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Research Article

Microbiocenosis of Anthropogenically Transformed Soils

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Abstract: A microbiological examination of the soils, polluted with different types of urban wastewater (Tashkent city), aiming determination of the microbial diversity and characterization of the bacterial community was carried out. The examination was conducted with use of classical microbiological methods with cultivation of samples on elective nutrient media. The soil sampling was carried out during winter season and period of plants' vegetation. As result of examination the qualitative and quantitative characteristics of the bacterial community were determined and the microbial diversity was established. The predominant microorganisms of this community, capable to active functioning at chloride concentrations of the environment for up to 10 % and possessing high remediation potential towards biological and chemical pollutants, have been isolated. Rare strains belonging to the genus Amycolatopsis, which, in contrast to typical representatives of this genus, have the ability to form a water-soluble blue pigment, have been isolated. It was established that typical representatives of microbial biota, such as heterotrophic microorganisms Bacillus, Pseudomonas and actinomycetes, possess significant remediation potential towards biological and chemical pollution. It was determined that pollution of the soil caused by anthropogenic factors at the end of the day leads to decrease in species diversity and changes in composition of the soil microbiocenosis. The results obtained convincingly testify perspectives of biomonitoring and possible use of microorganisms in the processes of soil rehabilitation. The introduction of pollutant-resistant microorganisms, which are capable to degrade them, may become a practical approach for soil cleansing in the future.

Keywords: Microbial Diversity, Microbiocenosis, Pollution, Anthropogenic Impact, Reflecting Soil Stability.

1. INTRODUCTION

The growth of cities and their increasing population density are inextricably bound to heavy anthropogenic impact on the environment and ecological situation. Growing population density contributes to the generation of more pollutants and waste, which promotes to the likelihood of increased impact of pollutants on the ecosystem as a whole. Globally, it is estimated that by 2050, approximately 68 % of the world's population will live in urban areas [1]. The importance of natural processes within the ecosystem, the necessity to study microbiocenoses with aim of possible impact and treatment of excess urban runoff, the study of possibility of using microorganisms to improve air and water quality are often overlooked within the context of urbanization [2].

The expansion of urban areas globally increases anthropogenic impacts on soil and underlines the important role of urban areas in securing the sustainable future. Thus, urban soil becomes increasingly important in providing a wide range of ecosystem conditions for life [3]. Cities around the world have begun to improve existing infrastructure with the latest technologies to manage storm water flow, to improve air quality and to provide additional social and economic benefits, at same time increasing levels of all kinds of waste. First of all, an increase in the level of pollutants affects soil microbiocenoses, since microorganisms are very sensitive indicators and immediately react to various changes in the environment, resulting in a high dynamic of microbiological indices [4-7].

The heavy anthropogenic impact and the high velocity of urbanization lead to a decrease in the

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ability of urban soil to recover from negative impacts. Morphological and structural changes in microbial populations and changes in their biochemical activity serve as a reflection of the anthropogenic impact on the ecosystem. Microbial reactions to the impact of anthropogenic factors are manifested quickly and quite clearly, which makes it possible to quickly identify the most vulnerable ecological zones, to predict their state while maintaining anthropogenic impact and to arrange the measures necessary to mitigate this impact [8].

Soil pollution leads to a deterioration of their properties, causes a decrease in biological activity, therefore, the identification of individual groups of microorganisms that can withstand toxic effects and the use of their adaptive capabilities can help cleanse soil from the products of technogenic pollution [9]. It is supposed that, even with a very strong bacterial presence, the process of self-cleaning of soil can take several months [10]. In a difficult ecological situation in the city, including cases of disturbed soil properties, saprotrophic microbial communities continue to function due to their high biological and ecological plasticity [11]. It was also established that humidity affects the quantity and activity of microbial biomass, controls the availability of oxygen for microorganisms, causes periods of microbial stress in water, and may also contribute to the destruction of organic matter, which leads to increased availability of carbon for soil microorganisms [8]. Thus, an attention should be focused on supporting and restorative processes that stimulate the functioning of soil and soil ecosystems [12, 13]. It is also possible that in order to assess the microbial community in the future, it would be necessary to determine the source of organic matter and to establish the relationship between vegetation, contaminated soil and microbial community.

In this regard, the purpose of this work was to identify the microbial diversity and to characterize the bacterial community inhabiting soils of the contaminated areas located at different distances from the Bozsu wastewater treatment plant (Tashkent).

