ CFU/g of soil, which was 3 times higher than in background soil samples ($5.1 \times 10^4$ and $6.5 \times 10^4$ CFU/g of soil, respectively). Several unusual sites were found in the residential part of the city with an extremely high number of ammonifiers: samples No. 1, 3 ($3.0 \times 10^8$ CFU/g of soil) and sample No. 11 ($5.0 \times 10^{10}$ CFU/g of soil). In the Northern and Eastern Industrial zone of the city, the content of ammonifiers was minimal.

The average content of denitrifying microorganisms in the urban soils of the Kogalym territory was 1.3 times lower than in background samples, amounting to $5.4 \times 10^4$ and $7.0 \times 10^4$ CFU/g of soil, respectively. This microbiological indicator did not vary much in the urban soils of the studied territory. Three sites in the residential central part of the city were characterized by elevated values: samples No. 17, 20 and 21 ($1\text{-}5 \times 10^6$ CFU/g of soil). As well as the number of ammonifying microorganisms, a reduced content of denitrifying microorganisms was found in industrial zones in the east and north-east of the Kogalym territory with a minimum amount in samples No. 28, 38, 39, 43, 44.

The average content of nitrogen-fixing microorganisms in urban soils was significantly higher (about 45 times) than in background samples, amounting to $4.5 \times 10^5$ and $1.0 \times 10^4$ CFU/g of soil, respectively. Several unusual sites were found in the residential part of the city with an increased number of nitrogen fixators: samples No. 2 and 14 ($5.0 \times 10^6$ CFU/g of soil); samples No. 12 and 19 ($1.0 \times 10^7$ CFU/g of soil), which may be associated with artificial landscaping of the urban area, the development of rhizosphere and symbiotic microorganisms. In the Eastern Industrial zone, there was a reduced content of nitrogen fixers with a minimum amount in samples No. 40, 41 and 45.

It's a well known fact that low concentrations of heavy metals in the soil can stimulate the growth and activity of metabolic processes in microorganisms and increase microbial biomass, and at higher concentrations they become toxic and can contribute to a decrease in microbial biomass, a decrease in the number of soil bacteria (Sorokin et al., 2009; Bagaeva et al., 2013). We hadn't found any strong divergence in the number of heterotrophic, ammonifying, denitrifying, nitrogen-fixing and nitrifying microorganisms in the microbocenoses of the studied urban soils, which could be observed when man-made pollutants were exposed to the soil. However, the ecological state of the soils in the Eastern Industrial District of Kogalym is not entirely favorable. The excess of mobile forms of Cu and Ni over the MPC was detected in most samples, as well as the maximally reduced number of nitrogen cycle microorganisms: ammonifying, denitrifying and nitrogen-fixing. The results obtained are consistent with the literature data that high doses of copper inhibit the processes of ammonification, denitrification and nitrification (Magalhães et al., 2007).

As a result of the study of the taxonomic diversity of soil microbocenoses of the city of Kogalym, the presence of bacteria of the phylum Bacillota, Actinomycetota, Pseudomonadota was shown in them. The main phylum was Bacillota, represented by bacteria of the genus *Bacillus* (10 species) and *Kurthia* (2 species). The phylum Actinomycetota was represented by bacteria of the genus *Aureobacterium* (2 species) and *Curtobacterium* (2 species). Only one species of bacteria of the genus *Pseudomonas* has been detected. Microorganisms of northern soils develop in a cold climate, in relatively nutrient-poor soils. G. A. Evdokimova showed that the adaptation of the microbiota to extreme natural conditions is expressed in limiting its diversity (Evdokimova, 2014). Heterotrophic microorganisms isolated from the urban soils of Kogalym were represented by 8 species, among which representatives of the genus *Bacillus* dominated, such as: *B. funiculus*, *B. niacini*, *B. okuhidensis*, *B. pseudalcaliphilus* and *B. psychrotolerans*. *Bacillus* are saprotrophic spore–forming bacteria that are typical inhabitants of soils, they actively participate in the decomposition of organic substrates and almost always dominate both polluted and uncontaminated soils with petroleum hydrocarbons and heavy metals (Dos Santos et al., 2011). Two representatives of the genus *Kurthia* were also identified: *K. gibsonii* and *K. zopfii* and one species of the genus *Aureobacterium* – *A. liquefaciens*. In the group of heterotrophs, the ratio of share of microorganisms was approximately the same (Figure 2a). The ratio of gram-positive spore rods and gram-positive spore rods was 2:1. Hydrocarbon-oxidizing microorganisms in the soil microbiocenoses of Kogalym were represented by 5 species, among which species of the genus *Bacillus* also dominated: *B. alcalophilus*, *B. funiculus*, *B. halodurans*, *B. niacini*, *B. psychrodurans*. The microorganisms *B. funiculus* and *B. niacini* were found in the group of heterotrophic bacteria. In addition to bacteria of the genus *Bacillus*, microorganisms of the species *Curtobacterium flaccumfaciens* were also detected, the frequency of occurrence of which was 17 %. In the HOM community, there was a slight prevalence of *B. funiculus* and a lower content of *B. alcalophilus*, other microorganisms were represented in equal proportions (Figure 2b).