2. MATERIALS AND METHODS

2.1 Sampling

The object of research were samples of serozem soil from zones of different levels of pollution, located both on the territory of the Bozsu wastewater treatment plant (BWTP) itself and directly adjacent to it. Five soil samples were collected and analyzed in total (Table 1). Upper soil layer (0 - 20 cm) was subjected to microbiological analysis. Soil samples were collected according to “the envelope” method from 25-point samples. The soil sampling was carried out in the winter and spring.

2.2 Chemical Analysis

The soil samples were air dried, ground and sieved through a sieve (1 mm) before analysis. The samples were dried to a constant weight in an oven at 105 °C. Water extracts were prepared for the analysis: 30 g of soil + 150 ml of distilled water. Acidity and electrical conductivity were measured with a pH-EC meter (Hannah, Germany) directly in aqueous extracts (suspensions) after 1 min stirring with a glass rod.

Anions and cations were measured in filtered samples, filtered through “blue ribbon” filter. Nitrates and phosphates were measured photometrically

Table 1. Collecting sites and characteristics of samples

Samples	Sampling coordinates	Characteristics of the sampling site
2 sample	41°15′44.07″N 69°07.44′57″E	50 m from sump (zone of high contamination level)
3 sample	41°15′45.13″N 69°07.49′01″E	100 m from sump (sludge)
5 sample	41°15′42.41″N 69°08.17′7″E	200 m from sump (zone of medium contamination level)
6 sample	41°15′42.98″N 69°08.21′18″E	250 m from sump (fertilizer)
7 sample	41°16′12.66″N 69°08.11′15″E	500 m from sump (zone of low contamination level)

(Riele 520). Mobile sodium and potassium, as well as the chlorides content, were measured using ion-selective electrodes (Elite-031 (K^+); Alice-112Na (Na^+); Elite-261 (Cl^-)) potentiometrically on an ion meter (Expert-001.3, Russia) (Table 2).

2.3 Nutrient Media

To isolate microorganisms and determine the microbial landscape in the processes of destruction of pesticides, the standard Beef Extract Peptone, Czapek-Dox, Sabouraud, Endo, Giltai, Ashby, Postgate nutrient media were used (HiMedia, India).

2.4 Microorganisms

The structure of bacterial complex was characterized using physiological, biochemical and morphological indicators of individual cultures [14]. Taxonomic identification of bacteria was carried out according to the Bergey's manual of determinative bacteriology [15]. Taxonomic identification of micromycetes was conducted according to morphological and cultural features [16, 17].

3. RESULTS AND DISCUSSIONS

Soil-borne microorganisms (bacteria, actinomycetes, microscopic fungi) play a leading role in the processes of self-regulation of the natural ecosystem. It is a well-known fact that the number of microorganisms in the soil is constantly changing. But, in any soil layer there is a certain natural level of microbiota, which can be considered as a pool, in other words, the reserve of soil-borne microorganisms, which is not provided with the energy substance necessary for continuous reproduction, but is in a state of maintenance. The size of such stock is not affected by seasonality; the pool is determined by the characteristics of the soil itself and environmental factors that affect the soil properties [18].

The study of the quantitative and qualitative composition of microbiocenoses is of particular interest in such studies. In qualitative terms, the microbial communities of the studied sites are distinguished by high biodiversity (Figure 1).

Soil intensively accumulates a significant part of the pollutants that enter it and retains them for a long time. Heavy metals are fixed most strongly in the upper humus-containing horizons, that is, these toxicants are accumulated in the most fertile layer. The soil cover in the city carries out different ecological functions. The main and most important features of the urban soil are fertility and its suitability. Microbiological activity of

Table 2. Chemical indices of soil samples

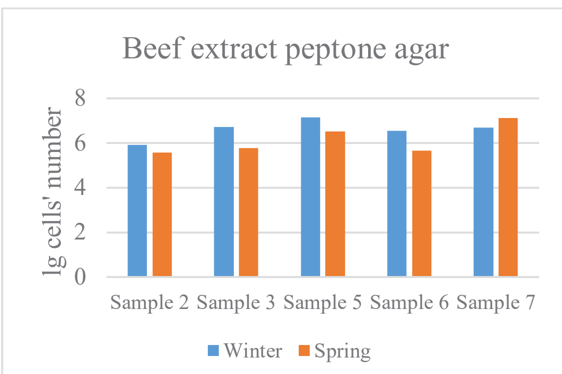
Sample	**pH**	**EC (mS/cm)**	**Mineral composition, % of dry weight**	**Losses during combustion (900 °C) (humus, carbonates, CO_2 and others), % of dry weight**	**NO_3^- (mg/kg dry weight)**	**PO_4^{3-} (mg/kg dry weight)**	**Cl^- (mg/kg dry weight)**	**Na^+, mobile forms (mg/kg dry weight)**	**K^+, mobile forms (mg/kg dry weight)**
2	7.8	0.27	86.00+0.010	14.00+0.010	14.92±0.83	8.21±0.07	1.08±0.12	45.80±0.26	651.67±12.58
3	7.9	0.33	85.76+0.039	14.24+0.039	5.23±0.19	8.13±0.02	11.22±0.58	26.33±0.35	72.50±0.50
5	7.9	0.13	86.29+0.050	13.71+0.050	6.11±0.28	2.08±0.01	3.35±0.10	7.92±0.13	225.00±0.50
6	7.8	0.2	84.37+0.019	15.63+0.019	5.61±0.02	8.31±0.03	11.32±0.43	12.38±0.03	44.43±0.47
7	7.9	0.13	86.68+0.034	13.32+0.034	5.48±0.26	4.77±0.01	4.80±0.21	13.52±0.23	327.00±1.00

soil determines the transformation, migration and accumulation of matter, energy and formation in the soil.

The results of studies reveal that bioremediation is usually associated with several key genera: Pseudomonadales, Actinomycetales, Flavobacteriales, Bacilleas and Clostridiales. It was established that in the soil microbiocenoses of all studied samples, the dominant position is occupied by the bacterial complex, among which prevail bacteria of the genera *Bacillus* and *Pseudomonas.* Coccal forms are rare, mainly representatives of genera Sarcina and Micrococcus. Thus, the carried-out survey revealed that diversity of microorganisms in the studied soil samples is somewhat reduced.

According to the results of the chemical analysis of the soil, these samples have a fairly high chloride and phosphate salinity (table 2). The carried-out studies made it possible to identify microorganisms that can grow at the salt concentration for up to 10 % and obtain salt-tolerant forms of bacteria exhibiting physiological activity at 5-7 % chloride; based on physiological and biochemical characteristics and MALDI-TOF analysis the isolated strains were identified as *Bacillus cereus* and *Pseudomonas aeruginosa* (Figure 2).

The survey of the contaminated areas revealed the presence of a high titer of nitrogen-fixing microorganisms, which were attributed to the genus Azotobacter (especially during spring) (Figures 3 and 4). It is necessary to note that oligonitrophils are quite common microorganisms in soils, which are capable to grow on a substrate with a low concentration of nitrogenous compounds and fix free atmospheric nitrogen (they take part in all the most important biological stages of organic decomposition).



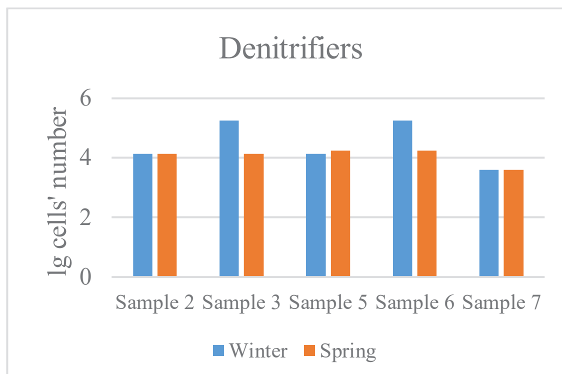


Fig. 1. Quantitative analysis of soil samples (total number of saprophytic and denitrifying microorganisms)