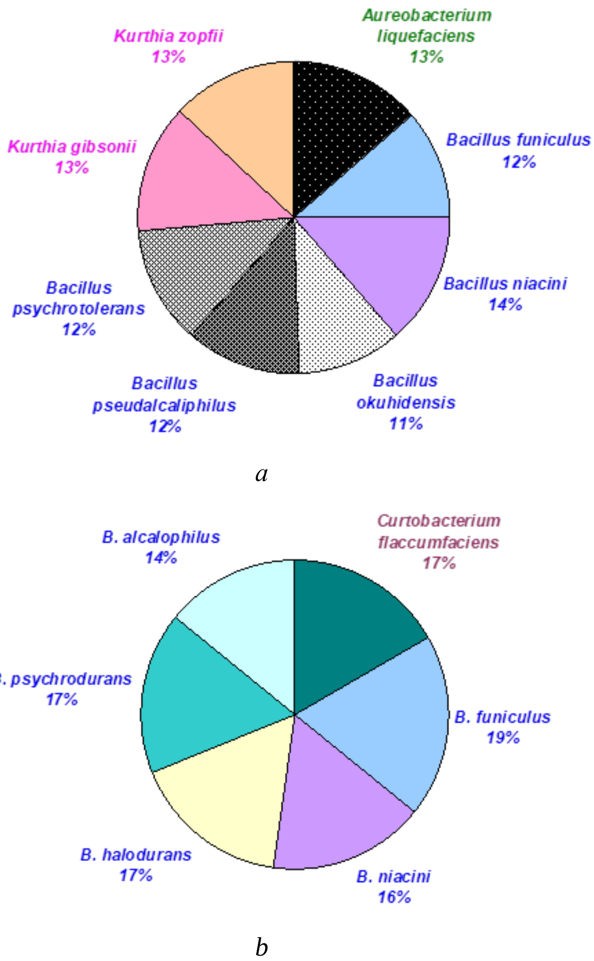



Figure 2: Taxonomic composition of heterotrophic (a) and hydrocarbon-oxidizing (b) microorganisms in the soils of the city of Kogalym.

Curtobacterium flaccumfaciens is described as a bacterium that stimulates plant growth. Such bacteria improve the growth of various crops and are used as a biofertilizer (Phour et al., 2020).

Bacteria of the genus *Bacillus* are widely known as active biodestructors of hydrocarbons capable of utilization of aliphatic and aromatic compounds with increased mineralization and alkalinity (Pleshakova et al., 2018).

The active reproduction of bacillus cells in soils and an increase in the diversity of the species composition of the spore-forming microbiota under conditions of oil pollution and other types of anthropogenic impact is noted (Pershina et al., 2016; Wolińska et al., 2016).

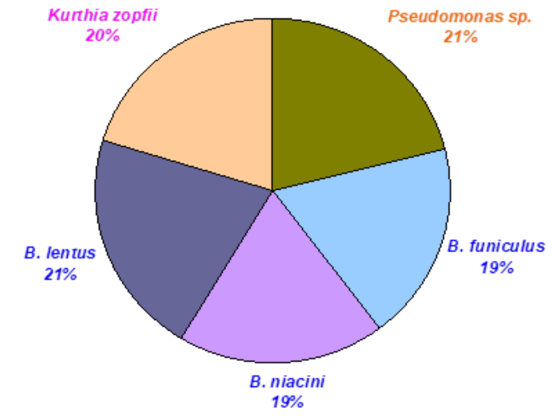
It has been proved that bacteria of the genus *Bacillus* isolated from coal dumps are resistant to heavy metals (Ni, Cr and As) and polycyclic aromatic hydrocarbons, and are also capable of degrading oil pollutants (Faskhutdinova et al., 2021).