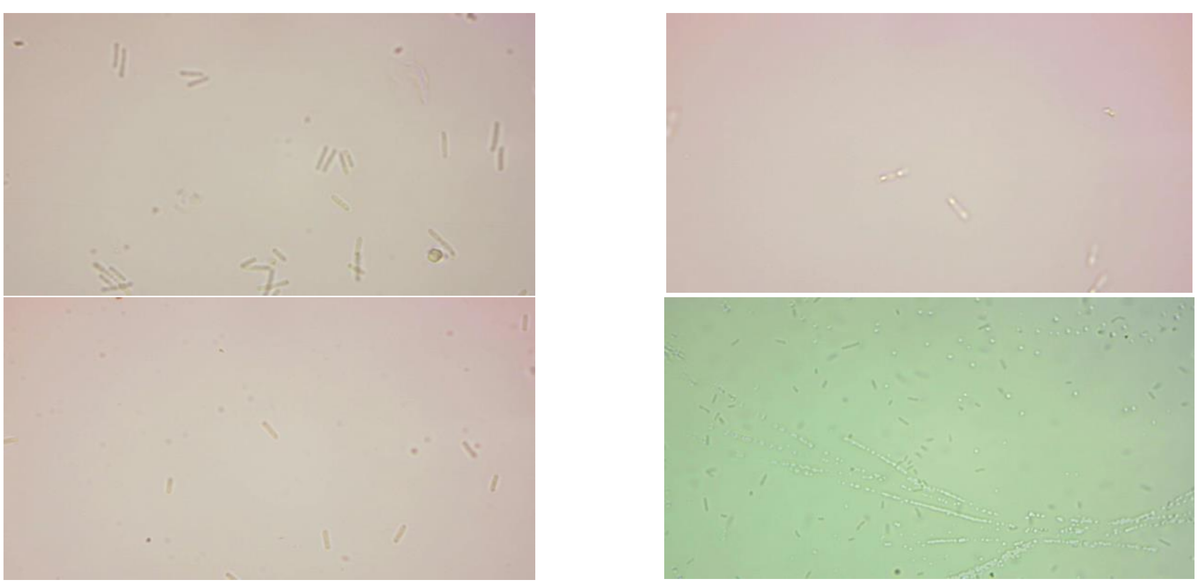


Fig. 2. Salt resistant strains *Bacillus cereus* and *Pseudomonas aeruginosa*

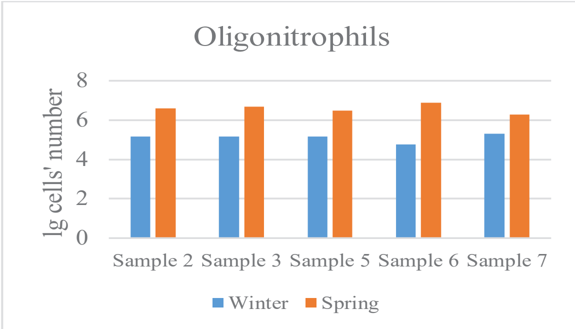


Fig. 3. Quantitative analysis of soil samples (oligonitrophils)

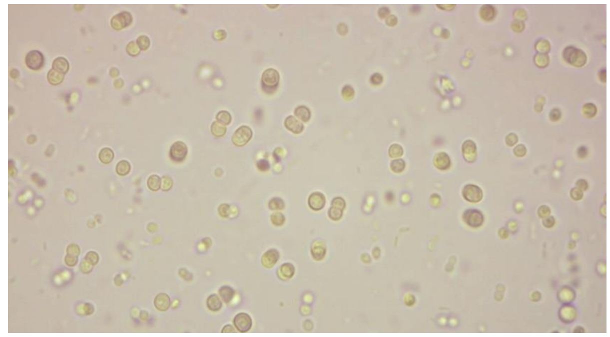


Fig. 4. *Azotobacter chroococcum* (x1000)

It should be noted that in polluted urban soil a significant change in the structure of microbial communities is noted, characterized by a decrease in the proportion of physiologically active bacterial cells, in comparison with background soils. It was established that micromycetes composes a significant part of the identified biocenoses; among fungi there were active forms of Trichoderma genus, as well as representatives of the genera Aspergillus and Penicillium, an increase in the titer of which, as a rule, is a specific reaction of the microbiota to urbanization. It is known that representatives of the genus Penicillium, as well as dark-colored populations of Azotobacter chroococcum, quite active in the soils of polluted urban zones, which is a specific response of the microbiota to urbanization [19].

Analysis of the development of sulfate-reducing microorganisms showed their presence in the soil samples in an insignificant amount. Only a slight increase was observed during the spring in the sample 5 (Figure 5).

Actinomycetes represent an essential link in the trophic chain of the ecosystem, carrying out the functions of microbes-decomposers. At the initial stage of the intake of organic matter actinomycetes destroy the hard-to-reach structural components of plant and animal origin and, together with bacteria, perform in the ecosystem the function of processing organic matter that has entered the soil. The main role of mycelial prokaryotes is the decomposition of complex polymers (lignin, chitin, xylan, cellulose, humic compounds) [20-23]. An increase in the number of soil-borne actinomycetes occurs at the late stages of microbial succession, when the biomass of fungi begins to decrease [24].

The participation of actinomycetes in the decomposition and synthesis of humic substances in the soil has been repeatedly noted [25, 26]. There is information about the use of humic acid polyphenols by actinomycetes in the presence of available carbon sources. Some representatives of the genera Nocardia, Micromonospora are able to oxidize humates, taking part in the mineralization of humic substances in the soil. Actinomycetes are involved in the accumulation of biologically active substances in the soil [27] and the formation of the nitrogen balance of soils [28]. It was established that many representatives of actinomycetes inhabit the studied soil samples, majority belonging to the genus Streptomyces (Figure 6).

The presence of various pigmented forms of actinomycetes, which is typical for soils with severe pollution, including heavy metal ions, should be noted separately (Figure 7).

There also were identified rare forms belonging to the genus Amycolatopsis, which possess the ability, in contrast to typical representatives of this genus, to form a water-soluble blue pigment (Figure 8).

It is known that sanitary and hygienic monitoring is directly related to the microbiological properties of the soil, since spores of fungi, actinomycetes, bacteria, as well as other life forms of microorganisms' resistant to insolation, are transported by air with soil dust. A significant change in the structure of microbial communities occurs, the ratio of taxa changes and new dominants appear in heavily polluted urban soils. Especially, in case of the complex household pollution, enterobacteria are detected in significant quantities (genera *Escherichia,* and *Enterobacter*).

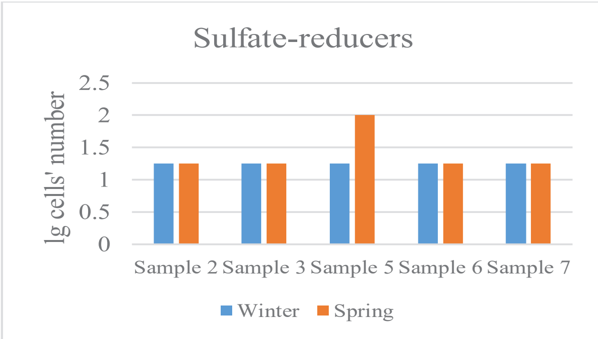


Fig. 5. Quantitative analysis of soil samples (sulfate-reducing microorganisms)

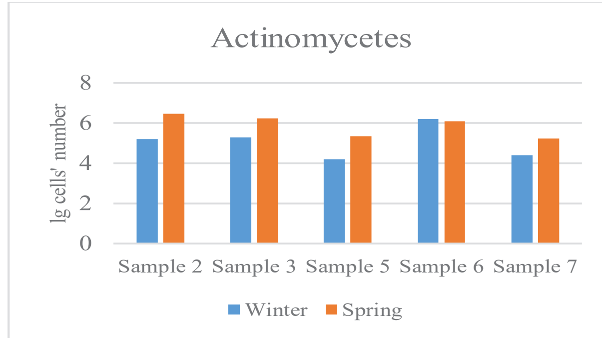


Fig. 6. Quantitative analysis of soil samples (actinomycetes)

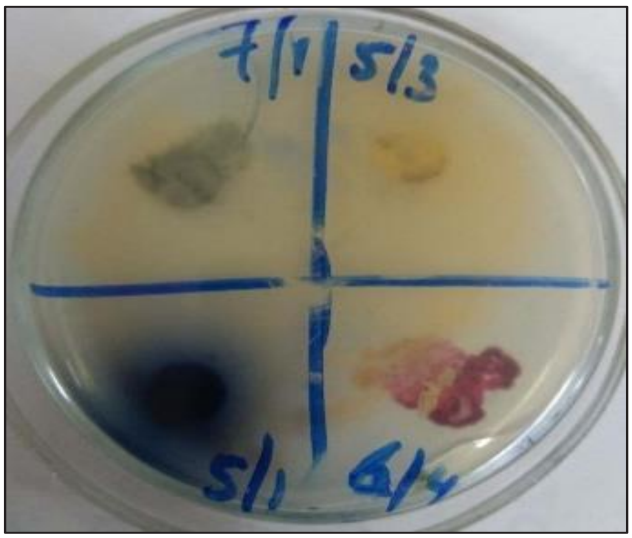


Fig. 7. Pigmented forms of actinomycetes isolated from the soils of BWTP contaminated with heavy metals