Most of the selected isolates grew well at a low temperature (+10 °C), at a pH of 9-10, used vaseline oil (1 and 5 %) as the only source of carbon and energy. *B. psychrodurans* also grew well at a 10 % concentration of vaseline oil in the culture medium. Therefore, the various types of bacilli isolated by us from the soils of Kogalym may have prospects for their use in biotechnologies for remediation of soils contaminated with oil and heavy metals, as well as for the disposal of oil and drilling sludge.

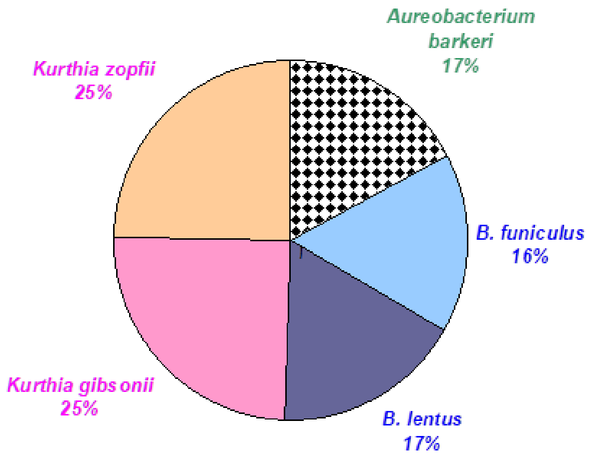
When considering the species diversity of ammonifying microorganisms (Figure 3a), the Bacillota phylum dominated, represented by bacteria of the genus *Bacillus* and *Kurthia* with the same frequency of occurrence (19-21 %). With a similar frequency (21 %), bacteria of the genus *Pseudomonas* were encountered, the species of which weren't identified.

The genus *Kurthia*, represented by the species *K. gibsonii* (25 %) and *K. zopfii* (25 %), dominated the group of denitrifying microorganisms in terms of the abundance index (Figure 3b). The frequency of occurrence of representatives of the genus *Bacillus* (*B. lentus* and *B. funiculus*) and the bacteria *Aureobacterium barkeri* was lower, amounting to 16-17 %.

The taxonomic structure of nitrogen-fixing and nitrifying microorganisms wasn't diverse. Nitrogen-fixing microorganisms were represented by two species: *K. gibsonii* (54 %) and *B. decolorationis* (46 %), among nitrifying microorganisms *B. alcalophilus* dominated.




*a*




*b*

Figure 3: Taxonomic composition of ammonifying (a) and denitrifying (b) microorganisms in the soils of the city of Kogalym.

## 4 CONCLUSIONS

The results of the biogeochemical survey of the territory of the city of Kogalym showed that the priority pollutants among the measured heavy metals are mobile forms of copper and nickel. The soils of the Eastern and Northern industrial zones of the city have the highest level of accumulation of these heavy metals. The maximally reduced number of nitrogen cycle microorganisms: ammonifying, denitrifying and nitrogen fixing in these industrial zones indicates a gradual change in the microbiological properties of urban soils, which may adversely affect the functioning of the soil cover as an integral part of the urban ecosystem.

The condition of such soils requires attention and constant monitoring, it is advisable to conduct their comprehensive monitoring.

As we have established, the soils of residential areas of the city of Kogalym are subjected to less man-made load, therefore their ecological condition is more stable compared to industrial territories within the city.

The present studies have shown that bacteria of certain genera belonging to the phylum Bacillota, Actinomycetota and Pseudomonadota predominated in the structure of microbial communities of the soils of the city of Kogalym. According to the abundance index, the group of heterotrophic and hydrocarbon-oxidizing microorganisms was dominated by bacteria of the genus *Bacillus*, which is probably due to their ability to form endospores and survive during a period of high environmental stress (Kumar et al., 2015). The following species had a high frequency of occurrence: *B. niacini*, *B. lentus*, *B. funiculus*, *B. decolorationis*, *B. halodurans*, *B. psychrodurans*. In the group of denitrifying microorganisms, the genus *Kurthia*, represented by the species *K. gibsonii* and *K. zopfii*, dominated in terms of the abundance index. Ammonifying microorganisms were represented in equal proportions by bacteria of the genus *Bacillus*, *Kurthia* and *Pseudomonas*. Bacteria of these genera are among the most resistant to soil contamination by various xenobiotics and can be used for bioremediation of disturbed soils.

The taxonomic diversity of the soil microbocenoses of the city of Kogalym indicates a significant bioremediation potential for the restoration and functioning of ecosystems, therefore, it is necessary to pay special attention to maintaining the stability and sustainability of microbial communities.

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