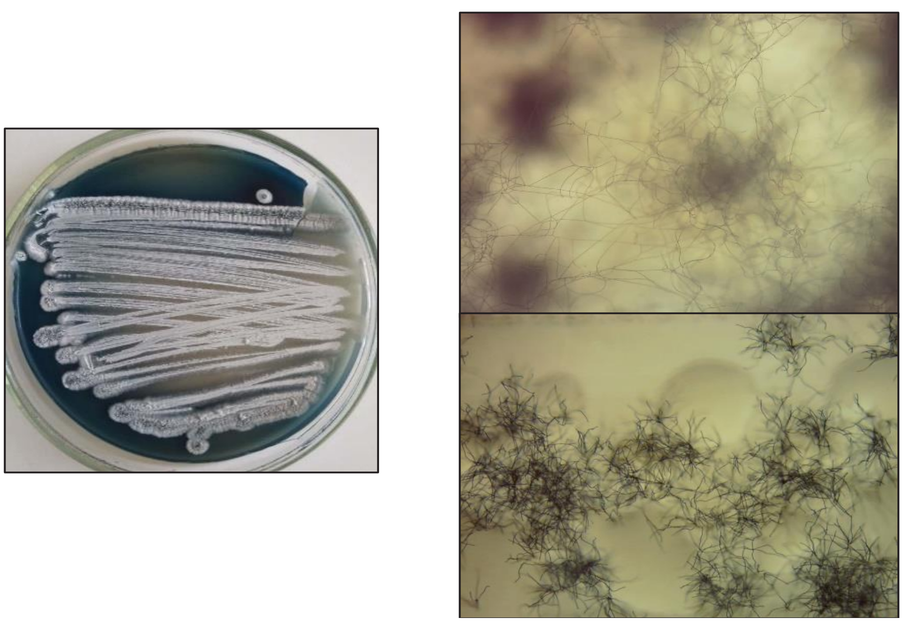


Fig. 8. Representative picture of *Amycolatopsis* genus (x400)

It is very important in microbiological studies to identify such a sanitary-indicative microorganism as Escherichia coli and other species belonging to *E. coli* bacteria group. Our study revealed that such bacteria were present in significant quantities in soil samples, especially sample 3 and 6; their titer reached 5.2 x10^3 and 1.5 x10^4 CFU/g soil in winter and increased up to 2.36x10^4 CFU/g soil during spring (Figures 9 and 10).

The presence of *E. coli* bacteria is an indication of possible contamination by pathogenic microorganisms. The source of origin of coliform bacteria may be not only the excrement of warm-blooded animals, but also vegetation and soil.

In many cases, the soil under study is a kind of depot and accumulator that ensures long-term preservation of both pathogens and soil-borne microorganisms. The duration of the preservation of pathogens in a viable state is determined by the self-cleaning ability of the soil: its combined biological, chemical, physical, suppressive and other properties. The untreated sewage, non-composted animal waste and human waste products used as local organic fertilizers pose a constant threat to soils. Their widespread disinfection and disposal should be an obligatory part of the regional social and environmental policy.

4. CONCLUSION

Anthropogenic changes in the soil can be traced based on the results of environmental monitoring of the complex of soil-borne microorganisms. The state of the soil of urban areas requires special attention, since the influence of anthropogenic processes on the soil system leads to a change in almost all of its components, from agrochemical and physical properties to microbiological and biochemical

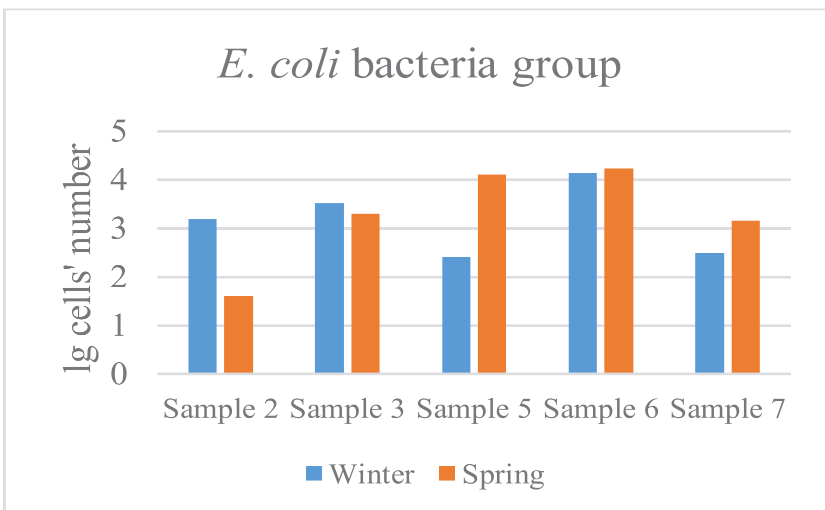


Fig. 9. Quantitative analysis of soil samples (*E. coli* bacteria group)

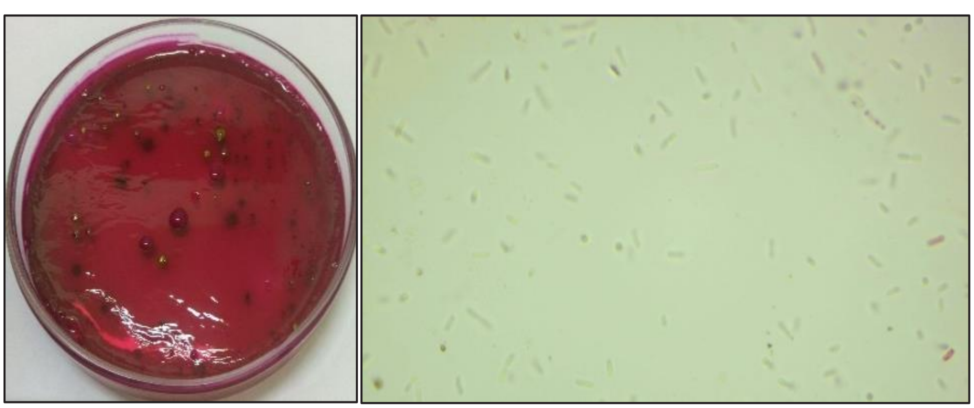


Fig. 10. Colonies of *E. coli* bacteria group and *E.coli* (x1000)

indicators, depriving the soil cover of the ability to perform important ecological functions.

As a result of the studies, it has been established that such typical representatives of microbiota as heterotrophic microorganisms related to genera *Bacillus* and *Pseudomonas*, and actinomycetes possess significant remediation potential. Moreover, soil contamination of the studied remediation areas caused by anthropogenic reasons ultimately leads to a decrease in species diversity and a change in the species composition of soil microbocenosis. A stable and resilient microbial community is of great importance for the restoration and functioning of the ecosystem, in this regard, it is necessary to pay special attention to the effective conservation of endangered ecological communities [29]. The results obtained undoubtedly reveal the prospects for biomonitoring and the possible use of microorganisms in soil cleaning processes. A practical approach in the future for cleaning soils from various kinds of contaminants may be the introduction of contamination-resistant microorganisms that can decompose them.

5. CONFLICT OF INTEREST

The authors declared no conflict of interest.

6. REFERENCES

1. J.A. Ocampo, A. Chowdhury, and D. Alarcón (Eds.). The World Economy Through the Lens of the United Nations. *Oxford University Press, Oxford, UK* (2018).
2. J.A. Aitkenhead-Peterson, M.K. Steele, and A. Volder. Services in natural and human dominated ecosystems. *Urban ecosystem ecology* 55: 373-390 (2010).
3. R. O'Riordan, J. Davies, C. Stevens, J.N. Quinton, and C. Boyko. The ecosystem services of urban soils: A review. *Geoderma* 395: 115076 (2021).
4. M. Kim., M.S. Hyun, G.M. Gadd, and H.J. Kim. A novel biomonitoring system using microbial fuel cells. *Journal of environmental monitoring* 9(12): 1323-1328 (2007).
5. B.R. Glick. Using soil bacteria to facilitate phytoremediation. *Biotechnology advances* 28(3): 367-374 (2010).
6. J.L. Joyner, J. Kerwin, M. Deeb, G. Lozefski, B. Prithiviraj, A. Paltseva, J. McLaughlin, P. Groffman, Z. Cheng, and T.R. Muth. Green infrastructure design influences communities of urban soil bacteria. *Frontiers in microbiology* 10: 982 (2019).
7. O.B. Ojuederie, O.S. Olanrewaju, and O.O. Babalola. Plant growth promoting rhizobacterial mitigation of drought stress in crop plants: Implications for sustainable agriculture. *Agronomy* 9(11): 712 (2019).
8. I. Brianskaia, V. Vasenev, and R. Brykova. Soil organic carbon stability of urban soils and of floodplain soils under different hydrothermal conditions. *In EGU General Assembly Conference Abstracts*, 663 (2020).
9. G. Satchanska, I. Ivanova, V. Groudeva, E.N. Pentcheva, T. Kerestedjian, and E. Golovinsky. Culture-dependent approach for determining microbial diversity in soils from KCM/AGRIA region. *Comptes rendus de l'Academie bulgare des Sciences* 58(4): 409-416 (2005).
10. H. Hung, R. Kallenborn, K. Breivik, Y. Su, E. Brorström-Lundén, K. Olafsdottir, J.M. Thorlacius, S. Leppänen, R. Bossi, H. Skov, and S. Manø. Atmospheric monitoring of organic pollutants in the Arctic under the Arctic Monitoring and Assessment Programme (AMAP): 1993–2006. *Science of the Total Environment* 408(15): 2854-2873 (2010).
11. M.A. Tobor-Kapłon, J. Bloem, and De P.C. Ruiter. Functional stability of microbial communities from long-term stressed soils to additional disturbance. *Environmental Toxicology and Chemistry: An International Journal* 25(8): 1993-1999 (2006).
12. E. Dominati, M. Patterson, and A. Mackay. A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecological economics* 69(9): 1858-1868 (2010).
13. P.C. Baveye, J. Baveye, and J. Gowdy. Soil "ecosystem" services and natural capital: critical appraisal of research on uncertain ground. *Frontiers in Environmental Science* 4: 41 (2016).
14. A.I. Netrusov, M.A. Yegorova, and L.M. Zakharchuk. Praktikum po mikrobiologii (Practical guide-book on microbiology). *Academia, Moscow, Russia* (2005) (in Russian).
15. D.H. Bergey, J.G. Holt, P. Krieg, and P.H. Sneath. Bergey's Manual of Determinative Bacteriology. *Williams and Wilkins, Baltimore, MD, USA* (1994).
16. D.A. Sutton, A.W. Fothergill, and M.G. Rinaldi. Guide to clinically significant fungi. *Williams and Wilkins, Baltimore, MD, USA* (1998).
17. M.A. Litvinov. Opredelitel' mikroskopicheskih pochvennyh gribov (Guide to microscopic soil-

borne fungi), *Ripol Klassik, Moscow, Russia* (2013) (in Russian).

18. T. Bahrampour, and V.S. Moghanlo. Evaluation of soil biological activity after soil contaminating by crude oil. *International Journal of Agriculture: Research and Review,* 2(6): 671-679 (2012).
19. V.S. Artamonova. Features of microbiological properties of soils in urbanized areas. *Sibirskiy ekologicheskiy zhurnal (in Russian)* 9(3): 349-354 (2002).
20. A.J. McCarthy, and S.T. Williams. Methods for Studying the Ecology of Actinomycetes. *Methods in Microbiology* 22: 533-563 (1990).
21. D. Jendrossek, G. Tomasi, and R.M. Kroppenstedt. Bacterial degradation of natural rubber: a privilege of actinomycetes?. *FEMS Microbiology letters* 150(2): 179-188 (1997).
22. N. Williamson, P. Brian, and E.M.H. Wellington. Molecular detection of bacterial and streptomycete chitinases in the environment. *Antonie Van Leeuwenhoek* 78(3): 315-321 (2000).
23. L.R. Lynd, P.J. Weimer, W.H. Van Zyl, and I.S. Pretorius. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and molecular biology reviews* 66(3): 506-577 (2002).
24. L.M. Polyanskaya, and D.G. Zvyagintsev. The content and composition of microbial biomass as an index of the ecological status of soil. *Eurasian Soil Science / Pochvovedenie* 38(6): 625-633 (2005).
25. V.A. Chernikov. Diagnostika gumusovogo sostoyaniya pochv po pokazatelyam strukturnogo sostava i fiziko-khimicheskim svoystvam (Diagnostics of the humus state of soils by indicators of the structural composition and physicochemical properties). *Agricultural Academy Press, Moscow, Russia* (1984) (in Russian).
26. C. Trigo, and A.S. Ball. Is the solubilized product from the degradation of lignocellulose by actinomycetes a precursor of humic substances?. *Microbiology* 140(11): 3145-3152 (1994).
27. W. Piepersberg. Pathway engineering in secondary metabolite-producing actinomycetes. *Critical reviews in biotechnology* 14(3): 251-285 (1994).
28. L.V. Kalakutskii, and L.S. Sharaya. Actinomycetes and higher plants. *Uspehi Mikrobiologii* 24: 26-65 (1990).
29. R.M.B. Harris, O. Carter, L. Gilfedder, L.L. Porfirio, G. Lee, and N.L. Bindoff. Noah's Ark conservation will not preserve threatened ecological communities under climate change. *PLoS One* 10(4): e0124014 (2015).